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No Evidence for Spread of *Plasmodium falciparum* Artemisinin Resistance to Savannakhet Province, Southern Laos

Mayfong Mayxay,* Maniphone Khanthavong, Odai Chanthongthip, Mallika Imwong, Sue J. Lee, Kasia Stepniewska, Bongkot Soonthornsata, Tiengkham Pongvongsa, Samlane Phompida, Bouasy Hongvanthong, Pascal Ringwald, Nicholas J. White, and Paul N. Newton

Wellcome Trust–Mahosot Hospital–Oxford University Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Laos; Faculty of Postgraduate Studies, University of Health Sciences, Vientiane, Laos; Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Nuffield Department of Medicine, University of Oxford, Oxford, United Kingdom; Centre of Malariology, Parasitology and Entomology, Ministry of Health, Vientiane, Laos; Savannakhet Provincial Malaria Station, Savannakhet Province, Laos; Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; Global Malaria Programme, World Health Organization, Geneva, Switzerland

Abstract. We conducted an open-label, randomized clinical trial to assess parasite clearance times (PCT) and the efficacy of 4 mg/kg (group 1, n = 22) and 2 mg/kg (group 2, n = 22) of oral artesunate for three days followed by artemether-lumefantrine in patients with uncomplicated *Plasmodium falciparum* malaria at Xepon Interdistrict Hospital, Savannakhet Province in southern Laos. Slides were read in duplicate. The overall mean (95% confidence interval; range) PCT in hours was 23.2 (21.2–25.3; 12–46) and 22.4 (20.3–24.5; 12–46) for the first and second microscopists, respectively ($P = 0.57$). Ten (23%) patients remained parasitemic on day 1 after treatment (4 [18%] in group 1 and 6 [27%] in group 2; $P = 0.47$). No patient had patent asexual parasitemia on the second and third days of treatment. The 42-day polymerase chain reaction–corrected cure rates were 100% in both treatment groups. Serious adverse events did not develop during or after treatment in any patients. In conclusion, no evidence of *P. falciparum in vivo* resistance to artesunate was found in southern Laos.

INTRODUCTION

Parasitologic responses to artesunate-containing treatment regimens for uncomplicated *Plasmodium falciparum* malaria have recently been shown to be slower in western Cambodia than elsewhere in the world. Both prolonged parasite clearance times (PCTs) and unusually high treatment failure rates after artesunate-mefloquine have been reported despite adequate antimalarial blood concentrations.[1–3] Although poor responses to artesunate have been described,[4] the recent reports suggest a consistent focal problem. There is also evidence for slightly prolonged PCTs and higher treatment failure rates after artesunate-mefloquine on the Thailand/Myanmar (Burma) border.[5,6] In pooled data from 18,699 patients who received artemisinin derivatives, of those who were non-hyperparasitemic, only 1.8–5% had PCTs > 72 hours.[7] Because the rate of parasite clearance is a good pharmacodynamic measure of efficacy of artemisinin-related compounds,[8,9] slow parasite clearance could indicate the emergence of significant resistance, although host immunity and antimalarial pharmacokinetic differences could confound the relationship.

Artemisinin-based combination therapies (ACTs) are central to current malaria treatment strategies,[10] and the spread of malaria parasites with significant resistance to the artemisinin derivatives would be a public health disaster, especially if they contaminated Africa. There is therefore an urgent need to investigate rapidly whether there is any evidence of resistance to artemisinin derivatives in southern Laos, which is only approximately 400 km from the Thailand/Cambodia border area of artemisinin resistance/tolerance. Although ACTs were introduced as national policy by the Government of Laos in 2005, artesunate monotherapy with variable patient adherence has been used since 1999. In addition, counterfeit artesunate containing subtherapeutic quantities of artemisinin has been reported in the area.[11]

The mean (95% confidence interval [CI]) PCT in a series of 861 patients with *P. falciparum* malaria recruited in three trials in southern Laos treated with artemisinin derivatives (artesunate plus mefloquine, n = 316), artemether-lumefantrine (n = 238), and dihydroartemisinin-piperaquine (n = 307) during 2002–2008 was 1.89 (1.86–1.92) days (range = 1–4 days). Only 2 (0.23%) patients had PCTs > 3 days (4 days) and 30 (3.48%) had PCTs > 2 days.[12–14] These results are reassuring. However, these studies involved daily blood sampling for parasite counts, rather than six hourly counts, which would be required to detect anything other than gross changes in PCT. Furthermore, the last available data were collected in 2008. We therefore conducted a randomized clinical trial comparing oral artesunate (2 mg/kg/day versus 4 mg/kg/day for 3 days) followed by a 3-day treatment course of artemether-lumefantrine to estimate the current efficacy of the drug for uncomplicated *P. falciparum* malaria in southern Laos.

