Chapter 4

Challenges in the Control and Elimination of *Plasmodium vivax* Malaria

Puji BS Asih, Din Syafruddin and John Kevin Baird

Additional information is available at the end of the chapter

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Abstract

The human malaria parasite *Plasmodium vivax* imposes unique challenges to its control and elimination. Primary among those is the hypnozoite reservoir of infection in endemic communities. It is the dominant source of incident malaria and exceedingly difficult to attack due to both inability to diagnose latent carriers and the potentially life-threatening toxicity of primaquine in patients with an inborn deficiency of G6PD, the only therapeutic option against hypnozoites. Large segments of endemic populations are not eligible for primaquine, and alternative strategies for managing the threat of relapse in any group have not been optimized or validated. Association of risk of primaquine failure against latent *P. vivax* with impaired alleles of P450 2D6 exacerbates the substantial pool of primaquine ineligibles. Resistance to chloroquine against acute *P. vivax* malaria commonly occurs; alternative therapies like ACTs are effective but seldom evaluated as a partner drug to primaquine in the essential radical cure. Many of the *Anopheles* mosquito vector of *P. vivax* in South and Southeast Asia, where >90% of infections occur, thrive in a diversity of habitats and exhibit wide ranges of feeding and breeding behavior. This chapter explores many of these challenges and possible approaches in controlling and eliminating endemic vivax malaria.

Keywords: *Plasmodium vivax*, malaria, latent malaria, hypnozoites, glucose-6-phosphtate dehydrogenase (G6PD), *Anopheles* mosquitoes, species-sanitation, control

1. Introduction

Human malaria caused by *Plasmodium vivax* currently has the widest geographical distribution among all malaria parasites with about 35% of the world population living at risk of this physically debilitating and sometime lethal infection [1–3]. **Figure 1** illustrates this

global distribution most heavily weighing upon South and Southeast Asia (SEA) [1]. In most endemic countries, chloroquine (CQ) remains the first-line therapy for acute vivax malaria after more than 70 years of continuous use. CQ-resistant *P. vivax*, documented nearly 30 years ago, now commonly occurs across much of SEA [4, 5]. Unlike the other dominant species causing human malaria, *P. falciparum*, some sporozoites (called bradysporozoites) of *P. vivax* develop into dormant forms in the liver called hypnozoites. This single feature—latency—defines and distinguishes the prevention, treatment, and control of vivax malaria. Other sporozoites (called tachysporozoites) immediately develop into actively dividing hepatic schizonts over the 7-day to 18-day incubation period and cause the primary parasitemia and acute attack of patent vivax malaria. Hypnozoites activate weeks, months, or even years later, causing a renewed clinical attack called relapses [6].

In natural endemic settings, it may not be known if any given patient presenting with patent acute vivax malaria is experiencing a tachysporozoite-borne primary attack or a bradysporozoite-borne relapse. This uncertainty poses a fundamental problem of interpretation of parasitemia that may follow therapy [5–8]. The origin of the parasitemia may be a consequence of new primary attack (reinfection), therapeutic failure against blood stages (recrudescence), or renewed latent malaria (relapse). These ambiguities may not be addressed by molecular genotyping techniques because relapses may be either homologous or heterologous to the primary infection event [9, 10]. Estimating the efficacy of blood schizonticidal therapy may thus be complex and difficult [11].

Another drug, a hypnozoitocide, is necessary to treat latent vivax malaria and prevent future attacks. Primaquine (PQ) has been the only available therapeutic option to kill hypnozoites since 1952. A single dose of 30 mg PQ within 48 hours of infection appears sufficient to kill stages of *P. vivax* or *P. falciparum* attempting to develop into hepatic schizonts or hypnozoites [12]. Beyond that period, presumably after formation of dormant hypnozoites, relatively large doses totaling 210 to 420 mg of PQ (delivered over 7 to 56 days) are required to prevent

