Chapter

Elimination of *Plasmodium vivax* Malaria: Problems and Solutions

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Abstract

Malaria is caused by multiple parasitic species of the genus *Plasmodium*. Although *P. falciparum* accounts for the highest mortality, *P. vivax* is the most geographically dispersed and the most common species outside of Africa. Several unique biological features make *P. vivax* less responsive to conventional control measures and allow it to persist even after elimination of *P. falciparum*. The ability of *P. vivax* to develop in diverse vectors at lower ambient temperatures bestows it a greater distribution range and resilience to ecological changes. Its tropism for reticulocytes often causes low-density infections below the levels detectable by routine diagnostic tests, demanding the development of more sensitive diagnostics. *P. vivax* produces gametocytes early enabling transmission before the manifestation of clinical symptoms, thus emphasizing the need for an integrated vector control strategy. More importantly, its dormant liver stage which engenders relapse is difficult to diagnose and treat. The deployment of available treatments for the liver hypnozoites, including primaquine and the recent U.S. Food and Drug Administration-approved tafenoquine, requires point-of-care diagnostics to detect glucose-6-phosphate dehydrogenase deficiency among endemic human populations. Here we review the continued challenges to effectively control *P. vivax* and explore integrated technologies and targeted strategies for the elimination of vivax malaria.

Keywords: *Plasmodium vivax*, relapse, transmission, G6PD, CYP2D6, radical cure

1. Introduction

Malaria has been an ancient scourge of humankind and efforts to mitigate the harm from malaria have been relentless. In 1955, the World Health Organization (WHO) launched the Global Malaria Eradication Program (GMEP), relying heavily on two essential tools: chloroquine (CQ), a safe and effective drug for malaria prevention and treatment, and the insecticide DDT for vector control. Despite the GMEP's enormous success in reducing malaria burden in many countries outside of sub-Saharan Africa, its failure to sustain the program resulted in malaria resurgence and discontinuation of this global campaign in 1969 [1]. The considerable gains achieved in many areas were soon lost and the world witnessed a sharp rise in malaria incidence in the following two decades. In India, for example, malaria prevalence reduced from an estimated 75 million cases to about 100,000 cases annually during the GMEP, only to rapidly expand to 6.5 million in 1976 [2, 3].

In recognition of this huge malaria burden, the Roll Back Malaria Partnership launched in 1998, marking a renewed attack on this disease resulting in a declining incidence of malaria globally. Empowered by a strong political will and enabled by financial commitment and new interventions, many national malaria control programs (NMCPs) now consider malaria elimination an attainable goal. WHO's "Global Technical Strategy for Malaria 2016-2030" provided goals for the next 15 years and specific guidelines for achieving these goals. Among them, "eliminating malaria in at least 35 countries" and "preventing the re-establishment of malaria in all countries that are malaria-free" specifically address the tasks to attain and sustain malaria elimination. Significant strides have been made toward malaria elimination in the past two decades with 19 countries attaining zero indigenous cases for 3 years or more between 2000 and 2018. These countries include Sri Lanka, Paraguay, and Uzbekistan, which were recently certified as malaria-free [4]. Despite these laudable achievements, formidable challenges still lie ahead for many endemic nations to achieve malaria elimination.

Of the six *Plasmodium* species naturally infecting humans, *P. falciparum* is usually considered the most virulent and is associated with the vast majority of deaths, while *P. vivax* is the most geographically widespread. In comparison, *P. ovale curtisi*, *P. ovale wallikeri*, and *P. malariae* are much less common, whereas the monkey malaria parasite *P. knowlesi* is primarily associated with zoonotic infections [5]. Since malaria elimination is the interruption of local malaria transmission (zero indigenous cases) in a defined geographic area, it is time to target the elimination of all malaria parasite species simultaneously to set the final stage for malaria eradication [6].

At the same time that malaria incidence is continually declining [4], malaria epidemiology is rapidly changing [7]. In countries pursuing elimination, structural changes in at-risk populations have resulted in malaria becoming geographically clustered in hard-to-reach pockets. "Border malaria" has become a shared phenomenon and malaria is increasingly an imported disease. Additionally, because of a divergent response by each species to control interventions, *P. vivax* has become the predominant parasite in malaria-endemic countries outside of Africa. Most pre-elimination countries—such as the members of the Asian Pacific Malaria Elimination Network (APMEN, www.apmen.org) — must be ready to face the ultimate challenge of eliminating vivax malaria, a potentially long and arduous process. In fact, because of the possibility of relapse, the WHO malaria-free certification requires no cases for three years [1]. Here we review the changing malaria epidemiology and discuss the challenges associated with vivax malaria elimination and solutions to address them.

