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***Plasmodium vivax* infects 130–435 million of the 2.6 billion people living at risk of infection. Recent studies suggest that vivax malaria can become lethal in a similar way to severe falciparum malaria. First-line therapies remain unchanged after 50 years. Despite evidence of failing chloroquine efficacy, little work has assessed the problem or explored alternative therapies. Primaquine treatment, the only therapeutic option against relapse, might also be failing. No licensed primary chemoprophylactic agent protects travelers from relapse. Misdiagnosis of species now affects clinical decisions resulting in inadequate therapy for *P. falciparum* and *P. vivax*. All of these factors demonstrate the lack of research on *P. vivax*.**

Investment and therapeutic options

Research investments reach across a broad spectrum of inquiry into the physiological, clinical, social and economic consequences of infectious disease burdens, as well as their treatment, prevention and control. Successful clinical management of potentially dangerous and prevalent infections surely represents the highest research priority. This review treats research on therapies as the principal indicator of broader research investments.

The quality of available therapies for dangerous infections reflects investments made in research on these diseases. The quality of therapy can be defined as the reliability of carefully analyzed clinical research demonstrating good safety, tolerability, efficacy and effectiveness. An even higher standard of quality provides understanding of metabolism, mechanisms of both therapeutic action and toxic side effects, and mechanisms of resistance by the target organisms. These facets of quality allow us to move forward with better therapies and, in turn, provide clinicians with options and a basis for making sound therapeutic management decisions. In other words, the hallmarks of infections that have received adequate attention and investment in research are the availability of drugs of predictable performance and of therapeutic options in the face of predictable or realized failure.

Against this backdrop *Plasmodium vivax* might be considered perhaps the most neglected, potentially dangerous and highly prevalent infection. The two current frontline therapies for *P. vivax*, chloroquine (CQ) and primaquine (PQ), have been in use since 1946 and 1950, respectively. These therapies are failing, but the risk of failure with either drug is not generally known. The mechanism of activity of either drug and the mechanisms of resistance remain unknown. The metabolism of PQ is little understood. PQ destroys red blood cells with an inborn deficiency of glucose-6-phosphate dehydrogenase (G6PD) – a disorder linked with endemic malaria and most prevalent where the drug is most needed – but the mechanism of toxicity or the metabolite responsible for it is unknown. There are some poorly characterized alternatives for CQ, and none for PQ. The strikingly poor quality of drugs available to treat vivax malaria reflects a lack of research investment in this infection.

If the marker of quality of therapeutics is applied to *P. falciparum*, the far more substantial investment in research on this parasite is evident. The past ten years have seen a much-needed and overdue proliferation of well-characterized therapeutic options for falciparum malaria. No one can argue against the higher research priority placed upon falciparum malaria therapies, given its greater lethality and perhaps prevalence. However, seeing the investment in treatment of falciparum malaria as directly linked and applicable to the problem of therapy of vivax malaria does not stand scrutiny. No fact supports this argument more clearly than the current state of therapeutic options for vivax malaria, which is detailed in this review.

A highly prevalent and potentially dangerous infection

Diseases that are highly prevalent but benign or dangerous but exceedingly rare can reasonably merit little research investment. However, *Plasmodium vivax* is neither rare nor benign. It occurs throughout the tropics, except in western and central sub-Saharan Africa where the absence of Duffy factor on the surface of red blood cells largely protects those populations. Guerra *et al.* [1] recently reported estimates of people living at risk of falciparum and vivax malaria at 2.5 and 2.6 billion people, respectively. Hay *et al.* [2] estimated 130–435 million infections by *P. vivax,* in contrast to the more widely cited figure of 70–80 million annual infections [3]. At even the lower estimate, this roughly equals the disease burden of dengue fever and exceeds the disease burden of typhoid by about fivefold.