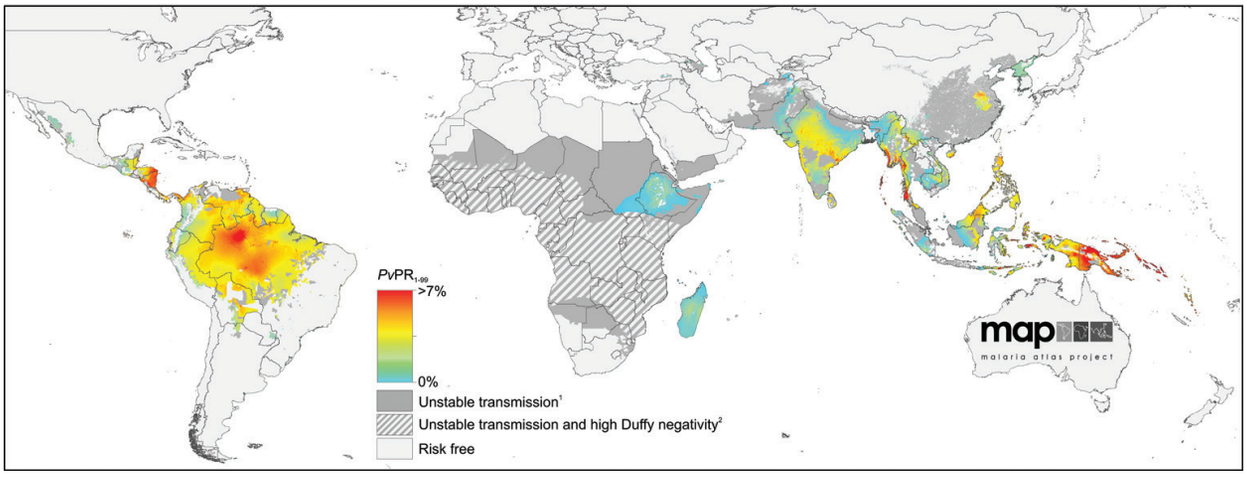

Figure 1. Distribution of *Plasmodium vivax* malaria in the world.

relapse [13]. Pharmacokinetic or pharmacodynamic interaction of blood schizonticidal and hypnozoitocidal therapies combined for the radical cure of vivax malaria has been observed and requires consideration in assessing the safety and efficacy of either or both in clinical use [14].

This chapter reviews the challenges vivax malaria poses in efforts to control and eliminate malaria in accordance with the Global Technical Strategy of the WHO [15] as they occur in many parts of the world. Experts advising the WHO formulated "*Plasmodium vivax* Control and Elimination: A Technical Brief" [16] highlighted the distinct character of this species in the context of control and elimination strategy. Conventional control aimed at diagnosing and treating of the acute attack and minimizing exposure to biting *Anopheles* mosquitoes will not suffice, largely due to the scale and importance of the latent hypnozoite reservoir in endemic communities. Decades of scientific, clinical, and public health neglect of this specific feature of vivax malaria leaves us poorly equipped to attack it safely and effectively. The biological basis of this problem is detailed with the aim of guiding discovery and development of sustainable solutions.

2. Biology of *Plasmodium vivax*

The broad and prolonged neglect of research on *P. vivax* has been highlighted by many researchers [17–20]. Although this lack of research certainly derives from complex and multiple factors, the misperception of this species as intrinsically benign perhaps dominates among them [21]. Today, we accept that a diagnosis of vivax malaria is sometimes associated with severe disease syndromes associated with fatal outcomes [16, 22]. The manner in which *P. vivax* threatens life with such typically low-grade parasitemias (usually tenfold lower than *P. falciparum*) is an important and relatively new question. Nonetheless, some researchers suggest that vivax malaria may be primarily an infection of hematopoietic tissues rather than of the vascular sinuses *per se* [21, 23, 24]. If most *P. vivax* biomass in the human host resides within tissues of the bone marrow and spleen, it would have far-reaching scientific, clinical, and public health implications with respect to measuring and combatting the threats imposed.

We already know the likely importance of the latent hypnozoite reservoir and sub-patent/asymptomatic parasitemias [25–27]. Adding an as-yet unacknowledged sequestered trophozoite reservoir—very few or no asexual parasites in vascular sinuses but many in the extravascular spaces of erythropoietic tissues—would greatly amplify concerns regarding the effectiveness of diagnosis and treatment in control and elimination. It is possible that most *P. vivax* parasites—certainly hypnozoites but perhaps also trophozoites—occur beyond the vascular sinuses in both asymptomatic and acutely ill patients and, therefore, also beyond the reach of standard diagnostics.

Although the Duffy antigen on the surface of the red blood cell has long been considered essential to *P. vivax* invasion—and its absence in many African populations thought to explain

the relative rarity of *P. vivax* on that continent—recent evidence from a variety of African locales has shown patent *P. vivax* parasitemia in patients who are negative for that molecule [28]. Moreover, *P. vivax* has been shown to be present in parts of Africa where it is not prevalent [29] and is indeed prevalent in other areas of that continent like Madagascar, the Horn, and across the northern Sahel [30].