2. Geographic distribution and epidemiology of vivax malaria

Outside of Africa, *P. vivax* is the most common parasite causing malaria. It accounts for 75, 50, and 29% of the malaria burden in the Americas, SE Asia, and the East Mediterranean, respectively [4]. The parasite's ability to complete its sporogonic development in mosquitoes at ambient temperatures as low as 16 °C and to lie dormant for seasonal transmission has extended its geographical range deep into the temperate zones. There is considerable spatial heterogeneity in *P. vivax* distribution at the global and local scales. SE Asia carries more than half of the global *P. vivax* burden (**Table 1**). In the Asian continent, India, Cambodia, and Myanmar have higher endogenous *P. vivax* burden, and transmission is concentrated along international borders [8]. Similarly, the southern part of South America has a relatively low burden with Paraguay and Argentina recently achieving malaria-free

	Africa	America	Eastern Mediterranean	SE Asia	Western Pacific	Total
P. vivax	704	700	1,414	3,947	690	7,500
All	213,000	929	4,900	7,900	1,980	228,000

Table 1.
Estimated cases of P. vivax *and all malaria (×1,000) by WHO region [4].*

status, whereas the northern part of the continent has a substantial *P. vivax* burden (**Figure 1**). In Africa, until recently, *P. vivax* was documented only in the Horn of Africa, and considered extremely rare or "absent" in Central and West Africa because of the dominance of the Duffy-negative blood group [10], a required receptor for erythrocyte invasion by *P. vivax* [11]. Increasing reports of *P. vivax* in Duffy-negative individuals suggests its capability to exploit Duffy-independent invasion pathways [12, 13]. In the last decade, the growing evidence of *P. vivax* transmission in all regions of Africa, including acute and asymptomatic cases, infected vectors, serological indicators, and infected international travelers, indicates more *P. vivax* transmission than previously thought [14, 15]. Although malaria control programs in Africa are justifiably focused on *P. falciparum* (given the striking morbidity and mortality associated with this species), *P. vivax* is becoming an emerging concern for malaria elimination from African nations.

Throughout history, *P. vivax* has shown extreme resilience to control measures [16], and in many areas where *P. falciparum* and *P. vivax* co-exist, *P. vivax* is becoming predominant [17, 18]. With this shift in species predominance come changes in the at-risk populations. In areas of *P. falciparum* and *P. vivax* sympatry, clinical episodes of vivax malaria rapidly decrease around 12 months of age, whereas *P. falciparum* cases continue to rise until about 3 years of age [19, 20]. Since exposure undoubtedly plays a role in the acquisition and maintenance of immunity, *P. vivax* recurrence may allow for repeated exposure from fewer infection events which may contribute to this age discrepancy. Additionally, primaquine (PQ) is not commonly given to children below 5 years, while chloroquine (CQ) underdosing is not unusual [21], resulting in repeated *P. vivax* attacks in young children. Recent studies in SE Asia showed that school-aged children had significantly increased

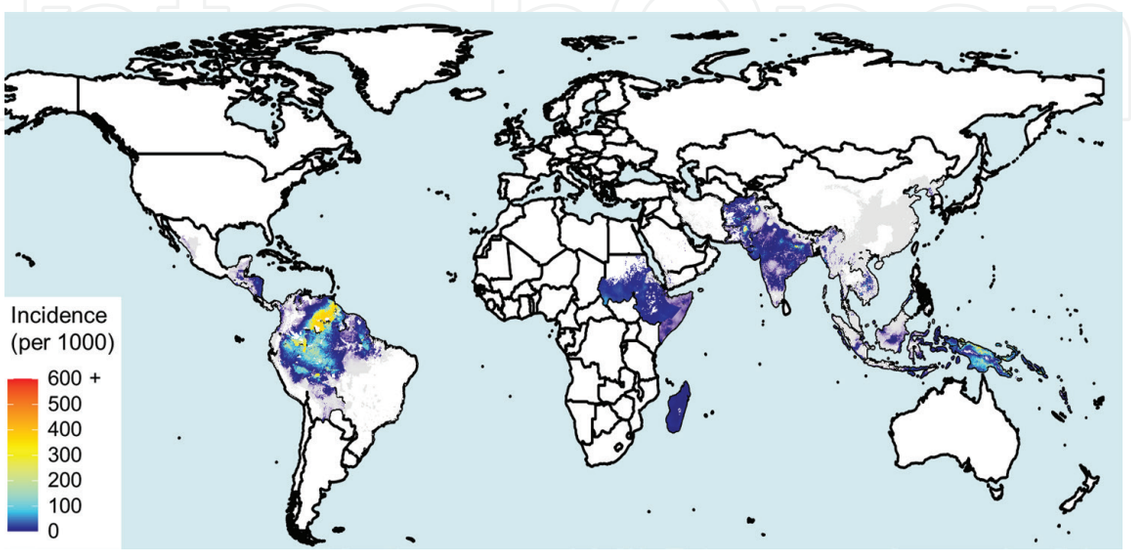

Figure 1.
Global distribution of P. vivax *malaria. Shading represents incidence in cases per 1000 people per year [8, 9]. Very low incidence areas are shaded in gray.*

odds of acquiring *P. vivax* infections [18, 22]. Because PQ is contraindicated for pregnant women, radical treatment of *P. vivax* remains difficult in this group. Consequentially, relapsing episodes of malaria during pregnancy can lead to congenital malaria [23–25]. Certain occupations such as soldiers and forest workers are also more vulnerable to malaria infections [18, 26]. A better understanding of local malaria epidemiology will be essential for implementing targeted control measures.

