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Vivax series:

Plasmodium vivax transmission: chances for control?

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***Plasmodium vivax* is a growing public health problem in many regions of the world as a result of re-emergence and increased transmission. This article reviews the unique biology related to *P. vivax* transmission and addresses potential problems associated with the control of this parasite, which depends on an in-depth knowledge of malaria transmission. The success of comprehensive control measures will require advanced laboratory and field research on this parasite, international awareness of the problem, and co-operation by members of the international malaria community to implement new knowledge and improve the management of transmission in each endemic area.**

Plasmodium vivax is responsible for >50% of all malaria cases outside Africa, and is endemic in the Middle East, Asia and Western Pacific, with a lower prevalence in Central and South America [1]. Vivax malaria usually causes benign uncomplicated malaria with relapses, and its clinical features differ from those of falciparum malaria (Table 1). However, infections occasionally result in severe clinical symptoms similar to *Plasmodium falciparum* [2].

Plasmodium vivax and *P. falciparum*, the two most prevalent species of malaria that infect humans, often co-exist in many parts of the world. In Thailand and many other malaria-endemic countries, *P. falciparum* and *P. vivax* are often transmitted by the same vector species [3–5]. Thus, the two parasite species are subject to similar control programs, which include collection of baseline information and implementation of various control measures (Box 1). However, control measures initiated based on knowledge about *P. falciparum* transmission might not be effective for the control of *P. vivax*.

The difficulty in controlling *P. vivax* has been exacerbated for several reasons (Box 1c). First, the developmental biology of *P. vivax* is unique in early gametocytogenesis and the generation of hypnozoites in the liver that are responsible for relapses of the disease. Second, the response of *P. vivax* to some antimalarials differs when compared with *P. falciparum*. Third, the behavior and physiology of mosquito vectors, especially cryptic species, are largely unknown in many regions where *P. vivax* is endemic. Finally, the lag of *P. vivax* behind *P. falciparum* research and funding has limited our potential to develop effective control measures tailored to *P. vivax*. This article reviews the unique characteristics of the *P. vivax* life cycle, potential problems with drug resistance, the importance of understanding *P. vivax* vectors in evaluating insecticide efficacy, the patterns of re-emergence or increasing prevalence of *P. vivax* in some regions, and current developments in transmission-blocking vaccines (TBV). The transmission of *P. vivax* can be controlled effectively through an increased knowledge and integration of research efforts in each of these areas.

Liver or exo-erythrocytic stages

A unique characteristic of *P. vivax* and *Plasmodium ovale* is the generation of dormant hypnozoites in the liver that are responsible for relapses. However, our knowledge of the biology of *Plasmodium* liver or exo-erythrocytic (EE) stages is far more scarce than that of the blood stages. In general, studies on *Plasmodium* EE stages have focused on *in vivo* and *in vitro* development of the parasites, molecular mechanisms of sporozoite–hepatocyte interactions during the sporozoite invasion phase, and isolation

Table 1. Clinical features of *Plasmodium falciparum* and *Plasmodium vivax*[a]

	P. falciparum	*P. vivax*
Prepatent period (days)	5.5	≥ 8
Incubation period (days)	9–14 (12)[b]	12–17 (15)[b] or up to 6–12 months
Fever periodicity (hours)	24, 36, 38	48
Erythrocytes parasitized	All	Reticulocytes
Merozoites per schizont	8–32	12–24
Relapses	No	Yes
Recrudescences	Yes	No
Drug resistance	Yes (to multiple drugs)	Yes (to chloroquine)

[a]Data obtained from Ref. [48].
[b]Mean incubation period is indicated in parentheses for *P. falciparum* and *P. vivax*.

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Box 1. Potential tools and problems in the control of *Plasmodium vivax* transmission

(a) Information required for strategic planning of malaria control programs

- Where does malaria transmission occur?
- When does malaria transmission occur (seasonal or year-round transmission)?
- What species of *Plasmodium* is the causative agent for malaria?
- What is the prevalence of *Plasmodium vivax* (VK210/247)?
- Who is the population at risk?
- What is the proportion of asymptomatic and/or symptomatic malaria cases?
- What is the drug sensitivity status of *P. vivax*?
- Which mosquito species is the main vector(s) of malaria transmission?
- What is the insecticide sensitivity status of the malaria vector(s)?