MATERIALS AND METHODS

Study site, patients, and clinical procedures. The study was conducted during June–September 2010 at Xepon (30 beds) Inter-District Hospital (16.69° N, 106.20° E, 208 meters above sea level) in southern Savannakhet Province, Laos, near the Laos/Vietnam border, approximately 665 km southeast of Vientiane, the capital of Laos. Xepon (88 villages, population = 48,000) is inhabited predominantly by rice farmers of the Lao Theung ethnic groups. Malaria transmission is seasonal with a peak during the rainy months of July and August.

Under the null hypothesis that the proportion of patients parasitemic on day 3 after ACT treatment is < 3%, the probability of finding more than six patients with a positive parasite

*Address correspondence to Mayfong Mayxay, Microbiology Laboratory Mahosot Hospital, Vientiane, Laos. E-mail: mayfong@tropmedres.ac

count on day 3 is $P < 0.05$ if 20 patients are recruited in each arm.[7]

Patients with uncomplicated *P. falciparum* malaria were enrolled in the study provided that they gave fully informed written consent, had *P. falciparum* mono-infections with an asexual parasite density of 10,000–175,000 parasites/μL, were ≥ 10 years of age (and for female patients were before menarche or after menopause), had a tympanic temperature ≥ 37.5°C or history of fever during the previous 24 hours, were able to swallow oral medication, and were willing to stay in the hospital until parasite clearance and complete 42-days of follow up. Patients were excluded if they had danger signs or severe *P. falciparum* malaria,[15] severe malnutrition, febrile conditions caused by diseases other than malaria or other known underlying chronic or severe diseases, had received antimalarial drugs in the previous 48 hours, had a history of hypersensitivity reactions or contraindications to study medicines, or had had a splenectomy.

On admission, blood samples were taken for parasite count and hematocrit, and three blood spots were collected onto filter paper for polymerase chain reaction (PCR) genotyping in the event of reappearance of *P. falciparum* during follow-up.[12–14] The PCR amplification was performed on paired samples for parasite genotyping to distinguish between reinfection and recrudescence by using three parasite loci (merozoite surface protein 1, merozoite surface protein 2, and glutamate-rich protein).[16]

Patients who met the enrollment criteria were randomized (the treatment allocation was kept in a sealed opaque envelope, which was opened only after the decision to recruit had been made) to oral artesunate (Batch AS091001; Guilin Pharmaceutical Company, Shanghai, China), either 4 mg/kg/day (group 1) or 2 mg/kg/day (group 2) for 3 days, followed by the ACT recommended by the national treatment guidelines; artemether-lumefantrine (20/120 mg) (Coartem®, Novartis, Basel, Switzerland), 1 dose twice a day for three days. Dosing by body weight was 1 tablet if < 15 kg, 2 tablets if 15–24 kg, 3 tablets if 25–34 kg, and 4 tablets if ≥ 35 kg. The artesunate used was quality controlled by Research Institute for Industrial Pharmacy (Potchefstroom, South Africa). Drug administration was directly observed and if vomiting occurred within one hour of dosing, medication was re-administered. All antimalarial drugs were stored at 6–10°C.

Tympanic temperature, parasite counts, and hematocrit were measured every 6 hours until parasite clearance and then daily until discharge and on days 7, 14, 21, 28, 35, and 42. A standard physical examination was performed on day 0 (before drug administration), and the patients were reviewed daily until parasites cleared, then weekly for 42 days from the start of treatment, or at other times if he or she felt unwell. At each visit, finger prick blood was taken for a malaria smear and hematocrit. Three blood spots were collected onto filter paper from those with reappearance of asexual parasitemia. Patients with *P. falciparum* recurrence were re-treated with artesunate, 4 mg/kg/day for 7 days plus mefloquine, 15 mg base/kg on day 1 and 10 mg base/kg on day 2 and followed up for an additional 42 days. Those who had *P. vivax* appearance during follow-up were treated with chloroquine (25 mg base/kg) for 3 days and further followed-up. The incidence of any adverse event was recorded.