3. Morbidity of vivax malaria

Biologically, *P. vivax* exhibits key differences from *P. falciparum* influencing its transmission, presentation and outcome [27]. Historically, *P. vivax* malaria has been mistakenly described as "benign tertian malaria". In fact, *P. vivax* infection causes a full spectrum of disease symptoms ranging from uncomplicated febrile illness to severe and fatal malaria. Severe *P. vivax* malaria is often associated with severe anemia, a common complication, as well as thrombocytopenia, acute respiratory distress, hepatic dysfunction, renal failure, seizures or coma, and shock [28, 29]. Severe anemia is the most common complication associated with *P. vivax* malaria [30–32]. *P. vivax* has a strong preference for $CD71^{high}$ reticulocytes [33] and aggravates anemia by targeting cells immediately after replacement [34, 35]. Recurrent *P. vivax* parasitemia further elevates the risk of severe anemia [36]. Although the risk of thrombocytopenia is prevalent in all forms of malaria, evidence suggests that it is more common in *P. vivax* than *P. falciparum* patients [37, 38]. In pregnant women, *P. vivax* infection is associated with a higher risk of anemia, abortion and low birth weight [39, 40]. Furthermore, the presence of co-morbidities may exacerbate *P. vivax* infections resulting in severe and life-threatening complications.

4. Relapse

One distinctive feature of *P. vivax* that enables the parasite to evade conventional control measures designed for *P. falciparum* is the formation of a dormant liver stage, termed hypnozoite [41]. Hypnozoites persist in a non-dividing fashion within the liver where they may be awakened weeks and months later causing relapse. Recent detections of *P. vivax* parasites in the bone marrow and spleen have raised the possibility that the extravascular merozoites might be an additional source of recurrence in addition to the relapse from hypnozoite activation [42, 43]. Two latency forms of *P. vivax* strains are recognized. The long-latency strains (e.g., the St. Elizabeth strain) prevalent in temperate zones have either a ~ 9-month latency period or a 2-week incubation time for the primary infection followed by a ~ 9-month interval before the relapse, allowing for parasite survival through the long winter season when mosquito vectors are absent [44, 45]. In contrast, for the short-latency tropical strains (e.g., the Chesson strain) from SE Asia and Oceania, relapses typically occur ~3 weeks after the primary infection [46]. Some areas, such as the Greater Mekong subregion host both types of strains [46, 47]. Besides, the sporozoite inoculation load can impact the latency period. The ratio of hypnozoites to sporozoites could vary by strain, and parasites with higher proportions of hypnozoites may be more inclined toward frequent relapses [48, 49]. Mechanisms of hypnozoite reactivation are elusive and may involve external stimuli such as drugs, another malaria infection, or other infectious diseases [50]. A meta-analysis of *P. falciparum* drug efficacy trials reveals a high risk of *P. vivax* parasitemia after treatment of falciparum malaria [51].

Relapse increases the morbidity associated with *P. vivax* infections. Relapses can contribute to 50–80% of the overall vivax infections in high transmission areas [52–55]; sometimes occurring repeatedly from the same infection, such as a case from Eritrea where one patient's three episodes of vivax malaria were caused by meiotic siblings suggesting the same infection event [56]. Additionally, *P. vivax* is frequently found in highly polyclonal infections, even in areas with relatively low malaria endemicity. One study from Cambodia found that around half of polyclonal infections might have resulted from relapse [57]. Long-distance parasite migration and introduction also favor hypnozoites. When introduced by an asymptomatic human host to an area with suitable *Anopheles* vector species, relapsed parasites could spark autochthonous infections and establish new transmission foci, as observed in Greece [58].

5. Diagnosis

Accurate and timely diagnosis of malaria by species is essential for the delivery of appropriate treatments. In clinical settings, acute vivax malaria is diagnosed by microscopy or rapid diagnostic tests (RDTs). Routine microscopy has a limit of detection (LOD) of around 50 parasites/μl of blood [59, 60]. Specific training is required, as misidentification of the infecting species is fairly common [59, 61]. RDTs are a fast, affordable and efficient method for malaria diagnosis with a LOD of ~200 parasites/μl, but the sensitivity varies among brands [62]. The tropism of *P. vivax* for reticulocytes can result in parasite densities much lower than the LODs of the conventional diagnostic methods. Recently, the creation of ultra-sensitive RDTs has lowered the LOD for *P. falciparum* infection [63, 64] but one targeting *P. vivax* is still lacking.

In malaria elimination settings, malaria prevalence is often assessed through active case detection in the form of cross-sectional surveillance. Most available clinical diagnostic tools are inadequate for detecting *P. vivax* asymptomatic reservoirs with very low parasitemia. Molecular methods although sensitive to low-density infections and useful in epidemiological surveillance are not feasible in field applications [19]. The presence of microscopically subpatent infections in endemic populations may render a mass screening and treatment-based strategy ineffective if screening is based on low-sensitivity tools [65].

Hypnozoites pose a significant challenge for the elimination of vivax malaria because they defy detection by any diagnostic methods. Recently, a screen for IgG responses to a panel of 342 *P. vivax* antigens in longitudinal clinical cohorts established that antibody responses to eight proteins detected *P. vivax* infections in the previous 9 months with 80% sensitivity and specificity [66]. Modeling demonstrates that treating a serologically positive population could potentially reduce *P. vivax* prevalence by 59–69%. While this new development still awaits prospective evaluation, it offers a promising surrogate marker for hypnozoite detection and treatment.