(b) Methods for malaria control

- To inhibit mosquito–human contact by using insecticide-treated materials or mosquito trapping.
- To eliminate mosquito vector: insecticides, larvicidal bacteria, larvivorous fish and zooprophylaxis (practically not acceptable by villagers).
- To inhibit parasite development in human (e.g. causative drug, vaccine).
- To inhibit transmission of parasite from human to mosquito (transmission-blocking drugs, transmission-blocking vaccines).
- To inhibit transmission of parasite from mosquito to human (prophylaxis drug, vaccine).

(c) Potential problems for the control of *Plasmodium vivax* transmission

- No rapid and sensitive method available for large-scale survey.
- Complication of drug treatment and monitoring of drug efficacy due to hypnozoite and relapse-stage parasites.
- Different treatment regimen, mostly with or without drugs against hypnozoites (i.e. primaquine) among treatment providers, especially community based and hospital based.
- Prevalence of asymptomatic cases among semi-immune populations.

of liver-stage antigen for the purpose of anti-infection vaccines. Recent studies on the invasion phase identified a hepatocyte receptor CD81 that is required for *P. falciparum* and *Plasmodium yoelii* sporozoite infectivity [6], defined the role of hepatocyte proteoglycans in mediating sporozoite targeting to the liver [7], and demonstrated that migration through host cells activates sporozoite infection of hepatocytes [8]. However, studies on liver-stage-specific gene expression and antigens are hampered largely by difficulties in obtaining EE parasite materials. So far, only one liver-stage antigen, liver-stage antigen 1 (LSA-1), is known to be expressed during EE development of *P. falciparum*, and could contribute to protective immunity to sporozoites [9,10]. LSA-3, which is expressed in the mosquito and liver-stage parasites, is another promising vaccine candidate because immunization with LSA-3 induced protection against *P. falciparum* challenges in chimpanzees [11].

The development of LSA-1 and LSA-3 vaccines has been based on *P. falciparum* and mouse models of malaria, but the lack of a *P. vivax* EE-stage model precludes the characterization of these antigens for *P. vivax*. Many questions remain on the biology of the hypnozoite stage, such as how hypnozoites develop in human hepatocytes, and which factors stimulate transformation from hypnozoite to mature EE schizonts. Current efforts to develop a hepatocyte cell line capable of supporting both *P. vivax* and *P. falciparum* infections will aid future research in this area (J. Sattabongkot, unpublished).

Potential problems with drug resistance

In recent years, the alarmingly rapid increase of multiple drug-resistance (MDR) *P. falciparum* strains has been a major concern for the future control of malaria and has been the focus of many malariologists [12]. By contrast, research on drug resistance in *P. vivax* has lagged behind. Many drug-resistant *P. falciparum* strains have originated in Southeast Asia. In Thailand, MDR *P. falciparum* strains are notably prevalent on the Thai–Cambodian and Thai–Myanmar borders. The antimalarial drug sulfadoxine–pyrimethamine (SP), which targets the parasite folate synthesis pathway, effectively targeted *P. falciparum* malaria until the widespread appearance of resistant parasites reduced its use [13]. SP has not been considered an effective drug against *P. vivax* malaria because of the immediate appearance of resistance in this parasite, which led to the belief that *P. vivax* is intrinsically resistant to SP [14,15]. A more plausible explanation is that heavy deployment of the antifolates against *P. falciparum* infection had selected resistant *P. vivax* strains. Indeed, the mechanism of resistance to SP in the two *Plasmodium* species appears to be identical [16–18], and sequence data suggest a sequential pathway for the acquisition of the *P. vivax* dihydrofolate reductase (DHFR) mutations [18]. In Thailand, where SP has not been in use for 20 years, *P. vivax* populations remain resistant to SP [19]. A recent study [20] showed that WR99210, a novel inhibitor of DHFR, is effective against most pyrimethamine (PM)-resistant *P. falciparum* DHFR and *P. vivax* DHFR. The suggestion that PM and WR99210 exert opposing selective forces on the *P. vivax* populations has generated novel interest in combination therapy with these drugs.

