DIRECTLY OBSERVED THERAPY WITH PRIMAQUINE TO REDUCE THE RECURRENCE RATE OF *PLASMODIUM VIVAX* INFECTION ALONG THE THAI-MYANMAR BORDER

Wanchai Maneeboonyang, Saranath Lawpoolsri, Supalarp Puangsa-art, Surapon Yimsamran, Nipon Thanyavanich, Pitak Wuthisen, Sutthiporn Prommongkol, Wuthichai Chaimongkul, Prasert Rukmanee, Natefa Rukmanee, Irwin F Chavez, Kasinee Buchachart, Srivicha Krudsood and Pratap Singhasivanon

Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

Abstract. This study was carried out from April 2005 to June 2006 to evaluate the recurrence of *P. vivax* malaria infection in relation to drug compliance along the Thai-Myanmar border in Ratchaburi, Thailand. Ninety-two patients with vivax malaria were sequentially assigned to 2 groups. Both groups received a standard dose of chloroquine (total dose = 2.5 g) for 3 days and primaquine (total dose = 210 mg) for 14 days. The experimental group received a full course of treatment using daily directly observed therapy (DOT) while subjects in the control group were given the medication with necessary instructions to take as self-administered therapy (SAT). Patients were followed up for 3 months on Days 14, 21, 28, 60 and 90. Five of 46 patients from the SAT group had recurrence of malaria on Days 21, 44, 60, 72 and 87. Recurrence was not observed among patients in the DOT group. Survival analysis also showed significant differences between the SAT and DOT groups ($p < 0.05$). The study suggests patient compliance with the 14-day primaquine treatment with DOT improve the outcome of *P.vivax* malaria treatment.

Keyword: *Plasmodium vivax*, recurrence rate, primaquine, drug compliance

INTRODUCTION

Plasmodium falciparum and *Plasmodium vivax* infections remain a public health problem in Thailand. Three decades ago, *P. falciparum* caused approximately 80% of all malaria infections. Effective anti-malarial drug treatment along with vector control has resulted in a dramatic decline in the incidence of *P. falciparum* during these decades. However, the reduction in the incidence of *P. vivax* was relatively small compared to *P. falciparum*, which led to an increase in *P. falciparum* to *P. vivax* ratio up to 1:1 in 1998 (Chareonviriyaphap *et al,* 2000; Silachamroon *et al*, 2003). In 2003, a total of 37,355 malaria cases were reported in Thailand, of which 18,295 were *P. vivax* infections. Mixed infections with *P. falciparum* and *P. vivax* are also common. About 30%

Correspondence: Wanchai Maneeboonyang, Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Bangkok 10400, Thailand. E-mail: tmwmn@mahidol.ac.th

of patients treated for *P. falciparum* infection developed vivax malaria after treatment (Looareesuwan *et al*, 1987).

Unlike *P. falciparum* malaria, *P. vivax* malaria rarely develops a complicated infection. However, *P. vivax* has a dormant stage (hypnozoite) that can persist in the human liver and cause reappearance of the parasite by invading the bloodstream weeks to years later. In about 60% of untreated or inadequately treated cases, clinical symptoms recur after a period of quiescence, which depends on the strain of the parasite. This phenomenon is called relapse.