6. Chemotherapy and drug resistance

Most antimalarial drugs in use are blood schizontocides that kill asexual blood-stage parasites, which are associated with clinical symptoms. The ability of *P. vivax* (and also *P. ovale* spp.) to form liver hypnozoites capable of causing relapses requires the addition of a hypnozoitocide to prevent relapses. For the radical cure of *P. vivax* malaria, CQ and PQ have been the companion therapies of choice for the treatment

of uncomplicated vivax malaria since the 1950s. Due to the development of CQ resistance in the island of New Guinea, CQ was abandoned and replaced with an artemisinin combination therapy (ACT) there [67].

6.1 Treatment of *P. vivax* blood-stage infections

For the treatment of blood-stage uncomplicated *P. vivax* malaria, WHO recommends the use of either an ACT or CQ in areas where parasites remain CQ sensitive (CQS) or an ACT in areas where *P. vivax* is known to be CQ resistant (CQR) [68]. ACTs are contraindicated in pregnant women in their first trimester; thus for this patient population, uncomplicated vivax malaria is treated with either CQ for CQS malaria or quinine for CQR malaria. WHO recommends parenteral therapy for severe malaria with either artesunate, artemether, or quinine (listed here in the order of preference) for at least 24 h regardless of the causative *Plasmodium* species [68]. No additional drugs are needed to block transmission (as compared to the recommended low-dose PQ for blocking the transmission of *P. falciparum*) because *P. vivax* gametocytes are sensitive to most antimalarial drugs.

6.1.1 Chloroquine and unified treatment with ACTs

CQ remains the mainstay treatment for *P. vivax* malaria in most endemic countries. If low-grade or sporadic CQ resistance is identified, optimizing the treatment regimen can improve the therapeutic efficacy of CQ. A recent meta-analysis of CQ efficacy studies indicates underdosing (<25 mg of CQ/kg) among a substantial proportion (>30%) of patients [21]. Increasing the recommended dose to 30 mg/kg, especially in children under 5 years, could reduce the risk of early recurrence by more than 40% if CQ is used alone. The safety and tolerability of the increased CQ dose are substantiated by earlier studies where CQ doses of 50 mg/kg were used to treat CQR *P. falciparum* [69]. In addition to underdosing, there is accumulating evidence of emerging CQR parasites in endemic sites [70, 71]. A meta-analysis of 129 clinical trials on CQ efficacy identified CQR *P. vivax* parasites in most vivax-endemic areas, though the prevalence of resistance varied geographically [72]. The epicenter of CQR *P. vivax* is located on the island of New Guinea, where the CQR parasite was first reported in 1989 [73]. Reports of high rates of recurrent parasitemia within 28 days in subsequent years [74–76]—consistent with the WHO definition for RI resistance [77]—led to the ultimate withdrawal of CQ from treating vivax malaria in New Guinea [67]. ACTs have shown high efficacy as a treatment replacement of CQ for uncomplicated vivax malaria in many endemic sites [78–80]. Dihydroartemisinin-piperaquine treatment had a significantly lower risk of *P. vivax* recurrence at day 42 than artemether-lumefantrine [81]. These higher rates of recurrence are probably due to different pharmacokinetic profiles of the partner drugs: lumefantrine has a much shorter half-life (~4 days) than piperaquine (28–35 days) and thus offers less protection against early relapse and/or reinfection. This can be mitigated by the inclusion of PQ in the treatment [81]. In areas co-endemic for both *P. vivax* and *P. falciparum*, the deployment of a unified ACT-based strategy for both parasites provides several advantages [78]. First, the excellent clinical efficacy of ACTs against vivax malaria makes them highly suitable for areas of known or suspected CQR vivax. Second, it offers operational ease in routine practice where species misdiagnosis is a frequent issue [18]. The World Malaria Report 2020 indicated an increasing number of countries adopting ACTs as first-line therapy for *P. vivax* [82]. The reluctance to change the treatment may be due to the perceived ease of treating vivax malaria and the economic burden associated with the switch to a much more expensive drug.

6.1.2 Chloroquine resistance

To ensure the high efficacy of first-line therapy, close monitoring is essential. For *P. falciparum* malaria, this is typically done by clinical efficacy studies, *in vitro* drug assays, and molecular surveillance of resistance-conferring genetic markers. Surveillance for *P. vivax* resistance relies heavily on *in vivo* assessment of the schizontocidal therapy, conducted through follow-ups of recurrent *P. vivax* parasitemia after initial treatment for 28 days. Extending the follow-up to 42 days will allow the identification of late recrudescence [21]. After the standard treatment with CQ (3-day regimen of 25 mg/kg CQ base), the blood concentration of the active drugs (CQ and desethyl CQ) reaches the minimum inhibitory concentration (MIC, ~100 ng/ml) around 28 days, and thus recurrent parasitemia before day 28, regardless of the origin of the parasites (recrudescence, relapse or new infection), is likely due to CQR parasites [83]. Given that drug resistance is defined as the growth of the parasite in the presence of the drug above the MIC, CQ resistance must be confirmed by measurement of residual blood CQ and desethyl CQ levels on the day of recurrence.