3. Chloroquine-resistant acute *P. vivax*

Resistance to CQ by the asexual stages of *P. vivax* has been documented in most endemic regions [4, 5]. Resistant strains dominate the malarious Western Pacific and Indonesian archipelago and nations there have adopted highly efficacious ACTs [11] as first-line therapy. With the possible exception of artesunate combined with sulfadoxine-pyrimethamine, all ACTs have shown superb efficacy in killing asexual blood stages of *P. vivax* [31]. The safety and efficacy of PQ against relapse when combined with partner blood schizonticides other than CQ, quinine, or dihydroartemisinin-piperaquine [32, 33] require validation in clinical trials [14]. Elsewhere, for now, resistance appears sporadically and at relatively low frequencies. Despite substantial efforts to identify molecular markers of *P. vivax* resistance to antimalarial drugs, none have yet been validated. *In vivo* testing in patients or relatively difficult *ex vivo* drug testing procedures remain necessary [34]. The monitoring of antimalarial efficacy offers possible relief from risk of failure due to parasite resistance to specific therapies, but this is carried out relatively infrequently.

4. Latent and sub-patent *P. vivax*

The latent and sub-patent parasitemia caused by *P. vivax* is difficult or impractical to detect using available technologies. These unnoticed or invisible infections probably represent a dominant majority in most endemic settings. Thus, the primary blow to therapeutic effectiveness (the proportion of patients needing a particular therapy and receiving high-quality drug in a full and adequately absorbed dose) is simply the inability to identify those in need of therapy.

The human host also imposes important barriers to the effectiveness of antimalarial therapies in the real world. Clinical contraindications, patient adherence, provider prescribing practices, provider and patient access to the drug, and its quality and availability; all further chip away the realizable effectiveness of any given antimalarial agent. The contraindications are particularly important in the case of *P. vivax* and the crucial therapy against relapses with PQ, the only current therapeutic option for that clinical indication. Primaquine (and all other 8-aminoquinoline compounds evaluated) invariably provokes an acute hemolytic anemia in patients receiving therapeutic doses against relapse and having an inherited X chromosome-linked deficiency in glucose-6-phosphate dehydrogenase (G6PD) enzymatic activity [35]. This abnormality affects approximately 400 million people or 8% of people residing in malaria endemic countries [36].

Safe access to PQ for radical cure of vivax malaria may require access to point-of-care diagnostics for G6PD deficiency [37]. Even with such testing, however, there remains the problem of treating those diagnosed as G6PD-deficient, pregnant or lactating women, and infants below the age of 6 months [38]. There are no optimized or validated means of preventing relapse without 8-aminoquinoline drugs, e.g., by chemopreventive or presumptive periodic preventive therapeutic strategies [39, 40]. The 8-aminoquinoline drug, called tafenoquine, is in late clinical development and will likely soon offer a single-dose option to PQ, virtually eliminating the important adherence problem with that therapy [41, 42].

Another potential problem in the human host may be the inability to metabolize PQ to its active hypnozoite-killing metabolite by cytochrome P450 2D6 (CYP2D6) [43]. Natural polymorphism in the gene expressing CYP2D6 leads to a range of metabolic activities ranging anywhere between far above normal and null. Patients in need of PQ anti-relapse therapy and having significantly impaired or null CYP2D6 activity may relapse even with full compliance to good quality drug. We do not yet know the extent of this problem with regard to the frequencies of CYP2D6 alleles associated with PQ therapeutic failure, but the significantly impaired CYP2D6 *10 allele (a particular genetic variant of CYP2D6 gene) is relatively common among Southeast Asians, at about 35% frequency [44]. It may be that many Asians will be unable to adequately metabolize PQ and achieve successful radical cure [45].

The ambiguity of geographically variable frequency and timing of relapse—along with reinfection and recrudescence in recurrent *P. vivax* malaria after PQ therapy—makes estimating PQ efficacy in endemic settings very difficult. This is true even with directly observed therapy using high-quality drug. After decades of recommending a 5-day regimen of PQ against relapse, on the basis of observed low rates of relapse following therapy, investigators in India ultimately included a relapse control group (placebo) and discovered that efficacy to be nil [46]—the low rate of relapse was naturally occurring. John et al. [47] systematically reviewed recurrence rates after standard 0.25 mg/kg daily for 14-day regimen with rates of recurrence averaging about 8% at 1 month, 10% at 2–3 months, 14% at 4–6 months, and 20% at 7–12 months. In two randomized controlled trials of PQ given at high dose (0.5 mg/kg) to 257 Indonesian soldiers infected by *P. vivax* in eastern Indonesia and followed for a year where reinfection was not possible, 35 (14%) experienced at least one relapse [32, 33]. Among the 21 subjects whose CYP2D6 genotype and phenotype were examined, 20 showed evidence of significant functional impairment of CYP2D6 [48].