Written informed consent was obtained from all participants. Ethical clearance for the study was granted by the Lao National Ethics Committee for Health Research, the Oxford University Tropical Medicine Research Ethics Committee, United Kingdom and the Research Ethics Review Committee of the World Health Organization. The trial registration number is ISRCTN12741594. The trial was monitored by the Clinical Trials Support Group of Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand.

Laboratory investigations and outcome measures. Parasite counts for thick and thin blood films, stained with Field's stain, and hematocrit were determined every six hours until parasite clearance for two consecutive slides and then daily until patients' hospital discharge and weekly after day 7.[12–14] Two qualified microscopists (Level 1/expert malaria microscopists certified by the World Health Organization, Geneva, Switzerland, and the Asian Collaborative Training Network for Malaria, Manila, the Philippines) read all the slides independently. The PCR amplification was performed on paired samples for parasite genotyping to distinguish between reinfection and recrudescence.[17]

The primary study outcome was the number of patients with PCT > 72 hours. The PCT was defined as the time in hours from the first treatment dose to the first of two consecutive thick blood films that were negative for asexual *P. falciparum* parasites after checking ≥ 200 oil-immersion fields. The secondary outcomes were the PCR-corrected adequate clinical and parasitologic response at day 42,[18] fever clearance times, time in hours from the start of treatment at which the tympanic temperature first decreased to < 37.5°C and remained below 37.5°C for 48 hours), the frequency of adverse events and fractional changes in hematocrit after antimalarial treatment.

Statistical analysis. Data were entered into a database by double independent data entry and cross-validated. Data were analyzed by using SPSS version 11.0 (SPSS Inc., Chicago, IL). Comparisons between the two treatment groups were made by using the Mann–Whitney U test, Student's *t*-test, chi-square test, and Fisher's exact test as appropriate. Parasite clearance rates were calculated as the slope of the linear portion of the log-normalized parasite clearance curve, and this was used to estimate the times to clearance of 50% and 90% of the parasite load for each patient. The parasite reduction ratio was defined as the proportion of admission parasites that remained after 24 hours.

RESULTS

Patient screening and enrollment are shown in Figure 1. Baseline characteristics of the two study groups were similar (Table 1). The male preponderance reflects the decision of the Institutional Review Board of the World Health Organization that women of childbearing age could not be recruited. Because three patients were found after randomization to have taken antimalarial drugs before recruitment, four additional patients were randomized so that 20 patients without prior antimalarial drug use were recruited into each arm. All patients (100%) completed 42 days of follow-up (Figure 1).

Admission parasitemia, parasite reading, and clearance. The overall admission geometric mean (95% CI) parasitemia/μL was 45,113 (35,473–57,372) (Table 1). The percentage (95% CI) agreement in reading slides (as positive or negative) between the first and second microscopists was 96.4% (94.4–97.6%) ($\kappa = 0.92$), which is classified as very good agreement.[19]