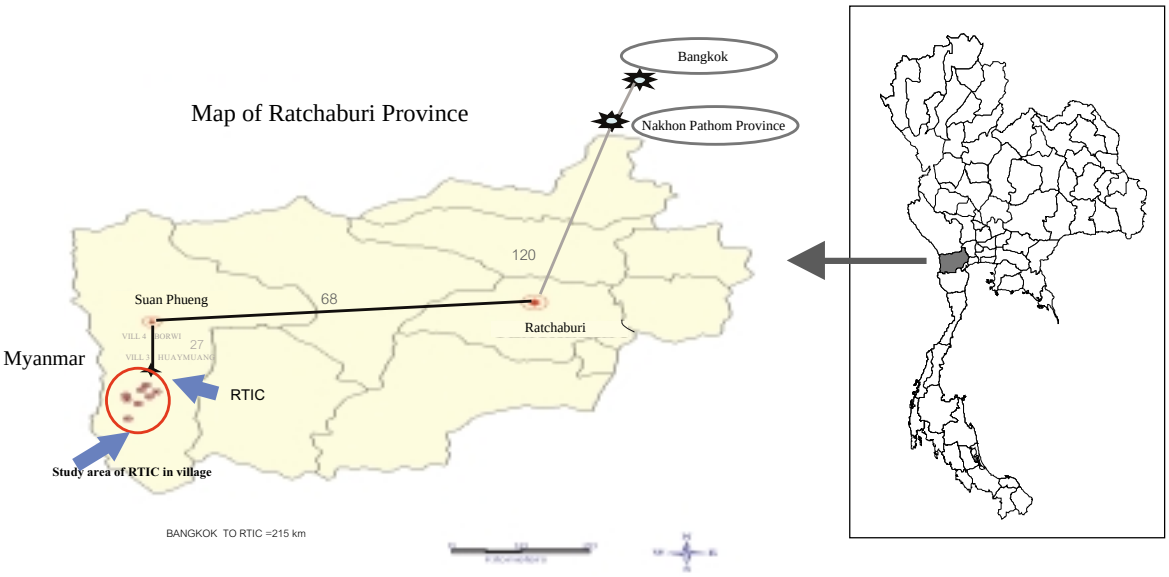
P. vivax infections has a great impact on society and the economy (Mendis *et al*, 2001), particularly, in cases of relapse. In a recent study among a population residing along the western border of Thailand, *P. vivax* was found in 54% (n=2,573) over a period of 2 years (Luxemburger *et al*, 1997). Reports from Thailand and India indicate *P. vivax* infection is associated with a lower hemoglobin level in pregnant patients and reduced birth weight (Nosten *et al*, 1999; Singh *et al*, 1999). An association between *P. vivax* infection and malnutrition in children has also been reported (Williams *et al*, 1997).

Currently, the standard treatment for *P. vivax* infection is a 3 day course of chloroquine with a total dose of 1,500 mg followed by primaquine 15 mg a day for 14 days. This regimen often causes resolution of acute symptoms and clearing of the parasitemia, although a significant number of relapses have been observed (Tan-Ariya *et al*, 1995; Looareesuwan *et al*, 1997). Primaquine is the only drug available to prevent relapses of *P. vivax* from hypnozoites in the liver. The efficacy of conventional doses is approximately 80% (Burnag *et al*, 1994; Doherty *et al*, 1997). Unlike *P. falciparum*, which began to show resistance to chloroquine in the 1950s, *P. vivax* remains sensitive to chloroquine throughout most of the world (Fryauff *et al*, 1997; Pukrittayakamee *et al*, 2000; Baird *et al*, 2002; Nandy *et al*, 2003). Although a few patients have shown a poor response to chloroquine, vivax malaria acquired in Thailand can still be successfully treated with chloroquine (Looareesuwan *et al*, 1999). There are a number of other factors that affect the vivax cure rate in a given area, such as the patient's level of immunity, the parasite strain, and the number of parasites present (Collins and Jeffery *et al*, 1996). Although several recent studies have shown failure of the standard vivax treatment, a few studies have assessed associations between the likelihood of treatment failure and behavioral factors of patient drug compliance. The objective of this study was to evaluate the reappearance of *P. vivax* malaria in relation to drug compliance in an area along the Thai-Myanmar border in Ratchaburi, Thailand.

MATERIALS AND METHODS

Study population and site

The study was conducted at Rajanagarindra Tropical Disease International Center (RTIC), Tanao Si Subdistrict, Suan Phueng District, Ratchaburi Province, Thailand (Fig 1). It is located 215 km to the west of Bangkok (Fig 1). Suan Phueng is a small district situated near the Thai-Myanmar border surrounded by the Tanao Si mountain ranges on the west. Tanao Si Subdistrict is located at the southern part of Suan Phueng and is composed of 7 villages. In 2004, it consisted of 523 households and had a population of 3,364. The population of Suan Phueng has grown over the past decade; the current



