DISTRIBUTION AND EMERGENCE OF CHLOROQUINE-RESISTANT *PLASMODIUM VIVAX*

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ABSTRACT: *Plasmodium vivax* is the second most geographically widespread human malaria parasite. The global burden of *P. vivax* malaria is approximately 70-80 million cases annually. Chloroquine given together with primaquine is currently the first-line treatment for *P. vivax*. *P. vivax* resistance to chloroquine has however been reported in several endemic areas, particularly Southeast Asia, the area with the greatest *P. vivax* burden. Emergence of chloroquine-resistant *P. vivax* (CRPV) imposes a significant impact on the control strategies and treatment policies in these endemic regions.

Keywords: *Plasmodium vivax*, chloroquine resistance, Distribution

INTRODUCTION

Plasmodium vivax is one of the five species of malaria parasite that infects humans. It is the second most prevalent malarial species which accounts for approximately 40% of total malaria cases worldwide in Middle East, Asia, Oceania, and Central and South America. Although the infection is less virulent than *Plasmodium falciparum* [1], it can be dormant in host's liver cells for months or even years, causing huge burdens on public health. Recently, *P. vivax* has been reported to also cause severe illness and death [2-4]. Chloroquine, the blood schizontocide, given at the dose of 25 mg base/kg bodyweight over 3 days, together with primaquine, the tissue schizontocide, given at the dose of 0.25 mg base/kg bodyweight over 14 days, is the treatment of choice for chloroquine sensitive *P. vivax* infection. The addition of primaquine doses is for the purpose of eliminating the *P. vivax* live stage, and thus decreasing the risk of relapse. *P. vivax* is becoming resistant to chloroquine (CQ) in some countries, *e.g.*, Myanmar, Papua New Guinea, Indonesia, and Peru. Effective and safe alternative drugs for CRPV are urgently needed.

PLASMODIUM VIVAX IN SOUTHEAST ASIA

Plasmodium vivax malaria affects several tropical and subtropical areas of the world, with different incidence rates. While the number of *P. falciparum* cases has been decreasing, the number of *P. vivax* cases has been increasing [5, 6]. In the Middle East, Asia, and the Western Pacific, *P. vivax* infection accounts for more than 50% of malaria cases, whereas, in Africa, Central and South America, it accounts for less than 20% of malaria cases [7]. The lower incidence of *P. vivax* in some areas is due to genetic background of the population, e.g., the Duffy negative trait in the West African population [8]. An increase in incidence of *P. vivax* and CRPV has been reported in several countries in Southeast Asia. Effective surveillance is an important component to monitor CRPV in these areas.

Brunei

The malaria burden in Brunei is relatively low. The endemic areas are commonly located in the hilly and mountainous interior, whereas the coastal areas are free from the disease [9]. This suggests that geographical location is an important factor in determining the malaria incidence in Brunei. Difference in malaria incidence has also been reported among various ethnic groups which could be due to difference in habits and customs [9]. *Anopheles leucosphyrus balabacensis* is recognized as the malaria vector in Brunei [9].

Cambodia

Malaria incidence in Cambodia was reported to have decreased during the last decade, although the cases treated by traditional healers or private sectors remain unrecorded [10]. Recently however, Cambodia and Myanmar have been reported to be

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the countries with the highest malaria burden in the world [11]. Malaria cases have been reported in several parts of Cambodia [12-14] including areas along the Cambodian-Thai border, where immigrant workers are at high risk. *P. vivax* infection is not the predominant malaria species in Cambodia, but it is distributed all over the country [15]. Resistance to chloroquine and antifolates were first reported in Cambodia in 1957, and 1965, respectively [16, 17]. Treatment with primaquine is not recommended due to high incidence of glucose-6-phosphate dehydrogenase (G6PD) deficiency in the population. This leads to concern over treatment and control policy for *P. vivax* in Cambodia in the future.

Indonesia

Indonesia is composed of seven main islands (island groups) which includes Sumatra, Java, Kalimantan, Sulawesi, Maluku, the Lesser Sundas, and Papua. Studies on malaria epidemiology, immunology, and drug resistance have been conducted in several geographical sites in the country [18-21]. The most common infection is *P. falciparum*, followed by *P. vivax* [22]. Emerging cases of CRPV have been reported in Irian Jaya and Nias district of the North Sumatra [18, 23]. Moreover, *P. vivax* associated coma has been reported in Papua New Guinea [2, 4, 24, 25].