Evidence supports the notion of providing presumptive anti-relapse therapy to all patients diagnosed with any species of malaria agents, especially *P. falciparum*. In a retrospective analysis of over 10,000 research subjects naturally infected by *P. falciparum* in Thailand or Myanmar, 912 were treated with rapidly excreted blood schizonticides, and within 2 months, just over 50% experienced a *P. vivax* attack [49]. The people infected by one species in any given community must be considered at high risk of harboring latent and perhaps sub-patent infections of the other co-endemic species. Species-specific therapies, especially in an age of dominant CQ resistance among the plasmodia, may not be sensible in an elimination context.

Effective diagnosis and treatment represents the cornerstone of current control and elimination strategies, and the obstacles described here require consideration in realizing gains against this tenacious endemic problem. Indeed, such gains have been achieved both historically and recently. At the turn of the twentieth century, endemic vivax malaria occurred across much of southeastern North America, northern and southern Europe, the Middle East, and northern Australia—areas where it no longer appears. Much of this success was achieved applying environmental modifications against local *Anopheles* vectors, but more recent elimination successes using principally diagnosis and treatment strategy have occurred in nations like Turkey, Azerbaijan, and Sri Lanka, as examples [50]. The same had been achieved on the Korean Peninsula during the 1970s, but endemic vivax malaria transmission reappeared during the 1990s and persists today [51]. Post-elimination vigilance that includes not only diagnosis and treatment services but also vector control may be essential to protecting and sustaining the elimination of endemic vivax malaria [50].

5. Vector control in vivax malaria

Vector control of endemic vivax malaria may not have immediate impacts due to the hypnozoite reservoir contributing >80% of acute attacks of vivax malaria in low or high endemic settings [52, 53]. Success in reducing malaria incidence and local transmission to zero in a malaria endemic area, particularly where sympatric *P. falciparum* and *P. vivax* occur, may require greater sustainability of vector control measures. Vivax malaria transmission will outlast falciparum malaria, and reestablishment of local transmission may occur without imported cases, i.e., by local hypnozoites. Prevention of the seeding of new hypnozoites in liver cells by biting *Anopheles* mosquitoes obviously may contribute positively to the control and elimination of vivax malaria in the long term, but no randomized controlled trials yet affirm this. In one large cluster-randomized trial in Myanmar, insecticide-treated bed netting (ITN) had no impact whatsoever on the risk of malaria [54], an outcome attributed to the dominant *Anopheles* vector, *A. dirus* s.s., feeding predominantly outdoors and early in evening or morning [55]. Relatively modest effects were reported from a similarly cluster-randomized trial in Vietnam, again attributed to mosquito behaviors unfavorable to control by this means [56]. The main Asian vector species tend to feed early in the evening and outdoors where they also rest [57], minimizing their exposure to household insecticides. In other studies of strategies for minimizing exposure to *Anopheles,* much greater impacts against falciparum malaria were demonstrated relative to those against vivax malaria [58–60].

Over the last decade, attempts of using spatial repellents (SRs) to minimize exposure to biting insects have shown some success in diverse settings [61]. Repellency is distinct from the killing action of insecticides in more than one way, i.e., no direct contact is required, and lacking lethality does not select for resistance. SRs are effective irrespective of indoor or late-night feeding and resting behavior like conventional netting or indoor spraying. SRs should be evaluated for added benefit in areas where traditional long-lasting insecticidal net (LLIN) or

indoor residual spraying (IRS) of insecticides interventions may not offer full protection or have reached their efficacy limits—especially in areas with residual transmission or in areas where elimination may be considered feasible. Control of disease in these areas will require new approaches, and possibly spatial repellency would be practical and effective [62, 63]. SRs may be useful as stand-alone tools of personal or household protection where other interventions may not reach. Also, they may be combined with conventional interventions to augment their impacts.