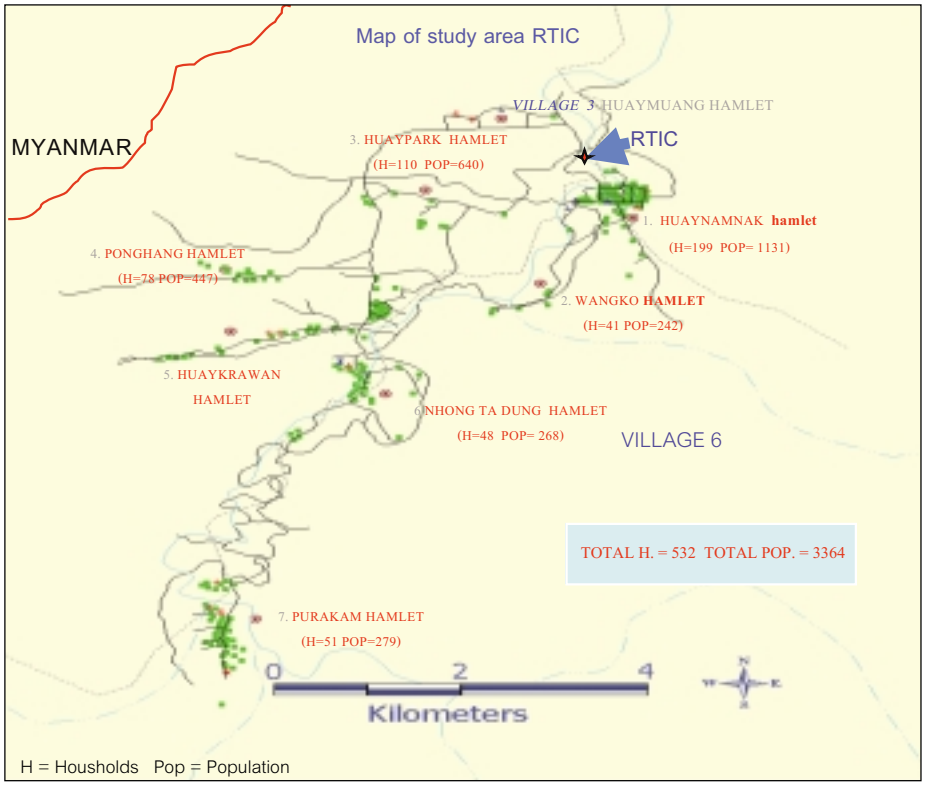


Fig 1–Study area, Tanao Si Subdistrict, Suan Phueng District, Ratchaburi, Thailand.

population is about 5,400 people and is made up of 4 ethnic groups: Karen (85%), Thai (14%), Mon and Burmese (1%). The majority of residents are hunters-gatherers with limited agricultural practice. The project protocol and objectives were explained to the population and informed consent was individually obtained from all study participants or their parents. Ethical permission for the study was granted by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Thailand.

Inclusion and exclusion criteria

The study recruited all patients living in villages 3, 4 and 6 of Tanao Si Subdistrict for at least one year who were diagnosed with vivax malaria confirmed by microscopy at the RTIC between April 2005 and June 2006. Only those willing to participate in the 3-month follow-up study and provide informed consent were included in the study. Exclusion criteria were pregnancy, mixed infection, history of G6PD deficiency, history of dark urine or significant hemoglobinuria and history of allergy or side-effects to anti malarial drugs.

Supervised and un-supervised primaquine treatment

All *P. vivax* cases in the study were treated with a standard drug regimen for *P. vivax* infection: a 3-day course of chloroquine at a total dose of 1,500 mg followed by primaquine 15 mg daily for 14 days. The dosage for children was adjusted according to age as recommended by the Malaria Control Program Thailand. If vomiting occurred within 1 hour of taking the drug, the patient was given the drug again. Patients were sequentially allocated into either the directly observed therapy (DOT) group, which received a full-course of the treatment with daily supervision by RTIC staff, or the self-administered therapy (SAT) group, which received a full-course of the treatment along with necessary instructions for self-administration. All patients were followed up for three months (Days 14, 21, 28, 60 and 90).