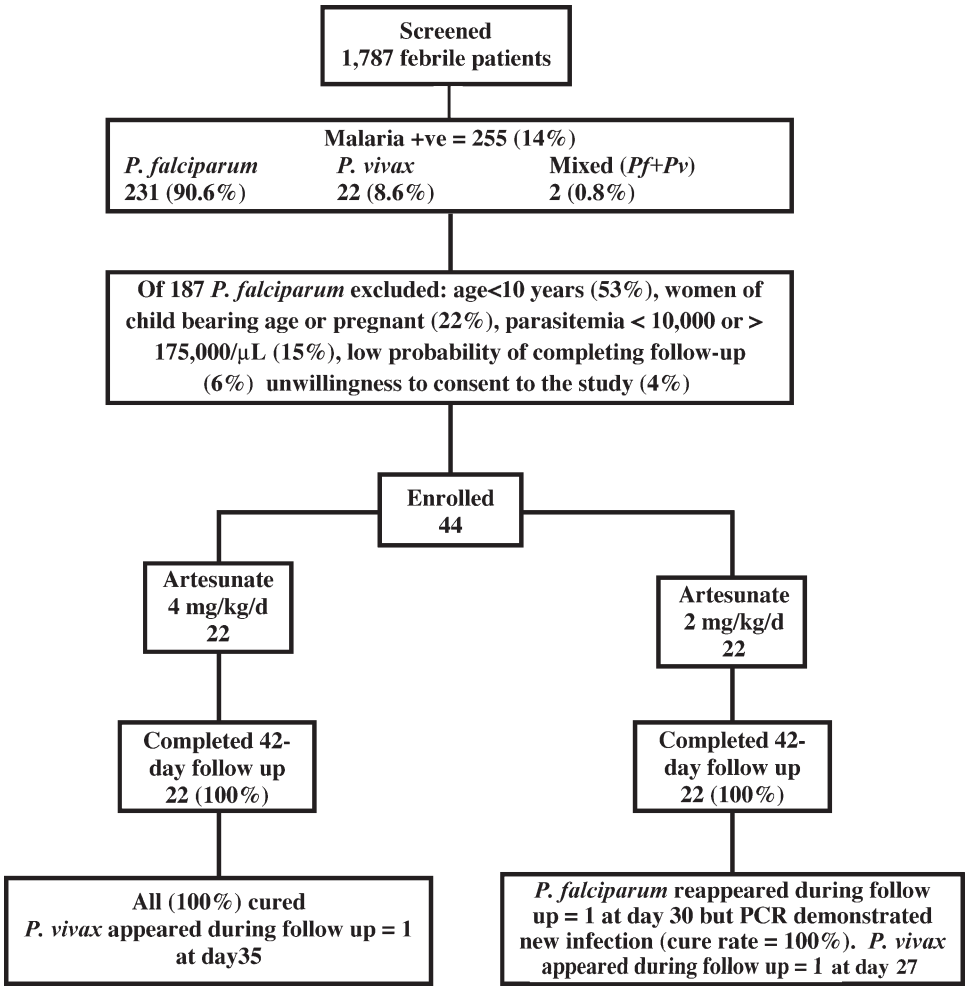

FIGURE 1. Participants in a study comparing 4 mg/kg/day versus 2 mg/kg/day of oral artesunate for treatment of *Plasmodium falciparum* Malaria in Laos. *Pf* = *P. falciparum*; *Pv* = *P. vivax*; PCR = polymerase chain reaction.

The slide-reading results from the first microscopist were used to calculate the slopes and parasite reduction ratios (Figure 2).

The overall mean (95% CI; range) PCTs (hours) were 23.2 (21.2–25.3; 12–46) and 22.4 (20.3–24.5; 12–46) for the first and second microscopists, respectively (P = 0.57). Ten (23%) patients remained parasitemic on day 1 after treatment (4 [18%] and 6 [27%] in group 1 and group 2, respectively; P = 0.47). No patient had patent asexual parasitemia on

TABLE 1

Admission demographics and clinical and laboratory details for patients enrolled in a study comparing 4 mg/kg/day versus 2 mg/kg/day of oral artesunate for three days followed by three days of artemether-lumefantrine for treatment of *Plasmodium falciparum* malaria in Laos*