Another vector control strategy for eliminating *P. vivax* in the Asian-Pacific region may be the method of environmental modification called "species sanitation." This approach offers prevention independently of the myriad problems and challenges of diagnosis and treatment or the limitations of insecticidal strategies. Species sanitation is simply sanitizing the environment against specific incriminated vector species by exploiting detailed knowledge of their bionomics (behavior and ecology) [64]. Malcolm Watson in British Malaya, along with Nicholas Swellengrebel and Raden Soesilo in the East Indies, invented, optimized, and validated species sanitation in malaria control [65]. A systematic analysis of 16 such interventions (most conducted before 1945) showed an average 88% reduction of malaria burden [66]. As new cases occur by relapse, reinfection, or importation, making the subsequent infection of mosquitoes improbable (by simply reducing their numbers) eventually suffocates transmission.

Although the implementation of LLIN, IRS, and species sanitation in different environmental settings rendered significant success rates [67], it is evident that the key determining factors for the success of any vector intervention selected is a thorough knowledge of the vector bionomics, local malaria transmission dynamics, and residual efficacy of choice insecticide. Knowledge of vector bionomics includes ascertaining breeding and resting preferences and feeding behavior of incriminated vector species. Transmission dynamics include information related to entomological inoculation rates, sibling species composition of vectors (based on reliable PCR identification assays), seasonality of malaria prevalence, and risk factors that may support the human-mosquito contact, while suitable insecticide means any available insecticide that renders knockdown effect and/or mortality to the incriminated vector population.

Another important issue to be considered is the ability of the *Anopheles* mosquitoes to adapt to the ongoing vector interventions by changing host-seeking behavior, such as from indoor to outdoor or *vice versa*, and selection of insecticide-resistant strain [68]. With current trends in globalization and population migration, deforestation, and resettlement of populations, reintroduction of malaria into areas that have been declared free from transmission is a clear and present risk. Therefore, no single intervention method may guarantee long-term efficacy; thus, regular monitoring of vector density and behavior should be a routine operation wherever this risk occurs. Most malaria control programs no longer have the entomological expertise needed to carry out these important tasks—addressing this problem may be the greatest and most important challenge within the context of a malaria elimination agenda.

6. Vaccination

A vaccine that prevents the seeding of human livers by both active schizonts and dormant hypnozoites of *P. vivax* would provide a conspicuously useful tool in eliminating this species. Mass or routine vaccination now seems impractical with non-sterilizing vaccines of short-lived immunity needing 3 or 4 doses. These may improve in the future, but even now a malaria vaccine could be applied in geographically or demographically narrowed settings to potentially great impacts. For example, high-risk and hard-to-reach populations like migrant workers or soldiers having sterile immunity to malaria (even if for just a season or two) may not only protect those people from harm but also greatly slow importation of malaria into receptive areas where transmission has been interrupted. Likewise, people living in areas prone to reintroduction of endemic malaria by high volumes of immigration from high-risk areas may be immunized and protected against very dangerous outbreaks and epidemics [69].

Today, there is no vaccine available that can prevent infection by *P. vivax* with high levels of sterilizing immune protection. That is also true for all other plasmodial species. The half-century-long efforts to develop a vaccine against *P. falciparum*—greatly aided by the ability to cultivate this species in continuous laboratory cultures since the late 1970s—culminated in the molecular subunit vaccine called RTS,S ASO1 (mimicking a protein-coating infectious sporozoites) [70] with the registered trademark name Mosquirix™ (GlaxoSmithKline). The vaccine did not prevent infection in the African infants and young children vaccinated but had the modest effects against higher parasitemias and signs of illness [67]. The modest efficacy combined with worrying and puzzling signals like increased risk of pneumococcal meningitis and significantly higher all-cause mortality among vaccinated females apparently explains the WHO position to withhold a favorable opinion on the vaccine until further studies involving targeted and limited rollout in several African nations are completed [71]. Molecular subunit vaccines targeting *P. vivax* molecules have not progressed beyond Phase 2a and show similar inability to achieve high levels of sterilizing protection [72].