Data collection

Demographic and clinical data, including age, gender, occupation, weight, clinical symptoms, duration of fever prior to treatment, history of previous anti-malarial drug use, and body temperature, were recorded. Thick and thin blood smears were obtained from finger pricks and stained with Giemsa to determine malaria results. Blood films were negative if no parasites were seen in 200 oil-immersion fields on a thick blood film. Parasite density (asexual parasite/µl of blood) was determined by counting the number per 200 white blood cells (thick film). Patients in the DOT group were visited by RTIC staff daily to supervise primaquine administration. All subjects in both DOT and SAT groups were visited by RTIC staff on Days 14, 21, 28, 60 and 90. All subjects were instructed to visit RTIC if they had any symptoms during the follow-up period. At each visit, a blood smear was taken to examine for vivax and falciparum parasitemia. On Day 14, patients in the SAT group were asked whether they took their drug properly or not. During the follow-up period, if there was a reappearance of malaria parasite, either *P. vivax* or *P. falciparum*, it was confirmed by microscopy, the day of parasite reappearance was also recorded. Subjects who had a reappearance of *P. vivax* parasitemia were treated with the same regimen as the first attack. In cases where falciparum malaria was diagnosed during the follow-up period, patients were treated with a standard regimen for falciparum malaria (for adults:

Table 1
Number and percentage of malaria infections by gender during April 2005 to June 2006.

Microscopic results	Male		Female		Total	
	n	%	*n*	%	*n*	%
Negative	2,316	89.4	1,509	92.8	3,825	90.7
Positive	274	10.6	117	7.2	391	9.3
P. falciparum	182	7.0	75	4.6	257	6.1
P. vivax	87	3.4	40	2.5	127	3.0
P. malariae	4	0.2	2	0.1	6	0.1
Mix (*P. falciparum* and *P. vivax*)	1	0.04	0	0	1	0.02
Total	2,590	100	1,626	100	4,216	100

artesunate 600 mg over 2 days and mefloquine 25 mg/kg as a single dose on the first day; for children: artesunate 12 mg base/kg/total dose over 3 days and mefloquine 25 mg base/kg as a single dose on the last day).

Data analysis

Baseline characteristics of patients in the SAT and DOT groups were compared. Statistical tests were chosen regarding the distribution and type of the data. Student's *t*-test and chi-square tests were selected for normally distributed continuous and categorical data, respectively. Non-parametric tests, such as the Mann-Whitney *U* test and Fisher's exact test, were performed for non-normally distributed data. Survival analysis was performed to compare time to *P. vivax* reappearance after treatment between the SAT and DOT groups.

RESULTS

During April 2005 to June 2006, a total of 4,216 blood samples were examined at the RTIC. Blood samples were taken from 2,590 males and 1,626 females. The blood examination results showed that 391 persons (9.3%) were infected with at least one of three human malaria species, (*P. falciparum*, *P. vivax*, *P. malariae*). The slide positive rate of these three malaria species were as follows: 257 cases (6.1%) for *P. falciparum*; 127 cases (3.0%) for *P. vivax*; 6 cases (0.1%) for *P. malariae* and 1 case (0.02%) of mixed infection with *P. falciparum* and *P. vivax* (Table 1). Of the cases with *P. vivax* infection, only 94 patients met inclusion criteria and were selected for this study. Two patients were excluded from analysis due to an initial misdiagnosis and lost to follow-up before treatment completion. Therefore, 92 *P. vivax* cases remained in the study, of which 46 cases were assigned to the DOT group and the other 46 cases were assigned to the SAT group (Table 2).

Demographic, clinical, and laboratory characteristics at baseline are shown in Table 2. Patients in both groups were comparable at baseline with respect to clinical and laboratory characteristics. The majority of the subjects in both the DOT and SAT groups were male. The mean ages of the patients were 21.7 and 17.6 years in the DOT and SAT group, respectively. The mean weight was 34.9 kg (± 18.7) in the

Table 2
Baseline demographic, clinical, and laboratory characteristics of patients by treatment group.