Variable	Treatment groups		
	All (n = 44)	4 mg/kg/day (n = 22)	2 mg/kg/day (n = 22)
Male sex, no (%)	38 (86)	20 (91)	18 (82)
Age, years, median (range)	28 (11–66)	28 (11–66)	25 (12–65)
Height, cm	155.4 (151.6–159.2)	158.9 (155.6–162.2)	151.9 (145.1–158.7)
Body weight, kg	49.8 (45.8–53.8)	51.7 (46.7–56.7)	47.9 (41.5–54.4)
Tympanic temperature, °C	38.5 (38.1–38.9)	38.3 (37.8–38.8)	38.7 (38.1–39.2)
Patients without fever on admission, no. (%)	9 (20)	6 (27)	3 (14)
Systolic blood pressure, mm Hg	109.1 (106.2–112.1)	108.6 (104.7–112.6)	109.5 (104.9–114.2)
Diastolic blood pressure, mm Hg	77.0 (74.5–79.6)	76.8 (73.1–80.5)	77.3 (73.4–81.2)
Pulse, beats/min	86.8 (84.6–88.9)	86.2 (82.4–89.9)	87.4 (84.8–89.9)
Respiratory rate/min	24.3 (23.3–25.4)	24.3 (22.7–25.8)	24.4 (22.8–25.9)
Splenomegaly, no. (%) patients	2 (4.5)	1 (4.5)	1 (4.5)
Hepatomegaly, no. (%) patients	1 (2.3)	0	1 (4.5)
Parasitemia, geometric mean (95% CI) parasites/μL	45,113 (35,473–57,372)	45,112 (30,853–65,948)	45,123 (32,509–62,632)
Hematocrit, %	42.1 (40.4–43.8)	42.9 (40.1–45.7)	42.1 (40.4–43.8)

*Values are mean (95% confidence interval) unless otherwise indicated.

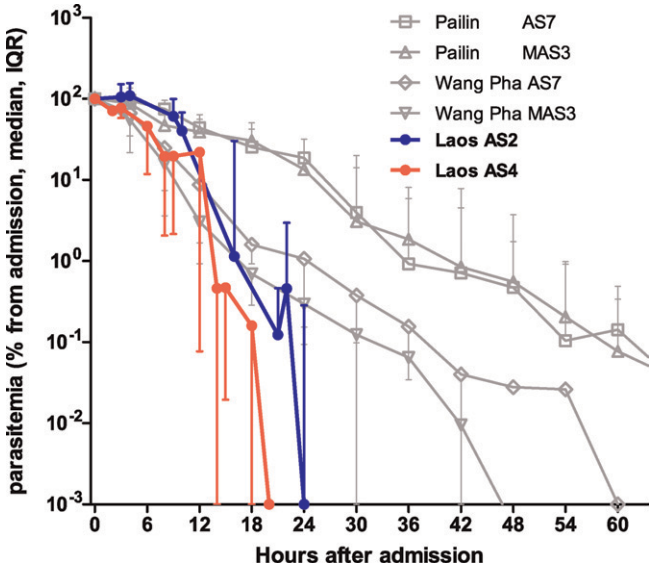

FIGURE 2. Parasite clearance curves for Lao patients compared with those from previous studies conducted in Pailin, Cambodia and Wang Pha, Thailand.[3] Lao data are $\log_{10}$-normalized median parasite densities over time among the 22 patients in each treatment group. Error bars indicate 50% of interquartile ranges (IQR).

the second and third days of treatment. The mean [95% CI] PCT (hours) was significantly shorter in group 1 than in group 2 (21.0 [18.7–23.3] versus 25.4 [22.1–28.8]; $P = 0.029$) for slides read by the first microscopist but not for the second microscopist ($P = 0.10$). For the 41 patients with no evidence of prior antimalarial drug use, the mean (95% CI; range) PCT (hours) was 23.6 (21.5–25.7; 14–46) and 22.5 (20.3–24.6; 12–46) for the first and second microscopists, respectively. Using the results of slide reading from the first microscopist, we determined that the estimated overall median (range) time (hours) to 50% parasitemia and the clearance rate of parasites was 5.1 (0.6–17.8) and 0.16 (0.09–0.26), respectively (Table 2). The mean [95% CI] PCT (hours) was longer in those ≤ 15 years of age (n = 7) than in those > 15 years of age (n = 37), but this was not statistically significant (26.1 [17.5–34.6] versus 22.7 [20.6–24.8]; $P = 0.23$). None of the patients had patent gametocytemia on admission, and gametocytemia did not develop after treatment.

Cure rates, fever clearance, and change in hematocrit. The mean (95% CI) multiplicity of infection was 1.33 (0.92–1.75) and this was found in 30% of the total samples for this study. One patient in group 2 had *P. falciparum* reappearance at day 30 and the PCR analysis indicated that this was a reinfection. Therefore, the 42-days PCR-corrected cure rates were 100% in both treatment groups. Two patients (one in each group) had *P. vivax* appearance at day 35 and day 27 and were successfully treated with oral chloroquine.

