New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance
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**Purpose of review**
Unlike *Plasmodium falciparum*, *Plasmodium vivax* rarely causes severe disease in healthy travellers or in temperate endemic regions and has been regarded as readily treatable with chloroquine. However, in tropical areas, recent reports have highlighted severe and fatal disease associated with *P. vivax* infection. We review the evidence for severe disease and the spread of drug-resistant *P. vivax* and speculate how these may be related.

**Recent findings**
Studies from Indonesia, Papua New Guinea, Thailand and India have shown that 21–27% of patients with severe malaria have *P. vivax* monoinfection. The clinical spectrum of these cases is broad with an overall mortality of 0.8–1.6%. Major manifestations include severe anaemia and respiratory distress, with infants being particularly vulnerable. Most reports of severe and fatal vivax malaria come from endemic regions where populations have limited access to healthcare, a high prevalence of comorbidity and where drug-resistant *P. vivax* strains and partially effective primaquine regimens significantly undermine the radical cure and control of this relapsing infection. The mechanisms underlying severe disease in vivax malaria remain poorly defined.

**Summary**
Severe, fatal and multidrug-resistant vivax malaria challenge our perception of *P. vivax* as a benign disease. Strategies to understand and address these phenomena are needed urgently if the global elimination of malaria is to succeed.

**Keywords**
antimalarials, drug resistance, malaria, *Plasmodium vivax*, severe malaria

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**Introduction**

*Plasmodium vivax* threatens almost 40% of the world’s population, causing an estimated 72–390 million clinical infections each year [1,2]. Outside of Africa, *Plasmodium falciparum* and *P. vivax* almost invariably coexist and are often equally prevalent [3], yet the public health importance of *P. vivax* is frequently overlooked [4]. Recent calls for the global elimination of malaria have brought renewed vigour to malaria control programmes, but with this a realisation that the challenges in controlling and eliminating *P. vivax* are far greater than those for *P. falciparum*. In areas where intensive control measures have been implemented, the relative proportion of malaria due to *P. vivax* usually increases when compared with *P. falciparum* [5]. Several important biological differences account for these observations, in particular, the development of dormant liver stages (hypnozoites) causing recurrent blood stage infections (relapses), the early appearance of gametocytes and the greater transmission potential of *P. vivax* at low parasite densities. In the past two decades, the situation has been compounded by the emergence and spread of drug-resistant strains of *P. vivax* in Asia, South America and, more recently, in Africa [6–8,*9,10–13*]. The global prevalence and consequences of drug-resistant *P. vivax* remain poorly documented, reflecting both the inherent difficulties associated with the in-vivo test for relapsing malarial and the belief, often unsubstantiated, that chloroquine continues to be effective.

Although usually considered a benign infection, there is a growing appreciation that vivax malaria causes significant morbidity and inflicts a huge socio-economic burden on endemic countries [1]. Reports of severe and fatal malaria from the island of New Guinea and from India have reinforced this [14**–17**] and demand a reappraisal of the pathogenesis of this infection. In this article, we review the evidence describing severe manifestations of vivax malaria and the literature on the extent and
Epidemiology of severe vivax malaria

In coendemic regions, a significant proportion of patients admitted to hospital with malaria have *P. vivax* mono-infection. Recent studies from Indonesia, Papua New Guinea (PNG), Thailand and India show this figure to vary from 20 to 40% [14**,15**,17**,18]. *P. falciparum* remains the dominant species associated with classically defined severe malaria but, depending on the setting, *P. vivax* can account for up to a quarter of all severe cases detected [14**–16**]. A broad range of severe manifestations has been reported, some of which have been associated with subsequent death. In Papua, Indonesia, the overall mortality among those hospitalized with *P. vivax* is 0.8–1.6%, similar to that observed with pure *P. falciparum* (1.6–2.2%) [14**,15**].

Infants are particularly vulnerable to infection with *P. vivax*. Indeed in southern Papua, where *P. falciparum* is the most prevalent species, more infants are hospitalized with vivax malaria than with falciparum malaria [19]. In this age group, *P. vivax* infection is associated with an increased risk of severe anaemia [odds ratio (OR) = 2.4] and thrombocytopenia (OR = 3.3) compared with infants with *P. falciparum*. The risk to infants starts with in-uterus exposure, which is associated with maternal anaemia, prematurity and low birth weight, all of which are known to contribute to overall infant mortality [20,21].