Variable	DOT	SAT	*p*-value
Male/female	28/18	30/16	0.67[a]
Age (years), Median (min-max)	12 (2-70)	10 (1-80)	0.35[b]
Weight (kg), Median (min-max)	36.6 (10-68.6)	25.5 (6-83.4)	0.66[b]
Past history of malaria infection (times), Median (min-max)	1 (0-7)	1 (0-30)	0.57[b]
Duration of symptoms prior to treatment (days), Median (min-max)	2 (0-7)	2 (0-8)	0.82[b]
Mean baseline temperature in ºC (SD)	37.9 (1.1)	37.7 (1.1)	0.32[c]
Hematocrit (%), Median (min-max)	38 (26-49)	38 (23-53)	0.72[b]
Geometric mean parasite count per 200 WBC	211.8	120.6	0.01[b]

[a] Chi-square test; [b] Man-Whitney *U* test ; [c] *t*-test

DOT group and 32.7 kg (± 19.5) in the SAT group. Patients in the SAT group were more likely to have more previous malaria infections than those in the DOT group (3.4 times in the SAT group *vs* 1.2 times in the DOT group). There were no differences in the duration of symptoms prior to treatment and the mean highest temperature in both groups. The mean hematocrit level was 37.6 % in the DOT group, and 38.6% in the SAT group. The geometric mean parasite count per 200 WBC at baseline among patients in the DOT group was significantly higher than the in the SAT group 8,470.5/200 WBC in the DOT group *vs* 4,825.7/200 WBC in the SAT group.

Patients in the DOT group were more likely to complete follow-up on Days 28, 60, and 90 (Table 3). During the 90-day follow-up, reappearance of *P. vivax* was observed in 5 patients in the SAT group on Days 21, 44, 60, 72, and 87. None of the patients in the DOT group had *P.vivax* reappearance during the follow-up. The majority of the reappearance occurred later than one month after initial treatment (Table 3). *P. falciparum* was detected in 3 and 5 patients in the DOT and SAT groups, respectively, during the follow-up period. The days of *P. falciparum* diagnosis in each group are shown in Table 3.

A Kaplan-Meier curve comparing time to reappearance among patients in the DOT group and in the SAT group is shown in Fig 2. The time until reappearance was significantly longer among patients in the DOT group, than among those in the SAT group ($p < 0.05$).

Characteristics of the five patients with reappearance compared with those of 41 patients without reappearance in the SAT group are shown in Table 4. Compared to patients without reappearance, those who had *P. vivax* reappearance were more likely to be of younger age, have a lower body weight, higher number of previous malaria infections and higher parasite count at baseline. However, these differences were not statistically significant, except the weight difference.

Table 3
Number of patients at different follow-up days, and malaria results during the 90-day follow-up by treatment group.

	DOT (*N*=46)	SAT (*N*=46)
No. of patients with 28-day follow-up	44	38
No. of patients with 60-day follow-up	38	30
No. of patients with 90-day follow-up	43	33
Reappearance of *P. vivax* parasitemia during		
Days 0-16	0	0
Days 17-28	0	1
Days 28-90	0	4
No. of patients positive for *P. falciparum* (days to *P. falciparum* diagnosis)	3 (Days 14,28,28)	5 (Days 9,14,14,28,28)

DISCUSSION

This study demonstrates the effect of treatment compliance with the 14 days of primaquine on the reappearance of *P. vivax* in a malaria endemic area along the Thai-Myanmar border. In Thailand, the standard regimen for treatment of *P. vivax* recommended by the Thai government is 3 days of chloroquine with total dose of 1,500 mg and primaquine 15 mg daily for 14 days, dosage adjusted for age. Treatment of *P. vivax* aims to not only cure the disease and symptoms caused by asexual blood stage parasites, but also to eliminate hypnozoites in the liver that cause relapse weeks or months after the first attack (Marlar-than *et al*, 1995; Dau *et al*, 1996; Phillips *et al*, 1996; Baird *et al*, 1997).