All patients had a history of fever during the 24 hours before enrollment and 35 (79.5%) of them were febrile at presentation, as defined by a tympanic temperature ≥ 37.5°C. After treatment, all patients were afebrile by day 2. The overall mean (95% CI) fever clearance time was 20.0 (16.2–23.8) hours, and this finding was not significantly different between the two groups. The admission mean (95% CI) hematocrit was significantly higher than those on other days ($P < 0.001$), but the fractional changes in hematocrit from admission over time did not differ significantly between the groups (Table 2).

Adverse events. Severe malaria or serious adverse events did not develop in any patients during or after treatment. The frequency of patients with symptoms and signs, before treatment, that may subsequently be confused with drug adverse events were similar between the two groups (Table 3).

TABLE 2

Outcome measures for the treatment of patients enrolled in a study comparing 4 mg/kg/day versus 2 mg/kg/day of oral artesunate for treatment of *Plasmodium falciparum* malaria in Laos*

Variable	Treatment groups			*P*
	All (n = 44)	4 mg/kg/day (n = 22)	2 mg/kg/day (n = 22)	
42-day cure rate, no. (%) patients	44 (100)	22 (100)	22 (100)	–
Fever clearance time, mean hours	20.0 (16.2–23.8)	20.6 (15.7–25.5)	19.5 (13.3–25.7)	0.77
Patients remained febrile at day 1, no. (%)	7 (16%)	2 (9%)	5 (23)	0.41
Patients remained febrile at day 2, no. (%)	0	0	0	–
Parasite clearance time by first microscopist	23.2 (21.2–25.3)	21.0 (18.7–23.3)	25.4 (22.1–28.8)	0.029
Parasite clearance time by second microscopist	22.4 (20.3–24.5)	20.8 (18.4–23.1)	24.1 (20.6–27.6)	0.10
Positive parasitemia at day 1, no. (%) patients	10 (23)	4 (18)	6 (27)	0.47
Positive parasitemia at day 2, no. (%) patients	0	0	0	–
Time to 50% clearance of parasite density, hours	5.1 (0.6–17.8)	4.4 (0.6–15.8)	6.4 (1.2–17.8)	0.01
Time to 90% clearance of parasite density, hours	9.7 (4.5–28.1)	9.0 (4.5–18.1)	11.6 (5.2–28.1)	0.02
Parasite reduction ratio at 24 hours	0 (0–10)	0 (0–0.6)	0 (0–10)	0.38
Parasite clearance rate (slope of curve for $\log_{10}$-normalized parasite clearance)†	0.16 (0.09–0.26)	0.17 (0.13–0.26)	0.16 (0.09–0.22)	0.02
P. vivax appearance after treatment of *P. falciparum*, no. (%) patients	2 (4.5)	1 (4.5)	1 (4.5)	–
Hematocrit				
Day 0	42.1 (40.4–43.8)	42.9 (40.1–45.7)‡	42.1 (40.4–43.8)‡	0.36
Day 7	37.1 (35.6–38.5)	37.4 (35.3–39.5)	37.1 (35.6–38.5)	0.63
Day 14	37.4 (35.9–38.8)	37.7 (35.4–39.9)	37.4 (35.9–38.8)	0.67
Day 21	38.3 (36.9–39.7)	38.5 (36.6–40.4)	38.3 (36.9–39.7)	0.75
Day 28	38.8 (37.6–39.9)	38.7 (37.0–40.4)	38.8 (37.6–39.9)	0.90
Day 35	39.2 (37.9–40.4)	39.7 (37.9–41.4)	39.2 (37.9–40.4)	0.39
Day 42	39.5 (38.3–40.7)	39.7 (38.0–41.4)	39.5 (38.3–40.7)	0.76

*Values are mean (95% confidence interval) unless otherwise indicated.
†Defined as the slope of linear portion of the $\log_{10}$-normalized parasite clearance curve.
‡Significant difference from other days ($P < 0.001$).