Severe manifestations

Almost all of the severe manifestations of falciparum malaria have also been described in vivax malaria [4,22*]. Severe anaemia (haemoglobin level < 5 g/dl) is the most common in Papua (67–80% of cases) [14**,15**] and accounted for a third of severe cases in an Indian series [17**]. A study from Venezuela also found anaemia to be more severe and frequent in *P. vivax* infections compared with *P. falciparum* [23]. Although the associated mortality in Papuan patients with severe anaemia alone was low (0.4%), it rose considerably when other manifestations of severity were present (10–39%) [15**]. Recurrent bouts of *P. vivax* infection, whether from reinfection, relapse or recrudescence, result in the removal of both uninfected and infected red cells from the peripheral circulation. Haemolysis, increased red cell fragility [24], dyserythropoiesis and retention of erythrocytes within the spleen [25*,26], all contribute to severe anaemia, though their relative contributions are poorly defined.

*P. vivax* frequently affects the lung with cough occurring in the majority of patients [27]. Respiratory distress was the primary manifestation of severity in PNG where it was reported in 5% of children infected with *P. vivax* [16**], but is described less frequently elsewhere. In adults, acute lung injury has been described as the cause of acute respiratory distress in returning travellers with vivax malaria, most of whom had a diagnosis of *P. falciparum* coinfection excluded by PCR [27,28]. Inflammatory increases in alveolar–capillary membrane permeability [27–29] as well as mononuclear cell accumulation in the pulmonary microvasculature are likely to underlie this syndrome [22*]. In endemic areas, the majority of vivax-associated respiratory distress occurs in children, an age group in which acute lung injury is rare [15**,16**]. This suggests that though acute lung injury appears to be a major cause of vivax-associated respiratory distress in adults, other causes such as anaemia, acidosis and concurrent respiratory tract infections are likely to contribute to vivax-associated respiratory distress in children.

Jaundice and renal failure have been reported in series of adults from India [17**] and northern Papua [14**]. Coma occurs in association with *P. vivax* but is rare compared with its frequency in severe falciparum malaria [30,31]. Severe thrombocytopenia and acidosis have also been reported [28].

Although *P. vivax*–*P. falciparum* coinfection has previously been associated with a reduced risk of severe malaria, the recent studies from across New Guinea have shown an increased risk of severe malaria in mixed infections, particularly severe anaemia [14**,15**].

Association or causation?

Reports of severe vivax malaria challenge our understanding of *P. vivax* pathophysiology [22*]. Processes central to the development of severe disease in falciparum malaria, including high parasite biomass, widespread sequestration of cytoadherent parasites and reduced deformability of infected and uninfected red cells, are not found in vivax malaria (though a role for limited or transient cytoadherence has been proposed [22*,26–28]). However, there are a number of caveats in interpreting these reports of severe vivax malaria. As with severe falciparum malaria, incidental parasitaemia, sepsis and comorbidities are likely to be important contributory factors [32]. Furthermore, only one of the large descriptive studies was able to rule out microscopy errors and occult mixed plasmodium infections [17**].

The geographical variation in the risk of severe vivax malaria is striking. In temperate regions where relapses are infrequent [33] and in areas where early diagnosis and radical cure are readily available, the risk of severe disease from single infections is low and largely confined to acute lung injury [27,28]. In contrast, severe and fatal vivax malaria is more frequent in poorly resourced endemic...
regions where populations have limited access to healthcare and suffer from a complex array of comorbidities, nutritional, genetic and immunological disorders, all of which may contribute to the pathology. In many of these areas, *P. vivax* can be a chronic relapsing disease, similar to that described in temperate climates in the preantibiotic era when vivax malaria was known to cause a debilitating febrile illness that could last for years with appreciable morbidity.

Heterogeneity in parasite virulence may also play a role in the clinical spectrum of disease. Indeed, differences in the virulence of *P. vivax* strains were well described in the malaria therapy era, with some strains reportedly leading to recovery without treatment, whereas others were associated with case-fatality rates of 10–14% [34]. Recent laboratory studies raise the possibility that such virulence may be associated with chloroquine-resistant (CQR) *P. vivax* which, compared with chloroquine-sensitive isolates, have faster parasite growth rates [35], a phenotype correlated with severe falciparum malaria [36]. The risk of severe falciparum malaria and malaria mortality has been previously associated with the rise of chloroquine resistance in *P. falciparum* [37]; a similar pattern may now be occurring in parallel with the rise in CQR *P. vivax*.