It has long been considered that vivax malaria is rarely fatal, and if it is fatal, it is considered to almost always be a consequence of rupture of the spleen [4]. Few texts attribute to *P. vivax* the syndromes occurring in severe and complicated *P. falciparum* malaria. However, recent studies using PCR diagnostic technologies revealed that patients diagnosed with vivax malaria and having cerebral malaria, acute respiratory distress syndrome (ARDS), liver dysfunction and renal failure had no evidence of falciparum malaria [5–8]. Table 1 summarizes 108 cases of severe and complicated vivax malaria since 1998, 17 of

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Table 1. Reports of severe and complicated *P. vivax* malaria since 1998

Location	Year	No. of cases	Presentation	Fatal cases	Refs
India	2003	11 adults	Renal failure = 4 Jaundice = 4 ARDS = 3 (1 fatal) Cerebral = 3 (1 fatal)	2	[5]
Pakistan	2000	1 adult	Cerebral	0	[6]
Venezuela	2005	1 adult	ARDS	0	[7]
India	2006	1 adult	ARDS	0	[8]
Afghanistan	2004	1 adult	ARDS	0	[9]
Singapore	2003	2 adults	ARDS (fatal)	1	[10]
Columbia	1998	1 adult	ARDS	0	[11]
Malaysia	2003	2 adults	ARDS = 1 DIC[a]/Renal Failure = 1 (fatal)	1	[12]
India	1999	2	Renal failure = 2	?	[13]
India	2002	16	Renal failure = 16	3	[14]
Turkey	2005	1 child	Convulsions	0	[15]
Indonesia	2000	21 children 17 adults	Cerebral = 3 (2 fatal) Siezures = 2 (1 fatal) Anemia = 24 Hyperparasitemia = 1 Renal failure = 4 Liver dysfunction = 10 (3 fatal) Acidosis = 3 (1 fatal) ARDS = 2 (1 fatal) Cardiac arrest = 1 (fatal)	9	[16]
Afghanistan	2003	1 adult	ARDS	0	[17]
India	2000	22	Liver dysfunction = 8 Cerebral = 1 (fatal) Anemia = 8 Thrombocytopenia = 4 Pancytopenia = 1	1	[18]
Brazil	2000	1 adult	Thrombocytopenia	0	[19]
New Guinea	2000	1 adult	ARDS	0	[20]
India	1998	1 adult	Thrombocytopenia	0	[21]
Kenya	2004	1 adult	Splenic rupture	0	[22]
Turkey	2003	2 adults	Splenic rupture	0	[23]
India	2002	1 adult	ARDS	0	[24]
India	2006	1 child	Renal failure	0	[25]
Total				17	

[a]DIC, disseminated intravascular coagulation.

which proved fatal [5–25], and only two reports of a total of three patients with ruptured spleens, who all survived [22,23]. A 2004 review of the literature [23] reports that only 11 well-documented cases of splenic rupture in vivax malaria were published in the English medical literature since 1960. Ruptured spleen is either tremendously underrepresented in the medical literature, or it is a minor contributor to the spectrum of severe disease caused by vivax malaria. A recent report by Anstey *et al.* [26] describes lung injury among 50 patients with vivax malaria, and these authors report findings suggestive of parasite adhesion within pulmonary microvasculature.

A retrospective analysis of nearly 6000 hospital admissions for malaria over three years at Jayapura in Papua, Indonesia revealed that a third of patients had a diagnosis of *P. vivax* (two-thirds of those with monoinfections) by microscopy [16]. In that setting, almost all diagnosed malaria was managed on an outpatient basis, and only the significantly ill were admitted. Among the 1135 patients admitted with a diagnosis of vivax malaria, 38 were classified as having severe malaria (predominantly cerebral malaria), ARDS, liver dysfunction or renal failure. The 25% case fatality rate with a diagnosis of vivax malaria and a classification of severe disease was identical to those among similar patients with falciparum malaria [16]. Ruptured spleen was not reported in any of the patients at Jayapura, despite 1952 admissions with a diagnosis of *P. vivax* (including 817 mixed infection diagnoses). Syndromes more closely resembling severe and complicated falciparum malaria dominated presentations of severe disease among patients with a microscopic diagnosis of vivax malaria.