Over the past decade, investigators applying live-attenuated sporozoites of *P. falciparum* have shown high levels of durable (~12 months) sterilizing protection in malaria-naïve adult volunteers in controlled human malaria infection (CHMI) experiments using a challenge strain homologous to the vaccine strain [73]. This approach relies on laboratory harvest of infectious sporozoites from laboratory-reared aseptic anopheline mosquitoes infected by *P. falciparum* maintained in the laboratory. Deriving live-attenuated sporozoites of *P. vivax* is possible [74] but exceedingly difficult, not strain-specific, and not sustainable as a source of vaccine. Nonetheless, immunization by irradiated sporozoites of the murine species *Plasmodium berghei* cross-protected against the murine species *Plasmodium yoelii* and *vice versa* in murine challenge models [75]. The possibility of sporozoites of *P. falciparum* cross-protecting against *P. vivax* challenge has not been examined directly, but proteomic analyses showed that these two human plasmodia species shared substantially more common probable T-cell epitopes than that between *P. berghei* and *P. yoelii*. A vaccine derived from laboratory-kept *P. falciparum*

systems offering protection against *P. vivax* would represent a quantum leap forward for vaccination against this species by effectively sidestepping the requirement for continuous laboratory cultivation for a live vaccine.

7. Challenges and recommendations

The greatest challenge in eliminating vivax malaria—the hypnozoite reservoir—may also be the greatest opportunity to accomplish the task. If >80% of incident malaria cases indeed derive from hypnozoites, then surely attacking and shrinking that reservoir would deliver substantial reductions in the burden of morbidity and mortality. Despite the availability of PQ for over 65 years, sustained and systematic assault on that reservoir has not been accomplished in the endemic tropics—largely due to the unsolved clinical problem of its hemolytic toxicity in G6PD-deficient patients.

Eliminating *P. vivax* malaria will require accepting the inadequacy of conventional falciparum malaria-focused control strategy, tactics, and tools and committing to the optimizing and validating of interventions suited to this stubborn parasite. This effectively means striving to solve the wrenchingly difficult problem of the hemolytic toxicity of PQ in G6PD-deficient patients by almost any means. The obstacles presented in managing populations and individual patients carrying this infection emphasize the great advantage of preventing it in the first place with an effective vector control strategy. In this context, species sanitation has proven highly effective against endemic Asian malarias a century ago [54] and would probably do so again.

Taking all these factors into considerations, we recommend the following measures for eliminating endemic vivax malaria:

1. Active case detection and early treatment are essential steps, fundamental to eliminating any endemic malaria; however, this measure alone will not lead to elimination—too many infections are latent, sub-patent, sequestered, and asymptomatic.
2. Adoption of safe and universal access to radical cure for cases of vivax malaria along with universal access to alternative means of relapse prevention for people ineligible for therapy with 8-aminoquinolines would accelerate progress to elimination. Achieving that will likely also require better diagnostics for both the parasite and G6PD deficiency than are currently available.
3. Adoption of radical cure with an 8-aminoquinoline and ACT with diagnosis of any species of malaria where *P. vivax* also occurs as a means of targeting likely carriers of hypnozoites.
4. Reduce new vivax infections/seeding of the liver with hypnozoites by substantially reducing human contact with malaria vectors, effectively stranding extant parasites in all stages of human infection—latent, sub-patent, patent, and eventually vanishing without *Anopheles* contact and onward transmission. Interrupting transmission by species sanitation measures may be the most durable and effective means of achieving this goal.

5. Examine the possibility of sterilizing immune protection against *P. vivax* provided by attenuated *P. falciparum* sporozoite vaccines providing an immediately highly relevant tool for eliminating endemic *P. vivax*.

8. Conclusion

Plasmodium vivax passes substantial challenges that may hinder achievement of global malaria elimination by 2030. The most challenging evidence is the lack of technology to detect the latent infection caused by hypnozoite. Therefore, the only tool to prevent *P. vivax* transmission originated from reactivation of hypnozoites is by vector control.

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List of acronyms

ACT	artemisinin-based combination therapy
CHMI	controlled human malaria infection
CQ	chloroquine
CYP2D6	cytochrome P450 2D6
G6PD	glucose-6-phosphate dehydrogenase
IRS	indoor residual spraying
ITN	insecticide-treated bed nets
LLIN	long-lasting insecticide treated nets
PQ	primaquine
SEA	Southeast Asia
SR	spatial repellent
WHO	World Health Organization

Author details

Puji BS Asih[1]*, Din Syafruddin[1,2] and John Kevin Baird[3,4]

*Address all correspondence to: puji@eijkman.go.id

1 Eijkman Institute of Molecular Biology, Jakarta, Indonesia

2 Hasanuddin University Medical Research Centre, Makassar, Indonesia

3 Eijkman Oxford Clinical Research Unit, Jakarta, Indonesia

4 Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom

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