Quinolines comprise the primary lines of antimalarial drugs. Since its initial deployment over 50 years ago, chloroquine (CQ) resistance was apparent in the late 1950s, and thus far has been reported from all *P. falciparum*-endemic areas. However, CQ is still the drug of choice for eliminating *P. vivax* blood stages. A recent study on the effectiveness of the available antimalarial drugs artesunate, artemether, CQ, mefloquine, quinine, halofantrine and primaquine (PQ) on treating vivax malaria in Thailand has not detected significant resistance to these drugs [19]. However, drug efficacy tests are often confounded by the relapse of vivax malaria. The relapse interval of Southeast Asian *P. vivax* strains coincides with the mean time when recrudescence occurs following drug treatment [21]. Sometimes relapses can be identified by the length of time between initial and subsequent infections. In some cases, however, it is not

possible to distinguish clearly a recrudescence from a relapse when using available genotyping methods because the strains causing relapse and recrudescence might have derived from the same initial infection. Consequently, CQ-resistant *P. vivax* parasites might be more common than those observed. After it was first reported in Papua New Guinea in 1989 [22], CQ-resistant *P. vivax* strains have been found in many regions of the world, including Indonesia [23], Myanmar [24], India [25], Guyana [26] and Brazil [27]. However, these are only sporadic reports, and the distribution, epidemiology and molecular mechanism of CQ resistance in *P. vivax* remain to be determined. The paucity of knowledge regarding these aspects is due, in part, to the absence of a standard method to evaluate CQ resistance, as well as a reliable system for culturing *P. vivax* parasites *in vitro*. Currently, mechanisms of *P. vivax* resistance to CQ are not well understood but appear to differ from those for *P. falciparum* [28]. In *P. falciparum,* mutations in the *P. falciparum* chloroquine-resistance transporter (*pfcrt*) gene are essential for CQ resistance, but such mutations are not found in the *pvcg* gene, a *pfcrt* homologue, of the CQ-resistant *P. vivax* strains. These differences in molecular mechanisms could partially account for the slower development of CQ resistance in *P. vivax* than in *P. falciparum*. Additional explanations could lie in the different biology of gametocytogenesis between the two parasite species. For example, *P. falciparum* gametocytes form after the appearance of symptoms and drug treatment, and hence favor the transmission of resistant genotypes [29]. By contrast, early *P. vivax* gametocytogenesis allows the parasite to be transmitted before the symptomatic stage of the disease and anti-blood-stage chemotherapy. Even if novel genes are involved in CQ resistance in *P. vivax*, they might not be readily identified because of the difficulties involved in performing genetic crossing and quantitative trait loci mapping of the resistant gene(s); for such studies, animal models of malaria are highly advantageous [30].

One important aspect of treating vivax malaria relates to the presence of hypnozoite stage in the liver and relapse of the disease. Several studies indicate that geographically different *P. vivax* strains exhibit different relapse patterns [31–33]. Tropical *P. vivax* strains normally begin to relapse within a month after the initial infection, whereas the hypnozoites of temperate strains usually have an incubation period of several months. Currently, PQ is the only drug available to eliminate EE stages because of its activity against hypnozoites. PQ remains an ideal companion drug to CQ, although it is less effective than CQ in clearing *P. vivax* blood stages [34]. Today, the combination of CQ for blood stages and PQ for liver stages remains the current drug regimen for vivax malaria in Thailand [35]. Failure to complete the 14-day PQ regimen recommended by WHO normally leads to a relapse of the disease [35]. The treatment regimens, effectiveness, safety and related problems with PQ therapy of vivax malaria have been comprehensively reviewed [36]. Like all drugs, development of resistance to PQ is inevitable and remains to be evaluated. Understanding the development of *P. vivax* hypnozoites in different regions will enable the development of more effective chemoprophylaxis and treatment. Development of a liver stage *in vitro* model for *P. vivax* malaria will be useful for drug studies such as the detection of PQ resistance and elucidation of resistance mechanisms. In conclusion, *P. vivax* drug resistance is of increasing concern and deserves increased research efforts.

Changes in *P. vivax* prevalence

The re-emergence of *P. vivax* in many malaria-endemic areas where the disease was eradicated many years ago has now become a major problem, such as in China and Korea [37,38]. In the USA, malaria transmission was eliminated by the late 1960s [39] (see: http://www.cdc.gov/epo/mmwr/preview/mmwrhtml/00042732.htm), but sporadic cases of locally acquired mosquito-transmitted malaria continue to occur. For instance, transmission of *P. vivax* in Virginia in 2002 illustrates the re-introduction of *P. vivax* in an area where susceptible mosquito vectors are present [40]. In most cases, the introduction of *P. vivax* into those areas is caused by the movement of people (travelers, immigrants or soldiers) from malaria-endemic areas [41,42].