All of the reports of severe and complicated vivax malaria described in Table 1 apart from five [5–8,11] lack evidence ruling out cryptic falciparum malaria or inaccurate microscopic diagnoses. Clinicians and laboratory workers managing severely ill patients in endemic areas rarely have the advantage of PCR diagnostics, and presentation of cerebral malaria, ARDS, liver dysfunction and renal failure often leads to the presumptive diagnosis of severe falciparum malaria. Even if the microscopist reports a parasitemia of *P. vivax*, the absence of *P. falciparum* in the smear might be dismissed either as a lack of expertise at the microscope (mixed infections are notoriously difficult to detect) or as sequestration of *P. falciparum*. The investigators for most of the reports summarized in Table 1 apparently overcame this bias and reported vivax malaria as the causative agent. Prospective studies using PCR diagnostics of severely ill malaria patients are required to gauge the true risk of severe

and fatal disease with vivax malaria. Perhaps more importantly, such studies must also rule out other infections likely to occur in a given area, for example bacterial meningitis, typhus, leptospirosis and dengue.

Well-documented studies reinforce the perception that vivax malaria is a relatively benign infection. Vivax malaria has been thoroughly studied in the clinical setting, albeit most often with a narrow range of strains represented, along with prompt diagnosis and treatment precluding serious disease [27,28]. Moreover, deaths due to *P. vivax* malaria among travelers occur rarely compared with those due to *P. falciparum* [29–31]. Finally, epidemic *P. falciparum* carries the well-known risk of high mortality, but there is no such record for epidemic *P. vivax*.

Most of what is known about morbidity and mortality linked to vivax malaria does not address settings of chronic and perhaps inadequately treated *P. vivax* malaria. Prevalent underlying disease states, like coinfections and nutritional deficiencies, add further uncertainty. The available data on severe *P. vivax* malaria suggest that there are conditions in which this parasite becomes lethal in a manner strikingly similar to that of *P. falciparum*. Many of the patients included in Table 1 come from settings where repeated exposures and relapse are likely, as well as risk for coinfection and poor nutrition. In the specific instance of the island of New Guinea, dominant resistance to CQ and to PQ (see below) may contribute to risk of severe disease.

The perception of vivax malaria as relatively benign may stem not only from an incomplete representation of settings of infection and disease, but perhaps also from an incomplete understanding of the taxonomy of the species. Some workers suggest that *P. vivax* is a complex of species [32]. Studies of naturally occurring morbidity and mortality caused by chronic exposure, inadequate therapy and relapse need to be done, particularly those supported with autopsy findings and thorough investigation of parasite genotypes.

Failing chloroquine therapy

Chloroquine has been first-line therapy for *P. vivax* since 1946. Although as little as 0.3 g CQ routinely cured CQ-sensitive *P. vivax*, the experts made no distinction between *P. vivax* and *P. falciparum* in recommending 1.5 g total adult dose for treatment of acute attacks of malaria [33]. This might, in part, explain the relatively late first-known appearance of CQ-resistant *P. vivax* in 1989 from Papua New Guinea [34]. The exquisite sensitivity of gametocytes of *P. vivax* to CQ (and the absence of such sensitivity in *P. falciparum*) might also help explain the prolonged effectiveness.