Ex vivo measurement of drug sensitivity has been conducted in many endemic regions, but it is not ideal for routinely monitoring antimalarial drug resistance in *P. vivax* because of the difficulties in setting up the *ex vivo* assays [84, 85]. Since a long-term *in vitro* culture system is not available for *P. vivax*, *ex vivo* assays are restricted to one-time assays using fresh field isolates, making further validation of results difficult. The *P. vivax* tropism for reticulocytes means that reinvasion does not happen frequently under field conditions. As a result, *ex vivo* drug exposure is limited to one intraerythrocytic cycle. The most commonly used method to quantify parasite growth is the modified Rieckman's microtest that compares schizont maturation rates [86, 87]. Another method quantifies the production of lactate dehydrogenase by the parasites [88]. Unlike *P. falciparum* clinical samples where parasites are all at the ring stage, *P. vivax* clinical isolates contain mixed stages with various degrees of synchronicity. Since *P. vivax* trophozoites are highly tolerant to CQ [89, 90], *ex vivo* assays for CQ need to be done using isolates with no less than 80% ring stages. Despite the variability of assay results between labs, *ex vivo* assays can complement *in vivo* studies to follow temporal changes of drug sensitivities in an endemic area [91].

Molecular surveillance of putative CQR markers in *P. vivax* populations, though conducted in multiple endemic sites, is hindered by the lack of understanding of the genetic basis of resistance [71]. Initial studies focused on the orthologs of the *pfcrt* and *pfmdr1*, the main determinants of CQ resistance in *P. falciparum*. Most studies fail to show a strong correlation between *pvcrt-o* mutations and the CQR phenotype. Some studies from the Brazilian Amazon indicated an association of CQ resistance with higher expression level and gene amplification of *pvcrt-o* [92, 93], whereas such a link was not validated in Papua Indonesia with high-grade CQ resistance [94]. The relationship between the upregulation of *pvcrt-o* expression and CQ resistance was recently supported by a genetic cross of *P. vivax* strains [95]. There are also considerable controversies about the main *pfmdr1* mutations Y976F and F1076L as potentially conferring CQ resistance [96–99], suggesting that *pvmdr1* may not be a major determinant for CQ resistance in *P. vivax*. Population genomics studies of *P. vivax* populations from areas with drastically different CQ resistance have identified genomic sites under strong selection [100, 101], but their significance in mediating drug resistance remains to be determined. When using *Plasmodium knowlesi* as an *in vitro* model, some of the markers did not seem to change the drug sensitivity phenotypes in transgenic parasites [102].

6.2 Treatment of *P. vivax* liver stages

6.2.1 Primaquine and tafenoquine in anti-relapse therapy

Relapses from hypnozoite reactivation are preventable by anti-relapse therapy with 8-aminoquinoline drugs. For the prevention of relapse, WHO recommends a dose of 0.25–0.5 mg/kg body weight of PQ daily for 14 days [68]. A high dose (0.5 mg/kg/day) is needed for tropical, frequently relapsing *P. vivax* strains such as the Chesson strain that is prevalent in East Asia and Oceania, whereas a lower dose (0.25 mg/kg/day) is recommended for temperate strains. Many nations adopt low-dose PQ for fear of possible harm to unscreened patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency, but suboptimal dosing may fail to prevent relapses in many endemic sites [46, 103]. Conversely, the high dose requires more detailed clinical investigations to document its efficacy [104].

The 14-day PQ treatment regimen is inevitably associated with poor adherence, which seriously compromises its public health benefit. It is estimated that the effectiveness of unsupervised PQ regimens in vivax patients from Papua, Indonesia, could be as low as 12% [105]. Unfortunately, a shortened 5-day regimen of 15 mg daily PQ did not efficaciously prevent relapse [106, 107]. However, a 7-day high PQ dose (1.0 mg/kg/day) regimen in Asia and Africa recently performed comparably to the 14-day PQ regimen (0.5 mg/kg/day), providing a possible solution to poor adherence [108, 109]. In 2018, another 8-aminoquinoline, tafenoquine (TQ), was approved by the U.S. Food and Drug Administration for radical cure of vivax malaria and malaria prophylaxis [110, 111]. TQ, administered as a single 300 mg dose, showed similar tolerability and efficacy to PQ in preventing relapse in vivax malaria [112, 113]. While TQ appears to be the best choice for travel medicine in people with normal G6PD activity, further clinical studies are needed before seeing its deployment in endemic regions.

In patients with known G6PD deficiency, PQ may be given at 0.75 mg/kg for eight weeks [68], but this should be under close medical supervision with ready access to blood transfusion services. This dosing regimen leverages the "total dose effect", discovered in the relapsing monkey malaria parasite *P. cynomolgi* [114]—it posits the same efficacious dose of PQ can be delivered in a range of schedules to achieve the same therapeutic effect. For the temperate and tropical strains, the total dose equals 210 and 420 mg PQ, respectively [115]. This regimen was tested in those carrying the G6PD A- variant experimentally infected with the Chesson strain and found to be safe and efficacious [116]. A recent trial of this regimen found that 5/18 (27.7%) G6PD deficient patients experienced >25% fractional drops in their hemoglobin concentrations, including one patient requiring transfusion [117, 118]. This study precludes the use of unsupervised weekly PQ in Cambodia (and perhaps other parts of the Greater Mekong subregion), where the regional prevalence of Viangchan (a class II G6PD variant) and other hemoglobinopathies such as hemoglobin E and β-thalassemia may predispose G6PD deficient patients to a greater risk of acute hemolytic anemia (AHA) when treated with PQ.