Lao People's Democratic Republic

Malaria is one of the most important parasitic diseases in the Lao PDR. There are several highly endemic areas especially in the rural areas of Attapeu, Khammouan, and Savannakhet provinces [26-29]. The important vector in the southern part of Lao PDR is *Anopheles dirus* [30-32], which is similar to that in eastern India, Myanmar, Thailand, China, Vietnam, and Cambodia [33]. *P. vivax* infection rate is approximately 30% [34, 35]. Chloroquine and sulfadoxine-pyrimethamine are not effective for treatment of *P. falciparum* since resistance to these drugs is widespread throughout the country [36]. This situation has an impact on *P. vivax* treatment in the country since chloroquine is the only first-line drug for *P. vivax* infection.

Malaysia

Malaria incidence in Malaysia has been declining over the past few years. The most infected population is children [37-39]. Most of the reported cases in peninsular Malaysia and Sabah are found in nature and largely concentrated among the hinterland Orang Asli population. The predominant malarial species are both *P. falciparum* and *P.vivax* [37, 38]. In 2010, *P. vivax* was the most prevalent human malaria parasite reported in Malaysia [40]. Nevertheless, *P. knowlesi* has been reported to be the most prevalent Plasmodium species in the interior division of Malaysia [41].

Myanmar

Myanmar shares borders with several neighboring countries including Thailand, Loas, China, India, and Bangladesh. Malaria in Myanmar is highly endemic in some border states, and imposes the highest malaria burden in the world [11]. In 1999, prevalence of *P. vivax* accounted for 16.8% of malaria cases [42], and increased to 58.2% with 12.7% cases of mixed infection with *P. falciparum* in 2007 [43]. Recently, *P. vivax* has been reported to be more prevalent than *P. falciparum* in Myanmar. This increase might be related to different vector species that are co-distributed in the endemic areas [43], and human migration within the country and between the neighboring countries that carry different alleles of *P. vivax*. Antimalarial drug resistance is of great concern in this country, largely due to widespread *P. vivax* resistance to chloroquine and sulfadoxine-pyrimethamine [44-46]. Moreover the geographical distribution of malaria is remarkably similar to the distribution of G6PD deficiency [47]. In Myanmar, 11% of the population who live in five states are G6PD deficient [48], of which G6PD-Mahidol is the dominant variant [48, 49]. Therefore, treatment of *P. vivax* malaria patients with the antimalarial drug primaquine or other 8-aminoquinolines may be associated with potential haemolytic anaemia.

Philippines

The Philippines is composed of 7,109 islands in the western Pacific Ocean with 79 provinces. Malaria is endemic in 65 of these provinces [50]. In 1970, malaria morbidity and mortality rates were reported to be 0.77 *per* 1,000, and 0.02 *per* 1,000, respectively [51]. Although both the morbidity and the mortality have decreased since the 1990s due to control strategies consisting of case finding and treatment and vector control, malaria remains one of the major public health problems in the Philippines [52]. Malaria in the Philippines is caused mainly by *P. falciparum* and *P. vivax*. The endemic areas are widespread across the country with varying incidences from one area to another, suggesting varying sensitivity of different parasite populations in different geographical areas. Major vectors include *An. minimus flavirostris*, *An. Mangyanus*, and *An. maculatus*.

Singapore

Malaria in Singapore has been controlled since 1982 [53], largely due to the construction of a

comprehensive drainage system [54]. During 1983-2007, the annual incidence of the imported cases ranged from 2.9 to 11.1 *per* 100,000 population, with a sharp decline since 1997 [55]. Most (90%) are imported cases originating from other Southeast Asian countries (Indonesia, Malaysia, Thailand, and Myanmar), and the Indian subcontinent (Bangladesh and India) [55]. *P. vivax* is the predominant species (66-78% of infection). There were 38 deaths, 92.1% from *P. falciparum* and 7.9% from *P. vivax* [55]. Interestingly, a total of 29 *P. vivax* malaria cases with no history of overseas travel were reported in 2009 in three different areas of Singapore [56]. Therefore, continuous surveillance of malaria transmission is required even in Singapore.

Thailand

In Thailand, a declining trend of malaria cases from 200,000 to 100,000 cases was observed from 1991 to 1996. During 1997-1999 however, the number of malaria cases increased to 128,833 due to the epidemic in the southern and eastern areas of the country. In 2011, a total of 31,991 malaria cases (Thais and foreigners) were reported; the ten provinces with highest incidence included seven provinces along the Thai-Myanmar border, two provinces along the Thai-Cambodia border, and one province along the Thai-Malaysia border [57]. Malaria transmission in Thailand is common in the forest areas especially along the bordering areas with Myanmar, Cambodia, Lao PDR, and Malaysia. Numbers of *P. vivax* and *P. falciparum* infections are similar. In recent years however, an increase in incidence of *P. vivax* infection has been observed. In 2000-2002, the proportion of *P. vivax* and *P. falciparum* cases were switched to 60:40 [58]. In Sa Kaeo province, located on the eastern border of Thailand sharing with Cambodia, *P. vivax* infection is currently the dominant malaria species, due to change in vector species [57, 59]. This raises concern over the contribution of epidemiologic change to the spread of resistance of CRPV in Thailand.