Studies in eastern Indonesia during the 1990s revealed a severe CQ resistance problem, with most treatments ending in failure [33]. More recent studies in eastern Indonesia reported essentially similar findings [35]. Surveys elsewhere in Indonesia showed low risk of failure in the western provinces and intermediate risk towards the center of the archipelago [33]. Case reports of resistance appeared from other countries in the region [33]. However, sustained survey work measuring risk of CQ failure in vivax malaria has been done only in Indonesia, Thailand and India. In Thailand, a thorough examination of several thousand infections revealed almost no evidence of resistance [36–40]. Among 869 subjects evaluated in three separate studies in India, none showed evidence of resistance to CQ [41–43]. Survey work in Turkey [44,45] and in Vietnam [46] showed moderate levels of resistance (15% and 22% failure, respectively). A recent study in northeastern Indonesian New Guinea, where failure for CQ alone approaches 100%, showed only 18% failure when CQ was supplemented with PQ [47]. Surveys from South America show from no resistance to low levels of resistance [48,49]. The problem of CQ-resistant *P. vivax* thus appears to have originated on the island of New Guinea and might be spreading outwards, but not yet within the reach of Thailand or India (see Table 2).

The reporting of systematic surveys to gauge the risk of CQ failure cannot be considered adequate in any region, including southeast Asia, and information from other regions is limited to only a few case reports – relatively little such risk assessment work appears to be in progress. Despite compelling evidence of failing efficacy, almost no research is under way to ascertain the risk of therapeutic failure of the first-line therapy for a potentially lethal infection that threatens 2.6 billion people.

Effective therapies for chloroquine-resistant *P. vivax*

A few reports describe the efficacy of approved drugs for the treatment of *P. vivax* [50–53], and some reports describe treatments for CQ-resistant *P. vivax* [54–56]. Mefloquine (MQ) combined with PQ was effective in Papua, Indonesia (98% efficacy) [47], and CQ combined with PQ was much more effective than CQ alone [47,54]. Some work in primate models also showed good efficacy of both MQ and the experimental 8-aminoquinoline, tafenoquine [57–59]. The failure to achieve continuous culture of *P. vivax* severely constrains the investigation of effective therapies, leaving clinical trials in humans and primate models as the only options for exploring alternatives. The expense and risk of such work usually requires solid preclinical studies as a foundation. Recent improvements with *in vitro* assays of drug activity against *P. vivax* (a rare example of a significant advance in research on this parasite) [60–63] at least offer the means of investigation of therapies in which the parasite can be obtained with relative ease. Despite evidence of resistance to CQ, and a reasonable forecast of further deterioration, clinical investigation of alternative treatments for vivax malaria resistant to CQ rarely occurs.

The puzzle of primaquine therapy

No standard drug for an infection can be more shrouded in mystery than PQ. Despite more than 50 years of continuous use in millions of people annually as the only drug available for its therapeutic indication, it is not known how PQ acts, how it should be taken, or if it acts when taken as directed [64].

PQ generates a complex mix of metabolites, each exhibiting often sharply distinct chemical and biological activities [65]. Which of these metabolites actually occur in the human liver and participate in killing hypnozoites, and how, is not clear. Nor is it clear which metabolites account

Table 2. Surveys for chloroquine-resistant *P. vivax* since 2000

Location	Year	Subjects (*n*)	Resistant (%)	Refs
Indonesia	2004	40	65	[35]
Turkey	2004	91	22	[44]
Indonesia[a]	2000	60	18	[47]
Vietnam	2001	113	16	[46]
Turkey	2001	112	15	[45]
Peru	2001	177	2	[48]
India	2004	287	0	[41]
Thailand[a]	2004	31	0	[39]
India[a]	2004	102	0	[42]
Colombia[a]	2004	210	0	[49]
Thailand	2003	161	0	[39]
India	2001	480	0	[43]

[a]Chloroquine combined with primaquine (greater efficacy).

for the hemolytic toxicity of the drug to people with G6PD deficiency. The largely hypothetical 6-methoxy metabolites of PQ do exhibit relatively strong activity against *P. berghei* liver stages [66], and when present in an environment favoring oxidized species (like redox-challenged G6PD-deficient red blood cell cytoplasm) become potentially toxic membrane disruptors [67,68].