**The importance of recurrent parasitaemia**
The radical cure of vivax malaria demands effective treatment of both the asexual stages of the parasite as well as the dormant hypnozoite stages. For the past 60 years, the only available treatment of *P. vivax* hypnozoites has been primaquine which has traditionally required a 14-day regimen that is associated with both poor adherence and effectiveness. The emergence of CQR *P. vivax* has exacerbated the problem. Studies in Papua, Indonesia reveal that 2% of patients receiving chloroquine monotherapy subsequently require admission to hospital [8], with 60–90% of patients having recurrent malaria within 28 days [8,9,38]. Such poor therapeutic efficacy is highly likely to contribute to the cause of severe disease, particularly that of severe anaemia. In addition, the haemodynamic and respiratory consequences of fever or anaemia or of both combined with other acute infections (for example pneumonia) or chronic diseases, common in endemic areas, may lead to decompensation with potentially fatal outcome from a *P. vivax* infection that might otherwise be uncomplicated [22].

**Drug-resistant Plasmodium vivax**
The first cases of CQR *P. vivax* were documented in 1989 [39], 30 years after reports of the emergence of CQR *P. falciparum*. This difference in the speed with which resistance develops is likely to reflect a number of intrinsic differences between the two species. Selective drug pressure, a crucial determinant of de-novo selection [40], is significantly lower in *P. vivax* infections, a consequence of asymptomatic parasitaemia and lower parasite biomass. In *P. falciparum* infections, gametocytogenesis occurs after the appearance of symptoms and is refractory to schizontocidal drugs, thus favouring the transmission of resistant genotypes. In contrast, *P. vivax* gametocytes appear early and are susceptible to schizontocidal agents, ensuring transmission occurs prior to drug selection.

**Diagnosis of drug resistance**
In the absence of empirical evidence to the contrary, it is often assumed that established chloroquine treatment protocols continue to be effective. Between 1966 and 2002, only 11% (47 out of 435) of published antimalarial drug trials assessed antimalarial efficacy in vivax malaria [41]. This reflects the challenges of conducting such studies and interpreting the clinical drug efficacy for *P. vivax*. Although three-loci genotyping has proved useful in defining recrudescence in *P. falciparum*, it has limited utility in *P. vivax* as recurrent infections with the same genotype can arise following both recrudescence and relapse, the latter occurring early in up to 80% of patients after treatment [9]. Clinical efficacy, therefore, has to be interpreted in light of the timing of recurrences, plasma drug concentrations, the half life of the drug and the early parasitolological response [6].

In-vitro drug susceptibility assays provide an alternative means of assessing drug susceptibility of *Plasmodium* spp. free from the confounders of host immunity, relapse and reinfection. However, the preferential invasion by *P. vivax* of young red blood cells limits its reproductive capacity and the ability to culture the parasite *ex vivo* [42]. Success has been achieved in evaluating the inhibitory effect of antimalarials on both asexual stages of the parasite from the human host after short-term cultivation [43–46]. However, isolates of *P. vivax* initially at the trophozoite stage are intrinsically resistant to chloroquine [35,47]. As *P. vivax* infections are typically asynchronous and the degree of asynchronicity varies between geographical locations, the in-vitro drug response needs to be interpreted according to the initial stage of the parasite and the duration of the assay [35]. Despite these difficulties, the current in-vitro susceptibility assay has shown utility in confirming the presence of emerging drug resistance [46,48–50], characterizing drug susceptibility profiles [50,51] and the screening of susceptibility to therapeutic agents [52].

**Geographical extent of chloroquine resistance**
The discovery of CQR *P. vivax* in PNG [39] was soon followed by reports from northern Papua, Indonesia [7,53]. Over the ensuing decades, high-grade CQR emerged in eastern Indonesia [8,38,54] and PNG [9]. The western
provinces of Indonesia have also reported reduced susceptibility, albeit to a lesser degree [55–57]. Low-level chloroquine resistance is present in Burma [12], Viet Nam [58,59], South Korea [60], Turkey [61], the horn of Africa [13], Madagascar [62], South America [10,63] and eastern India [64]. Although studies from Thailand have mostly recorded excellent cure rates [43,65], a recent large study on the western border found 10% recurrence at day 28 (F. Nosten, personal communication). Chloroquine appears to retain high efficacy against \textit{P. vivax} in most of India [66], Afghanistan [67], Pakistan [68] and Azerbaijan [69], though ongoing surveillance is warranted.