In Thailand, the standard regimen for *P. vivax* treatment remains effective (Burnag *et al*,1994; Pukrittayakamee *et al*, 2004). However, the reappearance of *P. vivax* after treatment is occasionally observed in the area. Although the re-infection cannot be excluded, a number of patients with reappearance may be cases of relapse. An important factor influencing the effectiveness of primaquine is patient compliance, because of the long duration of primaquine treatment needed to sufficiently eliminate the hypnozoites. Our findings show none of 46 patients who received primaquine treatment by DOT developed reappearance within 90 days, while 5 out of 46 patients who received SAT with primaquine developed a reappearance. This suggests patient compliance is critical for successful primaquine treatment in preventing relapse.

Patients in the SAT group who developed reappearance were children aged 2-15 years, which is consistent with other studies conducted in other areas (Prasad *et al*, 1991; Srivastava *et al*, 1996). The high failure rate among children is probably associated with vomiting after drug administration, inadequate dose of the drug after adjustment, or the concerns of their parents about taking the drug. The duration of symptoms before treatment, past history of malaria infection and parasite density at diagnosis are also potential factors for *P. vivax* reappearance after primaquine treatment (Srivastava *et al*, 1996; Duarate *et al*, 2001). Our findings suggest

Table 4
Characteristics of patients in the SAT group by *P. vivax* reappearance after receiving standard treatment (3 days chloroquine and 14 days primaquine).

Variable	SAT (*N*=41)	Patient reapp (*N*=5)	*p*-value
Male/female	27/14	3/2	0.576[a]
Age (years), Median (min-max)	12 (1-80)	5 (2-15)	0.10[b]
Weight (kg), Median (min-max)	29.3 (6-83.4)	17.6 (9.3-25.1)	0.046[b]
Past history of malaria infection (times), Median (SD)	1 (0-30)	3 (3-24)	0.351[b]
Duration of symptoms prior to treatment (days), Median (min-max)	2 (0-8)	1 (1-2)	0.074[a]
Temperature in ºC, Median (min-max)	37.5 (35.5-40.0)	38.0 (37.0-38.7)	0.571[b]
Hematocrit (%), Median (min-max)	38 (23-53)	38 (35-42)	0.710[b]
Geometric mean parasite count per 200 WBC	111.1	238.0	0.125[b]

[a] Fisher's exact test, [b] Man-Whitney *U* test

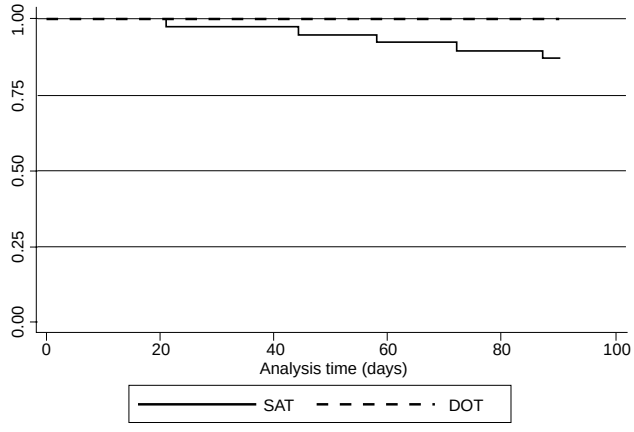


Fig 2–Kaplan-Meier survival curves comparing the DOT and SAT groups.

these factors may not be as important as patient compliance to drugs, because patients in the DOT group also had the same characteristics as those with reappearance, but none had reappearance.

An important limitation of the study was that none of the *P. vivax* reappearance cases observed in our study were further examined using molecular techniques to confirm whether the reappearance was due to re-infection or relapse. Therefore, we cannot distinguish re-infection from relapse. However, this study was conducted in an area of low malaria transmission; reinfection within 90 days is less likely. The parasite reappearance is also a result of parasite recrudescence due to chloroquine resistance; the recrudescence usually occurs within 16-28 days after treatment (Baird *et al*, 1997; Wilairatana *et al*, 1999; WHO, 2010). In our study, none of the reappearance episodes were observed within 16 days. In addition, the reappearance of *P. vivax* in 4 out of 5 patients occurred later than 28 days after treatment. This suggests the reappearance cases were less likely due to recrudescence or chloroquine resistance. However, the duration of relapse may be longer, depending on the strain of parasite (Doherty *et al*, 1997). A study in Thailand showed relapse occurs

mostly within 90 days after treatment (Looareesuwan *et al*, 1999). The duration of follow-up in our study should cover most relapse cases, although a small number of relapse cases might be missed.