6.2.2 G6PD deficiency and point-of-care testing

The root problem of PQ and TQ is hemolytic toxicity in patients with G6PD deficiency [115, 119, 120]. The *G6PD* gene is extraordinarily polymorphic with at least 217 known mutations, and their effects on the stability and catalytic efficiency of the enzyme vary greatly [121–123]. The residual enzyme activity varies from 5–10% of the normal levels in the G6PD A- variant from Africa to less than 1% of the normal levels in the G6PD Mediterranean variant. As a result, the clinical

spectrum of PQ toxicity can range from relatively mild and self-limiting in G6PD A- individuals to severe AHA in the G6PD Mediterranean individuals, while most variants from Southeast Asia (the Mahidol, Viangchan, and Canton variants) typically have intermediate levels of enzyme activity [124]. Evidence supporting protective advantages of the G6PD A- against *P. falciparum* [125–128] and G6PD Mahidol against *P. vivax* [129, 130] is consistent with the wide geographic distribution of G6PD deficiency and its overlap with malaria distribution [124]. G6PD deficiency affects around 8% of the global population, but its distribution is geographically heterogeneous and can range from 3 to 30% in tropical areas [131–133]. G6PD is X-linked; thus, male hemizygotes and female homozygotes have full expression of the G6PD deficiency, whereas heterozygous females display varying degrees of G6PD activity due to random X-chromosome inactivation (lyonization) resulting in red cell mosaicism. As a result, the male population displays two distinctive phenotypes, whereas the female population shows a full spectrum of G6PD activity, which has significant ramifications for the treatment with the 8-aminoquinoline drugs [134]. Because of this, cases of severe AHA have been identified in female heterozygotes receiving the high daily dose of PQ (1.0 mg/kg) even though these subjects tested as G6PD normal after screening with the qualitative fluorescent spot test (FST) [108, 109, 135]. Furthermore, even in vivax patients with a class III G6PD variant (e.g., the Mahidol variant considered mildly deficient), a low-dose PQ treatment (15 mg/kg/day) for three days could lead to AHA, requiring blood transfusions or even renal failure [117, 136, 137]. Therefore, for the goal of malaria elimination in areas with *P. vivax*, the deployment of point-of-care G6PD deficiency diagnostics is urgent [138]. Currently, FST is the most common method to screen for G6PD deficiency, which has minimum lab requirements of cold chain and electricity as well as trained personnel. The CareStart™ G6PD RDT (Access Bio) is a point-of-care screen for G6PD deficiency, but the cost (~15 USD) is prohibitive for large-scale implementation in low-resource endemic areas [139]. Of note, qualitative screening with the FST or RDT can detect G6PD deficiency below 30% of normal activity, but cannot reliably diagnose female heterozygotes with an intermediate deficiency (30–70% normal activity). Fortunately, rapidly eliminating PQ with its half-life of 6 h can be prescribed to patients with G6PD activity above 30% of normal activity [111]. However, TQ has a long half-life of 14 days, and the recommended threshold of G6PD activity is set at 70% of normal activity. Thus, for rolling out TQ in endemic areas, more stringent screening of G6PD activity with quantitative tests is needed [111, 140]. A recent cost-effectiveness analysis suggests that TQ may be deployed in endemic areas outside sub-Saharan Africa using a gender-specific strategy where G6PD-normal females can be prescribed a low-dose PQ for 14 days [141]. This approach again centers on the availability of a qualitative G6PD test.

6.2.3 Host cytochrome P450 (CYP) 2D6 activity

Another problem identified recently is that PQ efficacy depends on the host activity of the hepatic cytochrome P450 (CYP) 2D6. Failures of PQ to radically cure have been linked to reduced activity of CYP2D6 [142], which mediates activation of PQ to its active metabolite(s) [143, 144]. Follow-up studies in Indonesia have established CYP2D6-dependent metabolism of PQ as a key determinant of the efficacy against relapse [145]. Studies in Brazil similarly identified an association of the diplotype-based CYP2D6 activity score of ≤1.0 with increased risks of *P. vivax* recurrence within 180 days after PQ treatment [146, 147]. There are also cases of patients with impaired CYP2D6 activity suffering from multiple relapse attacks despite receiving adequate anti-relapse therapy with PQ [148, 149]. Even for the

single-low-dose PQ used as a transmission-reducing strategy for *P. falciparum*, the genetic variation in CYP2D6 affects the pharmacokinetics of PQ [150], and CYP2D6 poor/intermediate metabolism is associated with prolonged gametocyte carriage [151]. Thus, it is important to determine the extent to which reduced CYP2D6 activity is responsible for PQ failures in the radical cure of vivax malaria [152].