Another change of vivax malaria epidemiology is that *P. vivax* has become increasingly more prevalent in areas where it is sympatric with *P. falciparum* (see, for example, Ref. [43]). In Thailand, for example, *P. vivax* increased from $<20\%$ of total malaria cases in 1965 to $>50\%$ in 2002 (Figures 1,2). Such an increase in *P. vivax* prevalence is even more dramatic in certain geographical areas [43,44]. One reason for the increasing prevalence of *P. vivax* is that *P. falciparum* is an easier species to control with effective drug treatment because of the lack of hypnozoite stages. In addition, efforts to control drug-resistant *P. falciparum* have been very effective, hence altering the competition between *P. falciparum* and *P. vivax*. However, increasing *P. vivax* prevalence could also be due to changes in vector potential. In Sa Kaeo, a province in eastern Thailand bordering Cambodia, where *P. falciparum* used to predominate, almost all malaria cases comprise *P. vivax*, increasing from 666 cases in 1995 to 4381 cases in 1997 [44]. CQ-resistant strains of *P. vivax* were not detected in this area [45], so the relative changes in *P. falciparum* and *P. vivax* prevalence might be attributed to corresponding changes in the composition and abundance of anopheline mosquito vectors [46]. A two-year entomological study in Sa Kaeo (1998–1999) showed that a decrease in the abundance of *Anopheles dirus*, the main *P. falciparum* vector in this area, was accompanied by a concurrent increase in the abundance of members of the *Anopheles barbirostris/campestris* group [44]. It was suggested that these species might be important secondary vectors because of their high biting intensity, anthrophilicity and susceptibility to only *P. vivax* [44,46,47].

Other differences in parameters that determine the vectorial capacity of a given anopheline species might also be responsible for the temporal changes in the prevalence of *P. vivax*. Mosquito longevity is an important parameter to determine vectorial capacity for a malaria parasite [48]. Depending on the environmental and physiological factors, the average longevity of *Anopheles* mosquitoes in tropical countries varies from ten days to more than a month [48–50]. In experimental infections of colonized

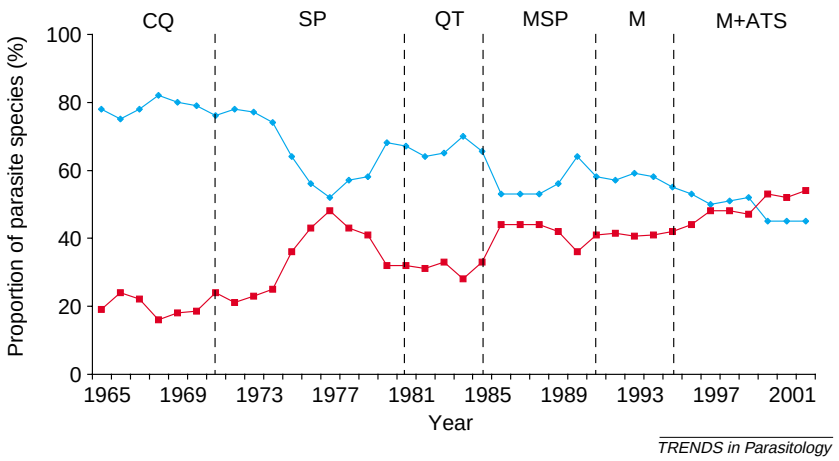

Figure 1. Antimalarial regimen used in Thailand from 1965 to 2002. Temporal dynamics of *Plasmodium falciparum* (blue) and *Plasmodium vivax* (red) proportions in Thailand, in relation to the drug policy. Broken lines indicate the time when a new drug policy (mostly for treating *P. falciparum* malaria) was initiated. For vivax malaria, the combination of CQ and PM remains effective. Data obtained from the Bureau of Vector Borne Diseases, Ministry of Public Health, Tiwanon Road Nonthaburi, 11000, Thailand. Abbreviations: CQ, chloroquine; MSP, mefloquine plus SP; SP, sulfadoxine and pyrimethamine; QT, quinine and tetracycline; M, mefloquine; M + ATS, mefloquine in combination with artesunate.