Setting aside the unknowns of metabolism, the pharmacokinetics of PQ is fairly well understood [69]. It is highly water soluble, rapidly absorbed and almost completely eliminated within 24 h. Its peak plasma levels occur within just an hour or two, and plasma levels drop by a half within two hours. The pharmacokinetic profile of a standard 14 day regimen resembles uniform fence posts – 14 independent spikes of presumably therapeutic levels of the drug. Preclinical and clinical studies revealed something unusual about these spikes – the sum of the areas under the curves (total dose) determined therapeutic efficacy independently of the dosing schedule [64,70]. If the daily dose of PQ is doubled and given for only 7 days, then it is equally effective. A 1.5-strength dose administered weekly over 8 weeks is effective too. As long as the sum length of the height of the 'fence posts' is the same (i.e. total dose), their individual height or space between them does not seem to affect the efficacy. The effect of PQ on the parasite seems to be cumulative. The mystery of this phenomenon almost certainly lies in our almost complete lack of understanding of the physiology of hypnozoites.

A series of clinical trials in the 1950s reported findings that challenged the notion that PQ is effective at all when given as monotherapy [71,72]. When challenged with sporozoites of the Chesson strain, volunteers were given either 210 or 315 mg PQ, either concurrently with standard CQ or quinine (QN) therapy, or after completing QN therapy. In effect, the investigators used combined therapy (CQ plus PQ or QN plus PQ) or two independent monotherapies (QN followed by PQ). At both of the total doses of PQ, QN therapy followed by PQ resulted in 80% relapse rates, whereas the combined therapies gave essentially effective cures. The participation of QN and CQ in the activity of PQ seems the most likely explanation for these findings, even though neither drug has any known effect on liver stages when given as monotherapy against blood stages. PQ might require a companion drug to prevent relapse by *P. vivax*. The good efficacy of PQ alone as a causal prophylactic [73,74] does not necessarily run counter to this evidence – daily PQ for primary prophylaxis almost certainly prevents the formation of hypnozoites rather than kills them.

The efficacy of PQ for preventing relapse is not understood. Definitive experimental challenge trials evaluating this activity have not been done in over 50 years. There is no widely accepted method for measuring the efficacy of PQ in clinical trials, and such measurements present onerous technical, logistical and ethical barriers. Except for a few rare exceptions, the trials are not done. PQ is the only drug available for preventing debilitating and possibly lethal relapses, and it is not known whether PQ is effective.

Preventing *P. vivax*

Every randomized, placebo-controlled trial of drugs for the prevention of malaria in travelers in the past 20 years has failed to address efficacy against late relapse by *P. vivax*. These trials tend to focus on demonstrating efficacy against *P. falciparum* while traveling. Indeed, study subjects are almost always residents of highly endemic areas, a fact driven by sample size and attack rate issues. The effect on late relapse is often deemed a separate issue covered by the practice of terminal prophylaxis with PQ, that is, presumptive standard therapy immediately following exposure (synonymous with post-exposure treatment or PET) [74]. Separating these issues has practical validity; however, one gets back to the issue of whether the presumptive therapy provides protection against relapsing vivax malaria in travelers. Schwartz and colleagues [75,76] looked at the effectiveness of various regimens of prophylaxis in Israeli travelers. Suppressive prophylaxis (the standards such as MQ or doxycycline) routinely failed to prevent late relapses of *P. vivax*, despite the prescribed use of PQ terminal prophylaxis. PQ as primary prophylaxis might be the only tool available that protects travelers from vivax malaria. Although now recommended by the US Centers for Disease Control and Prevention (CDC) and similar agencies of other governments, the current US registered label for PQ does not include an indication for this use [74]. Despite significant risk of *P. vivax* relapse among travelers, no currently approved drug or combination of drugs is known to be effective in preventing this risk. The only drug known to be effective, PQ, is not licensed for this use.