Vietnam

Malaria has been the major public health problem in Vietnam since the late 1970s [60]. In 1991, one million malaria cases and 4,646 deaths were reported. The burden of malaria however, was reduced dramatically from 37,416 cases and 50 deaths in 2003 [61] to 91,635 cases and 43 deaths in 2006 [62]. About half of all malaria cases and 80% of severe cases and malaria-related deaths occur in the central part of the country [62-64]. Concerted control measures have considerably reduced the prevalence of malaria in Vietnam and most of the reported cases are restricted to forested rural areas [61-63]. Co-infections of *P, knowlesi, P. falciparum,* and *P. vivax* have recently been reported in Khanh Phu, southern Vietnam [65]. A significant decrease in prevalence of the molecular marker of chloroquine resistance in *P. falciaprum pfcrt 76T* mutation, has been reported since the treatment policy has replaced chloroquine with artemisinin-based combination therapy (ACT). This could be associated with an increase in sensitivity of the parasite to chloroquine. The incidence of *pfdhfr* and *pfdhps* mutations associated with *P. falciparum* antifolate resistance however, remains high [66].

TIME TRENDS OF CHLOROQUINE RESISTANT *PLASMODIUM VIVAX* (Table 1)

Southeast Asia regions:

Indonesia

Case collection during 1992-2010 showed an increasing trend of CRPV in Indonesia. The incidence of CRPV in Indonesian New Guinea (Papua, formerly Irian Jaya), central, and western Indonesia is approximately 20-70% [67-71]. Sutanto et al., reported a high treatment failure rate of chloroquine of up to 78% in 2010 [71]. In contrast, a relatively low risk of CRPV of less than 20% was also reported during 1996-1999 (Java, Western Lombok and Gag Island) [72-74].

Myanmar

CRPV was first reported in Myanmar in 1993 [75]. Low efficacy of chloroquine for treatment of *P. vivax* (15% failure rate) was reported in 1995 in Mingaldon [76]. Reduction in chloroquine efficacy was later confirmed in 1996 in Dawei (34% failure rate) [77].

Malaysia

The survey of chloroquine treatment efficacy for *P. vivax* in 1976 revealed no evidence of resistance [78]. The first report of CRPV was in 1996 [79]. Subsequent study by Jamiah and colleagues in 1998 reported 10% CRPV cases [80].

Vietnam

In 2000, no CRPV case was reported in Vietnam [81], but two years later, 23% CRPV cases were reported [82].

Philippines

There has been only one observation on the efficacy of chloroquine in *P. vivax* in the Phillipines since 1996 with no evidence of CRPV [83].

Table 1 Failure rates of chloroquine (CQ) when used alone or in combination with primaquine (PQ) against *Plasmodium vivax* in Southeast Asia

Country	Region	Treatment	Follow-Up	Sample size	Failure	References
Indonesia	Indonesian	CQ	14 d	46	22%	[70]
	New Guinea	CQ	28 d	50	78%	[67]
		CQ+PQ	28 d	78	15%	[67]
	Sumatra	CQ	28 d	21	14%	[68]
		CQ	28 d	28	21%	[69]
		CQ	28 d	32	78%	[71]
	Gag Island	CQ	28 d	38	0%	[74]
	Western Lombok	CQ	28 d	20	0%	[73]
	Menoreh Hill, Java	CQ	28 d	14	0%	[72]
Myanmar	Mingaldon	CQ	28 d	50	14%	[76]
	Dawei	CQ	28 d	235	34%	[77]
Malaysia	Peninsular	CQ + PQ	28 d	222	0%	[78]
	Multiple areas	CQ	28 d	60	10%	[80]
Vietnam	Natrang	CQ	28 d	23	0%	[81]
	Multiple areas	CQ	28 d	120	23%	[82]
Philippines	Palawan	CQ	28 d	21	0%	[83]
Thailand	Trad	CQ	98 d	77	0%	[84]
	Chantaburi	CQ + PQ	> 30 d	57	0%	[85]
	Western border of Thailand	CQ + PQ	28 d	886	0.5%	[86]
	Sa Kaeo	CQ + PQ	28 d	26	0%	[58]
	Tak	CQ + PQ	42 d	130	0%	[87]
	Chanthaburi, Yala, Mae Hong Son, Kanchanaburi	CQ + PQ	28 d	201	2.5%	[88]

Thailand

Interestingly, no CRPV case has been reported in Thailand, a country with highly multidrug resistant *P. falciparum* [58, 84-86]. Two recent clinical studies with supported data on plasma concentrations of chloroquine and desethylchloroquine (an active metabolite of chloroquine) confirmed high efficacy of chloroquine for the treatment of *P. vivax* in Thailand. The first study conducted in western Thai-Myanmar border in a total of 130 patients showed a 42-day cure rate of 100% [87]. In another study conducted in a total of 201 cases, a 28-day cure rate of 97.5% was observed [88]. Confirmation of genuine CRPV by discriminating from treatment failure from other factors particularly host genetic factors should be performed. In addition, containment and surveillance program for CRPV should be systematically implemented.