Our findings suggest the effectiveness of 14 days of primaquine treatment can be influenced by patient compliance with the drug. Most malaria endemic areas are where people are poor and have low education (Thang *et al*, 2008; Vitor-silva *et al*, 2009). Language barriers and poor literacy are significant factors affecting patient compliance. In these areas, patients may not correctly understand the drug treatment instructions. Therefore, DOT with 14 days of primaquine may increase the effectiveness of primaquine treatment, especially in remote malarious areas.

ACKNOWLEDGEMENTS

The authors wish to thank the people from villages 3, 4 and 6 of Tanao Si Subdistrict, Suan Phueng District, Ratchaburi Province and the staff of the Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University for their cooperation in this research. This study was supported by funds from the Faculty of Tropical Medicine, Mahidol University in 2005.

REFERENCES

Baird JK, Leksana B, Masbar S, *et al*. Diagnosis of resistance to chloroquine by *Plasmodium vivax*: timing of recurrence and whole blood chloroquine levels. *Am J Trop Med Hyg* 1997; 56: 621- 6.

Baird JK, Tiwari T, Martin GJ, *et al*. Chloroquine for the treatment of uncomplicated malaria in Guyana. *Ann Trop Med Parasitol* 2002; 96: 339-48.

Baird JK, Waidy I, Frayauff DJ, *et al*. *In vivo* resistance to chloroquine by *Plasmodium vivax* and *Plasmodium falciparum* at Nibire, Irian Jaya, Indonesia. *Am J Trop Med Hyg* 1997; 56: 627-31.

Burnag D, Karbwang J, Thanavibul A, *et al*. High dose of primaquine in primaquine resistant vivax malaria. *Trans R Soc Trop Med Hyg* 1994; 88: 218-9.

Chareonviriyaphap T, Bangs MJ, Ratanatham S. Status of malaria in Thailand. *Southeast Asian J Trop Med Public Health* 2000; 31: 225-37.

Colins WE, Jeffery GM. Primaquine resistance in *Plasmodium vivax*. *Am J Trop Med Hyg* 1996; 55: 243-9.

Dau VK, Kar PK, Sharmar VP. Chloroquine resistant *Plasmodium vivax* malaria in India. *Trop Med Int Health* 1996; 1: 816-9.

Doherty JF, Day JH, Warhurst DC, Chiodini PL. Treatment of *Plasmodium vivax* malaria-time for a change? *Trans R Soc Trop Med Hyg* 1997; 91: 76.

Duarte EC, Pang LW, Ribeiro LC, Fontes CJF. Association of subtherapeutic dosages of a standard drug regimen with failures in preventing relapses of vivax malaria. *Am J Trop Med Hyg* 2001; 65: 471-6.

Fryauff DJ, Baird JK, Candradikusuma D, *et al*. Survey of in vivo sensitivity to chloroquine by *Plasmodium falciparum* and *P. vivax* in Lombok, Indonesia. *Am J Trop Med Hyg* 1997; 56: 241-4.

Looareesuwan S, Buchachart K, Wilairatana P, *et al*. Primaquine-tolerant vivax malaria in Thailand. *Ann Trop Med Parasitol* 1997; 91: 939-43.

Looareesuwan S, White NJ, Chittamas S, Bunnag D, Harinasuta T. High rate of *Plasmodium vivax* relapse following treatment of *falciparum* malaria Thailand. *Lancet* 1987; ii: 1052-4.

Looareesuwan S, Wilairatana P, Glanarongran R, *et al*. Atovaquone and proguanil hydrochloride followed by primaquine for treatment of *Plasmodium vivax* malaria in Thailand. *Trans R Soc Trop Med Hyg* 1999; 93: 637-40.