CYP2D6 is involved in the metabolism of as many as 25% of drugs in clinical use and is also highly polymorphic [153, 154]. Over 150 CYP2D6 allelic variants have been found and grouped into four phenotypic classes of non-functional, low, normal, and increased metabolizers, with respective activity scores of 0, 0.5, 1.0, and 2.0 per allele, corresponding to diplotype activity scores of 0, 0.5, 1.0, 1.5, 2.0, and > 2.0 [155]. Individuals with diplotype activity scores of ≤1.0 are considered to be poor PQ metabolizers [156]. The proportion of poor PQ metabolizers varies geographically. In the Brazilian Amazon, ~25% of the population was found to have reduced CYP2D6 activity [146, 147]. In Cambodia, 52 and 1% of subjects were found to have intermediate and poor metabolism, respectively [157]. Most surveys are based on genotyping results, whereas direct measurement of the CYP2D6 activity using CYP2D6 substrate metabolism (dextromethorphan to dextrorphan conversion) could more accurately determine the phenotype [145]. While CYP2D6 genotypes are not routinely screened in malaria-endemic areas, knowledge of the extent of this problem will help plan for vivax elimination.

6.2.4 Primaquine resistance

PQ-resistant *P. vivax* hypnozoites have never been unequivocally demonstrated. PQ efficacy studies are complicated to conduct and possibly one reason PQ resistant parasites have not been identified. PQ alone has excellent anti-relapse activity [158], but co-administration of a schizontocide (e.g., quinine, CQ, or an ACT) has been shown to significantly potentiate PQ's anti-relapse activity [159]. This effect has been recently verified using an *in vitro P. cynomolgi* hepatic system, wherein CQ could enhance PQ's activity against schizonts and hypnozoites by ~18-fold [160]. Any therapeutic failures of PQ in *P. vivax* radical cure could plausibly result from reasons other than PQ resistance. For example, treatment may fail because of improper PQ dose, short duration of treatment, or poor adherence to the treatment regimen [80, 161, 162]. Further, with the current genotyping strategy, it is not possible to reliably determine whether a recurrent infection after day 28 is due to relapse or reinfection. In endemic areas, patients can harbor multiple genotypically different hypnozoites and a relapse infection may be from reactivation of a heterologous hypnozoite clone [163, 164]. Likewise, a genotype different from that of the primary infection may be from either relapse or reinfection. PQ efficacy studies require longer follow-up, making it difficult to exclude the possibility of reinfection in endemic areas. In studies where the possibility of reinfection can be excluded [158, 165], PQ failure requires further scrutiny, especially with the newly identified CYP2D6 effect. In 21 Indonesian patients who experienced therapeutic failure of PQ against *P. vivax* relapse, 20 were classified as CYP2D6 impaired, whereas only one with normal CYP2D6 activity and adequate PQ exposure may represent true resistance to PQ [145]. Ultimately, PQ resistance may still be rare in most endemic areas, though continued surveillance is recommended.

7. Vector control

P. vivax produces transmissible gametocytes early in infection before the development of clinical symptoms [166–168], allowing ready transmission through

mosquito vectors with more efficient transmission in certain species [169, 170]. There are 71 *Anopheles* species/species complexes that are potential vectors for vivax malaria [171], and vector control is a critical component for integrated control of vivax malaria [172]. Long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) are the key vector-based malaria interventions that are highly effective in sub-Saharan Africa [173, 174]. However, these measures are either under-utilized with low coverage or less effective in certain regions [82, 175]. Since many vector species exhibit early evening and outdoor biting preferences, LLINs and IRS alone are not sufficient for interrupting malaria transmission [176]. In malaria elimination settings, residual transmission often occurs in a forested habitat that lacks core mosquito control coverage [177, 178], requiring targeted vector control efforts for special populations. Further, the emergence and spread of insecticide resistance compromising the efficacy of mosquito control measures needs continual monitoring [179–181]. Successful malaria elimination programs in various regions of the world have all included vector control as one of their pillar strategies [16]. Thus, novel vector control approaches are desperately needed including larval control strategies [182, 183], incorporation of ivermectin in the mass drug administration (MDA) program to reduce the life span of mosquitoes [184, 185], topical and spatial repellents [186, 187], genetically manipulated mosquitoes for population replacement [188], and next-generation LLINs and IRS [189].

8. Technologies and strategies for supporting elimination

8.1 Experience gleaned from successful stories

Despite the unique challenges posed by *P. vivax*, elimination is achievable with integrated control measures. There are nearly 40 countries and territories that have been WHO certified as malaria-free, with 10 of those achieving certification since the turn of the century. Although they all used a combination of strategies including vector control, case management, and mass drug administration, different regions emphasized specific sets of tools at different stages of elimination. The Maldives, the first country in SE Asia to reach malaria-free status, relied heavily on vector control [190]. For Sri Lanka's battle against malaria, strong surveillance, case detection, and patient isolation with treatment were key to its highly targeted elimination strategy [191]. Sri Lanka had anti-relapse treatment as a component of its elimination plan, especially for highly mobile military members, engendering the elimination of *P. vivax* almost simultaneously with *P. falciparum* [191]. In the republics of the former Soviet Union, preventive therapy and MDA with PQ, seasonal CQ chemoprophylaxis, and IRS, were instrumental for malaria elimination [16, 192]. China eliminated indigenous malaria cases in 2017 after the declaration of the National Malaria Elimination Action Plan in 2010 [193]. In the final stage of malaria elimination, China adopted targeted MDA to eliminate vivax transmission in central provinces and developed a rigorous 1–3-7 malaria surveillance strategy [194, 195].