Locations outside Southeast Asia:

The information on CRPV in Oceania is limited. The risk of therapeutic failure following chloroquine treatment is of great concern based on the supporting data from Indonesian New Guinea [70]. Surveys of therapeutic responses to chloroquine throughout Oceania are needed.

In South Asia, seven cases of CRPV were reported from India during 1995-2003 [73, 89 - 92].

In the Middle East, the cure rate of 100% was reported in 39 patients [93].

In South America, there was no evidence of CRPV in Guyana [94, 95], Colombia [96, 97], Brazil [98-100], and Peru [101] during the year 1985-2003. In 2001 however, Soto et al. reported 11% of CRPV in Colombia [97], and Ruebush et al. reported 2% of CRPV in Peru [101]. These indicate that CRPV apparently occurs in the New World but still at a low frequency.

In Africa, the CRPV was reported in two studies from Ethiopia, with a 28-day failure rates of 4.8% [102] and 3.6% [103].

ASSOCIATION BETWEEN *PLASMODIUM VIVAX* SEVERITY AND CRPV

Although there is no clear criteria for classification of severity of *P. vivax* malaria, a number of severe cases and even deaths attributable to *P. vivax* infection have been reported [104, 105]. The association between CRPV and severity of *P. vivax* also remains unclear. The clinical complications associated with severity of *P. vivax* malaria needs to be identified to achieve clearer understanding of the link between of CRPV and disease severity in

various *P. vivax* endemic areas.

IN VITRO SENSITIVITY OF *PLASMODIUM VIVAX*

Current antimalarial drug sensitivity assays are focused on *P. falciparum*, the most lethal malaria species. These assays were developed based on the successfully established *P. falciparum* continuous culture system [106], and since then, became an important tool for the WHO standard for assessing the antimalarial drug susceptibility. Nevertheless, the development of such systems for susceptibility testing and monitoring of other malarial species including *P. vivax* is limited. Accumulating evidence on treatment failure after administration of the standard chloroquine regimens of *P. vivax* since the first report in 1989 [107], and subsequently increasing reports from differently geographical areas [18, 20, 76, 82, 108, 109], stimulating the interest in the development of *in vitro* drug sensitivity assay for *P. vivax*. Although *in vivo* monitoring is a direct measure of the clinical efficacy of antimalarials, it is often difficult to carry out and subject to individual variation according to patient's immune status. Thus, *in vitro* assays, which provide drug susceptibility data excluding confounding factors like host immunity, are still essential and provide a valuable adjunct to *in vivo* assay.

The lack of a stable and continuous *in vitro* culture of *P. vivax* makes it difficult to determine the parasite's susceptibility to antimalarials and to develop effective drugs for this malarial species. An *in vitro* maturation of the parasite to schizonts in a single asexual life cycle is the minimal requirement for drug susceptibility assay. Unlike *P. falciparum*, *P. vivax* prefers to invade reticulocytes (immature erythrocytes), which represent only approximately 1% of erythrocytes in the blood circulating system [110], and all blood stage forms of *P. vivax* are found only in the peripheral circulation. The requirement for reticulocytes for the invasion of *P. vivax* blood stage parasite development is thus the major obstacle for continuous *in vitro* cultivation of *P. vivax* [111, 112]. The short-term culture system that enables the maturation of *P. vivax* from blood obtained from infected patients was developed in 1989 based on the microscopic count of mature schizonts [113]. Attempts to establish a long-term continuous *in vitro* culture system for *P. vivax* have thus far been unsuccessful [114]. Supplementation of the culture medium with reticulocyte-enriched blood either from monkeys treated with a hemolytic drug [115], or from hemochromatosis patients [112], was reported to support the long-term continuous culture of *P. vivax*. However, the procedure was so far not well developed due to its labor-intensiveness and the unavailability of reticulocyte-enriched human blood from hemochromatosis patients. Contamination with leukocytes as well as yet undefined nutritional needs are also additional limiting factors [111, 116, 117]. The improvement of this method using reticulocytes from either cord blood [118], or reticulocytes derived from haematopoietic stem cells isolated from cord blood [119], modified medium and culture conditions combining static and shaking periods are challenging. The development of a convenient short-term culture method based on schizont maturation inhibition was developed by Russel et al. [120], which is considered useful for *P. vivax* drug resistance monitoring [121]

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