Luxemburger C, Ricci F, Nosten F, Raimond D, Bathet S, White NJ. The epidemiology of severe malaria in an area of low transmission in Thailand. *Trans R Soc Trop Med Hyg* 1997; 91: 256-62.

Marlar-Than, Myat-Phone-kyaw, Aye-Yu-Soe, Khaing-Khaing-Gyi, Ma-Sabai, Myint-Oo. Development of resistance to chloroquine by *Plasmodium vivax* in Myanmar. *Trans R Soc Trop Med Hyg* 1995; 89: 307-8.

Mendis K, Sina BJ, Marchesini P, Carter R. The neglected burden of *Plasmodium vivax* malaria. *Am J Trop Med Hyg* 2001; 64: 97-106.

Nandy A, Addy M, Maji AK, Bandyopadhyay AK. Monitoring the chloroquine sensitivity of *Plasmodium vivax* from Calcutta and Orissa, India. *Ann Trop Med Parasitol* 2003; 97: 215-20.

Nosten F, McGready R, Simpson JA, *et al*. Effects of *Plasmodium vivax* malaria in pregnancy. *Lancet* 1999; 354: 546-9.

Phillips EJ, Keystone JS, Kain KC. Failure of combined chloroquine and high-dose primaquine therapy for *Plasmodium vivax* malaria acquired in Guyana, South America. *Clin Infect Dis* 1996; 23:1171-3.

Prasad RN, Virk KJ, Sharma VP. Relapse/reinfection patterns of *Plasmodium vivax* infection: A four year study. *Southeast Asian J Trop Med Public Health* 1991; 22: 499-503.

Pukrittayakamee S, Chantra A, Simpson JA, *et al*. Therapeutic responses to different antimalarial drugs in vivax malaria. *Antimicrob Agents Chemother* 2000; 44: 1680-5.

Pukrittayakamee S, Imwong M, Looareesuwan S, White NJ. Therapeutic responses to antimalarial and antibacterial drugs in vivax malaria. *Acta Trop* 2004; 89: 351-6.

Silachamroon U, Krudsood S, Treepratsuk S, Wilairatana P, Chalermrult K, Mint HY.Clinical trial of oral Artesunate with or without high-dose Primaquine for the treatment of vivax malaria in Thailand. *Am J Trop Med Hyg* 2003; 69: 14-8.

Singh N, Shukla MM, Sharma VP. Epidemiology of malaria in pregnancy in central India. *Bull World Health Organ* 1999; 77: 567-72.

Srivastava HC, Sharma SK, Bhatt RM, Sharma VP. Studies on *Plasmodium vivax* relapse pattern in Kheda district, Gujarat. *Indian J Malariol* 1996; 33: 173-9.

Tan-Ariya P, Na-Bangchang K, Tin T, Limpaibul L, Brockelman CR, Karbwang J. Clinical response and susceptibility in vitro of *Plasmodium vivax* to the standard regimen of chloroquine in Thailand. *Trans R Soc Trop Med Hyg* 1995; 89: 426-9.

Thang ND, Erhart A, Speybroeck N, *et al*. Malaria in Central Vietnam ; analysis of risk factors by multivariate analysis and classification tree models. *Malar J* 2008; 7: 28.

Vitor-silva S, Reyes-Lecca RC, Pinheiro TRA, *et al*. Malaria is associated with poor school performance in an endemic area of the Brazillan. *Malar J* 2009; 8: 230.

World Health Organization (WHO). Guidelines for the treatment of malaria. 2nd ed. Geneva: WHO, 2010. [Cited 2010 Apr 8]. Available from: URL: http://whqlibdoc.who.int/publications/2010/9789241547925_eng.pdf

Wilairatana P, Silachamroon U, Krudsood S, *et al*. Efficacy of primaquine regimens for primaquine-resistant *Plasmodium vivax* malaria in Thailand. *Am J Trop Med Hyg* 1999; 61: 973-7.

Williams TM, Maitland K, Phelps L, *et al*. *Plasmodium vivax:*a cause of malnutrition in young children. *Oxford J* 1997; 90: 751-7.