An. dirus, P. vivax sporozoites invade the salivary glands more than two days earlier than *P. falciparum* sporozoites, i.e. ≥11 days after the infected bite for *P. vivax* versus ≥13 days for *P. falciparum* (J. Sattabongkot, unpublished). Thus, if sporogonic development of *P. vivax* is faster than *P. falciparum* in natural vectors compared with laboratory- reared colonies of anopheline vectors, its transmission might predominate in certain areas where the vectors have a shorter lifespan. Further studies are necessary to re-evaluate the vector status in Thailand and other areas where *P. vivax* prevalence has increased.

Vector control with chemical insecticides

A thorough knowledge of mosquito vectors is essential to understanding the epidemiology of *P. vivax* transmission, and hence planning and implementing effective vector control programs [51–53]. Usually, control strategies must cope with a complex vector system, which includes a few primary vectors and several secondary vectors, whose impact on transmission varies depending on the region. In Southeast Asia, the main primary vectors, for example, *An. dirus s.l.* and *An. minimus s.l.*, are complexes of morphologically identical sibling species, which exhibit different behavioral patterns [54,55].

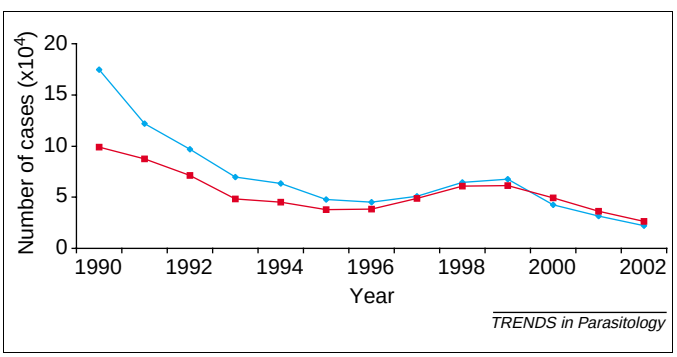

Figure 2. Malaria cases by species in Thailand. Trends of *Plasmodium falciparum* (blue) and *Plasmodium vivax* (red) in Thailand from 1990 to 2002. Data obtained from the Bureau of Vector Borne Diseases, Ministry of Public Health, Tiwanon Road Nonthaburi, 11000, Thailand.

The evaluation of insecticide efficacy in malaria-endemic areas depends on correct species identification, knowledge of vector sensitivity to insecticides [51–53], and the availability and usage of insecticides and insecticide-treated materials (ITMs) to the community [56]. Not all mosquito species are equally sensitive to the same chemicals; thus, it is important to measure the effect of insecticides against the targeted malaria vectors, especially where species complexes exist [57]. Insecticides and ITMs are highly effective measures to control *P. vivax* in areas where highly anthropophilic and endophagic vectors are present. In parts of Thailand, however, the poor efficacy of ITMs has been attributed to the exophilic and exophagic nature of the vector *An. minimus s.l.* [58].

The following two examples highlight the significance of evaluating insecticide efficacy where cryptic vectors probably exist. Studies were conducted on the Pacific coast of Nicaragua [59] and on the north coast of Peru [56]. Vivax malaria comprised 99% and 100% of all cases, respectively, and the main vector was *An. albimanus* (99% and >90%, respectively). In Nicaragua, the protective efficacy of lambdacyhalothrin-treated materials was 68% when the coverage of the ITMs in the village was 50% [59]. In Peru, however, ITMs provided insignificant protective efficacy [56]. The difference between the two studies was attributed to differences in vector-biting times and degree of endophagy: >50% of the mosquito vectors at the Peru study site bit people before bed time (before 21:00 h), while 70% of the vectors at the Nicaragua study site bit later (after 21:00 h). At both locations, the majority of mosquitoes (64% and 77%, respectively) were collected indoors. The different behavior of *An. albimanus* at these locations could be explained by different sibling species of the *An. albimanus s.l.* complex [60].

Transmission-blocking vaccines

Transmission-blocking vaccines (TBVs) are being developed to interrupt malaria transmission in the mosquito vector. TBV candidates are surface molecules expressed from gametes to ookinetes, which include the gamete and

early zygote surface proteins Ps48/45 and Ps230, and the late zygote and ookinete surface proteins Ps25 and Ps28 [61]. TBVs target the sexual stages of the malaria parasite as they are ingested in a bloodmeal by a mosquito, therefore low levels of antigenic polymorphisms are expected as a result of reduced selection pressure by the host immune system when compared with antigens expressed at pre-erythrocytic and erythrocytic stages of the parasites [62,63]. In principle, TBVs can be applied as follows: (i) for regional elimination of malaria in a similar way to the mass drug administration (MDA) program carried out on an island [64]; (ii) reduction in malaria transmission that will reduce child mortality even in areas of high endemicity [65]; (iii) prevention or control of malaria epidemics usually occurring as a result of natural or man-made disasters in tropical areas [66]; and (iv) protection of other vaccines or drugs against the spread of resistant parasites [67]. Although it would be difficult to introduce a TBV alone, it is a part of an integrated control program.