Correct diagnosis of *P. vivax*

The successful development and distribution of the artemisinin-based combined therapies (ACT) for treatment of *P. falciparum* and the almost universal continuing use of CQ for vivax malaria create an important need for reliable diagnostic methods. Patients who have *P. falciparum* malaria that is diagnosed as vivax malaria might receive ineffective therapy. The health or even life of a patient might hinge upon the competency of the microscopist making the diagnosis.

Diagnosis of malaria by microscopy has been almost completely neglected since the heyday of the Global Eradication Campaign. Most microscopists working in clinics, hospitals and even research institutions received their training in school, and perhaps have been through refresher training. However, there is no standard method

for measuring the competency of microscopists. In the field, the reliability of microscopists is usually measured by age and experience, simply because no other useful metric exists.

How often is *P. falciparum* misdiagnosed as *P. vivax*? The risk of a potentially grave error appears high even for expert microscopists. Mayxay *et al.* [77] found 8% of *P. vivax*-infected Thai patients in Bangkok treated with CQ later developed falciparum malaria. Studies in Asia and the Americas typically report 13%–19% of microscopically *P. vivax*-positive specimens also contained *P. falciparum* detected by PCR assay [78]. Among well-trained clinical malaria microscopists in Thailand, 61 of 446 (14%) true *P. falciparum* infections were diagnosed as *P. vivax* malaria [79]. During refresher training of 223 practising microscopists in Indonesia over 15 years, the error rate for species diagnosis of *P. falciparum* was 23% (K.B., unpublished).

In the setting of CQ-resistant *P. falciparum*, these error rates might estimate the risk of inappropriate therapy. Rapid diagnostic tests (RDT) do not solve this problem either. The risk of false negative *P. falciparum* with RDT (varying from 2% at >5000 parasites μL^{-1} to 25% at <500 parasites μL^{-1} [80]) can be considered the risk of receiving ineffective chloroquine therapy where *P. vivax, P. malariae* or *P. ovale* also occur.

Concluding remarks

Several key factors have contributed to the neglect of vivax malaria in research. Until very recently, the global burden of disease has almost certainly been underestimated by a very large margin [2,3]. The broadly held perception of vivax malaria as a benign infection diminished its standing with regard to research priorities. Finally, the inability to maintain *P. vivax* in continuous culture *in vitro* undoubtedly hindered research in the laboratory, and forced most research investments into relatively inaccessible, costly and difficult endeavors in endemic areas.

The highlighted gaps in understanding put forth in this review represent front-line research questions (Box 1). Although expanding laboratory work and interest would undoubtedly yield valuable tools against vivax malaria, the urgent need is for clinical and epidemiological studies. Those studies would give greater direction and impact to laboratory studies. Grasping when, how and how often vivax malaria causes severe illness and what drugs most effectively cure the severely ill, represent the first important steps in addressing the neglect of this parasite.

Box 1. Oustanding questions for *P. vivax* research

- Is *P. vivax* a complex of species?
- Does *P. vivax* cause a falciparum-like severe illness syndrome?
- Does *P. vivax* have virulent strains?
- What are the demographic or clinical risk factors for severe disease?
- What is the mechanism of chloroquine activity?
- How widespread is resistance to chloroquine?
- What is the mechanism of resistance to chloroquine?
- What are effective alternative therapies for chloroquine-resistant strains?
- What is the mechanism of primaquine activity against hypnozoites?
- Is primaquine monotherapy effective against hypnozoites?
- What is the mechanism of primaquine toxicity in G6PD deficiency?
- How can G6PD deficiency be determined at the point of care?
- How widespread is resistance to primaquine?
- How should resistance to primaquine be documented?
- What is the mechanism of resistance to primaquine?
- What are effective alternative therapies against relapse?
- How can late relapse in travelers be prevented?
- How can *P. vivax* be diagnosed accurately?

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