8.2 Strategies for vivax elimination

Management of clinical vivax malaria. Accurate diagnosis using sensitive methods is critical for proper treatment of vivax cases. More sensitive diagnostics such as the uRDTs under development may fill such a need. For the treatment of blood stages, a unified treatment with ACTs is highly recommended. The deployment of point-of-care diagnostics for G6PD deficiency will ensure the wider

prescription of the anti-relapse drug PQ. For those patients with G6PD deficiency, monthly presumptive treatment or prophylaxis with a drug with a long half-life such as naphthoquine may be adopted, as it has proven to be safe and 100% effective for preventing relapse malaria parasite [196, 197].

Targeted MDA. As mass screenings and treatment-based strategies are ineffective for the final elimination phase [65], residual transmission requires targeted MDA to eliminate asymptomatic and submicroscopic parasite reservoirs. For the success of MDA, better knowledge of malaria epidemiology and strong community engagement are needed. In areas such as the Greater Mekong subregion, G6PD deficiency and CYP2D6 poor metabolizers are prevalent and may account for 30% of the population. In addition, point-of-care diagnostics for G6PD deficiency are not available, which seriously undermines the feasibility of PQ-based MDA [156]. In these regions, periodic MDA with an ACT or prophylaxis drug combination with a long eliminating half-life may be an effective alternative [196–199]. Incorporation of ivermectin in the MDA program can reduce the life span of adult mosquitoes and in turn, the transmission of the parasite [184, 185].

Vector control. Traditional control methods such as LLIN and IRS need to be implemented with high coverage. This can be supplemented with novel vector control approaches such as larval control [183]. Topical and spatial repellents [186, 187] may be used for populations at higher risk of outdoor transmission.

Surveillance system. The establishment of a stringent malaria surveillance system in the NMCPs that allows timely responses to new malaria incidence plays a crucial role in malaria elimination. This has proved highly important for many, if not all, successful malaria elimination stories. Within this system, training and capacity building are necessary to establish a malaria control network responding effectively to emerging malaria cases.

8.3 Sustaining elimination

With increased international and cross-border travel, imported malaria cases re-introduce malaria in countries where malaria has been eliminated [193, 200], potentially leading to local transmission [58]. Weakening malaria control programs have been linked to almost all resurgence events such as one that occurred in central China [201], and resource concerns are a large contributing factor [202]. Targeted elimination programs (including regular screening and extensive vector management) can be costly, and there remains a concern that countries who have achieved elimination status may be tempted to reduce their targeted vigilance in order to prioritize funding for other endeavors [203]. However, vector control programs are vital to multiple infectious disease programs, which makes them key components of an integrated response. Additionally, countries should continue to train medical workers for the diagnosis and treatment of malaria and remain vigilant to malaria re-introduction from international travelers or mobile communities. Experience from the malaria program in South Korea demonstrated the significance of good case management practice combined with stringent surveillance for reducing the resurgent malaria threat [204]. In many malaria-free nations, chemoprophylaxis is suggested for international travelers [191, 205], and introduced cases are met with an investigation to eliminate the possibility of endemic spread [206].

9. Conclusions

Several unique biological characters of the *P. vivax* parasite are responsible for its wide distribution and persistence in the face of escalating control efforts.

Problems	Solutions
Relapse from reactivation of dormant hypnozoites; long-latency strains allow for parasite survival beyond winter season	Treatment of hypnozoite stage; sustained vector control to prevent transmission
Anti-relapse treatment with PQ or TQ increases risk of acute hemolytic anemia in G6PD deficient patients with varying degrees in male vs. female	Deployment of point-of-care G6PD RDTs and gender-sensitive treatment strategies that account for differing phenotypic presentation of G6PD deficiency.
Impaired activity of CYP2D6 is associated with poor PQ efficacy	Screening for impairment in CYP2D6 activity in regions of PQ treatment failure can inform targeted treatment strategy
Lack of radical treatment for patients with G6PD deficiency, low CYP2D6 activity, and PQ contraindication	Prophylaxis with a safe drug with a long half-life (e.g., naphthoquine)
Suboptimal dosing in CQ treatment of uncomplicated malaria	Standardizing dose to 30 mg/kg, especially in children under five years can prevent early recurrence
CQ resistance in many endemic sites	Treatment with ACTs
Lack of RDTs for detecting asymptomatic reservoirs with low parasitemia	Detecting antibodies to specific *P. vivax* proteins followed by treatment may reduce *P. vivax* prevalence
Residual transmission from asymptomatic and submicroscopic parasite reservoirs	Targeted MDA following epidemiological assessment and community engagement
Vector control strategies such as LLINs and IRS have low coverage, decreased adherence and limited efficacy in some regions	Increasing coverage of LLINs and IRS. Implementing novel vector control approaches to decrease mosquito density, lifespan, and outdoor transmission
Single vertical strategy to eliminate malaria remains unsuccessful	Combined strategies of strong surveillance, early case detection, patient isolation with treatment, sustained vector control.

Table 2.
Summary of the problems and solutions in eliminating P. vivax malaria.

Table 2 summarizes the challenges in controlling and eliminating vivax malaria and potential solutions. Because of the geographical variation of the vivax malaria situation, different endemic countries are likely to emphasize one or a few control strategies. As an infectious disease involving the human-parasite-vector triad, it requires integrated approaches targeting all components of these interactions for the ultimate elimination of vivax malaria.

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