### Genetic basis of \textit{Plasmodium vivax} drug resistance

Preliminary studies on drug resistance in \textit{P. vivax} have focused on the orthologues of the transporter genes \textit{pfcr} and \textit{pfmdr1}, known to be key determinants in \textit{P. falciparum} resistance. Although chloroquine resistance does not appear to be associated with polymorphisms in \textit{pcr} [48,62,70], studies from Indonesia, Thailand and PNG found that a Y976F \textit{pvmdrl} polymorphism correlated with reduced susceptibility to chloroquine both \textit{in vitro} [48] and \textit{in vivo} [71]. However, as chloroquine resistance can occur in isolates with wild-type \textit{pvmdrl} [48,72] and cure can be achieved in the presence of the 976 mutation [62], \textit{pvmdrl} mutations are likely to be at best only minor determinants of resistance.

In contrast to chloroquine resistance, amplification of the \textit{pvmdrl} gene is associated with a two-fold reduction in \textit{in vitro} susceptibility to mefloquine [51]. Imwong \textit{et al.} found amplification of \textit{pvmdrl} in \textit{P. vivax} isolates and correlated this with the clinical use of mefloquine in Thailand and Myanmar [73]. Hence, even though chloroquine remains the standard treatment for \textit{P. vivax} in this region, the use of mefloquine for \textit{P. falciparum} and mixed infections is enough to exert selective pressure on \textit{pvmdrl}. Sequential acquisition of mutations in \textit{pcedfr} and \textit{pcdhps} is also observed where \textit{P. vivax} is exposed to intense antifolate pressure [71,74–76].

### Treatment options for chloroquine-resistant \textit{Plasmodium vivax}

Mefloquine and atovaquone–proguanil retain excellent efficacy against highly CQR \textit{P. vivax} [77–79], whereas amodiaquine monotherapy appears to be compromised [49]. Of the artesimemin combination therapies (ACTs), dihydroartemisinin–piperaine produces the lowest failure rates at day 28 [9*,80,81,82*]. In contrast, artesunate-sulfadoxine/pyrimehtamine is associated with recurrence rates in excess of 10% at day 28 [9*,76], and this rises to over 20% for artemether–lumefantrine [9*,82*]. As none of these antimalarial drugs has any efficacy on the hypnozoite stages of \textit{P. vivax}, the difference in recurrence rates is likely to be a consequence of the different terminal elimination half-lives of lumefantrine (~4 days), amodiaquine (18 days) and piperaine (28–35 days) exerting different posttreatment prophylactic effects on relapsing infection. Further studies to rationalize regimens containing asexual and hypnozoite activity are needed [83].

### Conclusion

\textit{P. vivax} produces an acute febrile illness with the potential to become chronic and relapsing if inadequately treated. In comparison with the more pathogenic \textit{P. falciparum}, \textit{P. vivax} rarely causes severe disease in healthy individuals from nonendemic countries or temperate regions. In contrast, recent reports from tropical endemic communities highlight a high risk of severe disease and an association with mortality. In some regions, the risk of severe disease caused by vivax malaria in patients reaching hospital approaches that of falciparum malaria. However, reports of vivax-associated severe disease need to be interpreted with caution as they are likely to be confounded by microscopy errors, coinfection with \textit{P. falciparum} and comorbidities such as bacterial sepsis and malnutrition. The crucial question remains: how much of the severe disease and mortality is associated with vivax infection and how much is actually attributable to \textit{P. vivax}? Confirmation of the latter demands a reappraisal of our understanding of the pathobiology of the parasite and exclusion of comorbidities. Even if \textit{P. vivax} is a secondary or contributory factor in the cause of severe disease, this still has important public health implications for poorly resourced endemic regions. Evidence is mounting that delay in diagnosis of \textit{P. vivax}, the application of partially effective treatment regimens and our inability to reliably cure the dormant stages of the parasite all cause significant host morbidity and contribute to the socio-economic burden of this neglected parasite. In this context, the emergence and spread of high-grade drug-resistant \textit{P. vivax} poses a major threat to endemic countries that must be quantified and contained if there is to be any realistic prospect of the global elimination of malaria.

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### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 504–505).

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