Why do we need to develop TBV for *P. vivax* malaria? First, the control of this prevalent malaria species also requires an effective integrated program, where TBV is an important component. Second, early gametocytogenesis and transmission of *P. vivax* before disease symptoms develop make it harder to control using gametocytocidal drugs. TBVs appear to be the best strategy for disrupting *P. vivax* transmission in a competent vector. Third, in areas where both *P. falciparum* and *P. vivax* are prevalent, a reduction in one parasite population will probably result in the rise of the other species as a consequence of cross-species interactions [68,69]. Therefore, for many malarious regions outside of Africa, development of effective TBV requires coverage against both *P. falciparum* and *P. vivax*. The *P. vivax* TBV candidates, Pvs25 and Pvs28, have been isolated from *P. vivax* Salvador I (Sal I) strain [70]. Mice vaccinated with the yeast-produced Pvs25 and Pvs28 adsorbed to aluminum hydroxide developed strong antibody responses against the immunogens. The development of oocysts in mosquitoes was completely inhibited when these antisera were ingested with the *P. vivax* Sal I-strain-infected chimpanzee blood [71]. Antibodies raised against *P. vivax* Sal I-based vaccines overcome the genetic polymorphism of Pvs25 and Pvs28 present in natural isolates of *P. vivax*, suggesting the wide range applicability of Sal I-based vaccines [72]. Phase I clinical trials of Pvs25-based TBV are now in progress (A. Saul, pers. commun.).

Chances for control?

The unexpected re-emergence of *P. vivax* in many areas following a series of successful eradication programs serves as an important lesson in the history of failed malaria control programs [40–42]. These reports raise questions of how efficient malaria control programs are and to what extent successful control of *P. vivax* transmission can be achieved. A recent report of successful eradication of *P. vivax* on Aneityum, a Vanuatu island with a small number of inhabitants, is promising [64]. Using an integrated control strategy of MDA, permethrin-treated bednets, larvivorous fish and enthusiastic community participation, malaria transmission on Aneityum was eliminated within nine years after the control program was initiated. ITMs have been used even after malaria was eliminated from this island, and active surveillance for imported cases prevents the re-emergence of malaria.

The successful control of *P. vivax* transmission in larger malaria-endemic areas will depend on a precise definition of local epidemiology and consistent government support. Thailand has implemented three steps to control malaria: (i) early diagnosis and prompt treatment; (ii) promoting health education to populations at risk; (iii) vector control by indoor residual spraying and ITMs; and (vi) monitoring insecticide resistance. These steps have enabled substantial success in reducing malaria transmission in the central part of Thailand. Control of malaria transmission along the Thai border is more complicated because of the migration of people and different control policies on both sides of the border. Re-organization of health centers responsible for malaria control and political problems along the border also impact the success of the overall control program. MDA and effective surveillance are difficult to enforce because of the complicated nature of *P. vivax* relapses and the lack of rapid, reliable methods to screen for asymptomatic *P. vivax* infections (see, for example, Ref. [73]).

Control of *P. vivax* transmission is possible, but increased research efforts are required to: (i) understand the biology of *P. vivax* EE stages; (ii) understand the mechanisms of resistance of *P. vivax* to antimalarials (e.g. PQ) and develop new-generation antimalarials with activity against hypnozoites; (iii) understand the behavior of local anopheline vectors and malaria transmission patterns to optimize vector control using insecticides and ITMs; and (iv) develop anti-infection and TBV for *P. vivax* (e.g. based on LSA-1 and/or LSA-3, and Pvs25 and/or Pvs28, respectively).

Baseline information of *P. vivax* transmission in each area must be identified before planning effective control programs, and control methods should address local transmission epidemiology. To avoid a re-emergence of new cases, programs for eradication and post-control, such as active surveillance, need to be planned and continued for as long as malaria transmission occurs in neighboring areas.

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