TABLE 3

Possible adverse events found in patients enrolled in a study comparing 4 mg/kg/day versus 2 mg/kg/day of oral artesunate for the treatment of *Plasmodium falciparum* malaria in Laos

Symptoms and signs	Before treatment (n = 44), no. (%)	After treatment (at any time)	
		4 mg/kg/day (n = 22), no. (%)	2 mg/kg/day (n = 22), no. (%)
Weakness	43 (98)	0	1 (4.5)
Anorexia	38 (86)	1 (4.5)	3 (14)
Nausea	27 (61)	0	1 (4.5)
Vomiting	18 (41)	0	1 (4.5)
Abdominal pain	11 (25)	0	1 (4.5)
Diarrhea	7 (16)	0	1 (4.5)
Headache	44 (100)	1 (4.5)	3 (14)
Insomnia	27 (61)	1 (4.5)	1 (4.5)
Nightmares	6 (14)	0	0
Palpitations	25 (57)	0	1 (4.5)
Dyspnea	12 (27)	0	1 (4.5)
Dizziness	36 (82)	0	1 (4.5)
Vertigo	14 (32)	0	0
Tinnitus	13 (29.5)	0	0

DISCUSSION

The study showed no evidence of *P. falciparum* resistance to artesunate, as defined by prolonged PCT, approximately 400 km, as the crow-flies, from the focus of *P. falciparum* resistance to artemisinin derivatives in western Cambodia. None of the study patients were parasitemic on days 2 or 3 after treatment with either 4 mg/kg/day or 2 mg/kg/day of oral artesunate for 3 days, which is reassuring for the continued use of the currently recommended ACT regimen in Laos. The mean PCT in southern Laos (approximately 23 hours) is much shorter than that found in Pailin in western Cambodia (approximately 84 hours) and in Wang Pha in northwestern Thailand (48 hours) (Figure 2),[3] although the admission parasitemias in Laos were higher than those in Wang Pha but similar to those in Pailin. In contrast to patients in Laos, 55% of patients in Pailin and 8% in Wang Pha remained parasitemic at day 3 after starting treatment.

This study suggests that *P. falciparum* parasites resistant to artemisinins have not spread from the western Cambodia to the southern Laos/Vietnam border area. Importantly, artemisinin-resistant parasites have not been described from eastern Cambodia, south of Laos, and there is currently relatively little recorded population flow across the southern Lao/eastern Cambodia border (approximately 1,500–3,000 persons/year compared with an estimated 30,000–100,000 persons/year who moved across the southern Laos/Vietnam border) (data for 2005–2009).[20] With improvement of road communications, population flow across the Laos/Cambodia border will inevitably increase. Oral artesunate monotherapy, including fake or substandard quality artesunate, were in use in southern Laos for at least six years before 2005 when the Lao Government changed the national policy for uncomplicated *P. falciparum* malaria treatment to ACT.[11]

The absence of artemisinin resistant *P. falciparum* from at least one site in southern Laos is reassuring. However, prevention of conditions that would facilitate survival of artemisinin-resistant parasites in southern Laos, such as removal of all artesunate monotherapy, ensuring that ACTs are of good quality and the encouragement of adherence to correct dosage regimens, are urgently needed. Regular monitoring of the clinical efficacy of artemisinin-derivatives in southern Laos, especially closer to the Cambodian border, is required.

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Authors' addresses: Mayfong Mayxay, Wellcome Trust–Mahosot Hospital–Oxford University Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Laos, Faculty of Postgraduate Studies, University of Health Sciences, Vientiane, Laos, and Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7LJ, United Kingdom, E-mail: mayfong@tropmedres.ac. Maniphone Khanthavong, Samlane Phompida, and Bouasy Hongvanthong, Centre of Malariology, Parasitology and Entomology, Ministry of Health, Vientiane, Laos, E-mail: cmpelao@gmail.com. Odai Chanthongthip and Bongkot Soonthornsata, Wellcome Trust–Mahosot Hospital–Oxford University Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Laos, E-mail: kaebongkot@hotmail.com. Mallika Imwong, Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, E-mail: noi@tropmedres.ac. Sue J. Lee and Kasia Stepniewska, Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7LJ, United Kingdom; and Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, E-mails: sue@tropmedres.ac and kasia@tropmedres.ac. Tiengkham Pongvongsa, Savannakhet Provincial Malaria Station, Savannakhet Province, Laos, E-mail: tpongvongsa@hotmail.com. Pascal Ringwald, Drug Resistance and Containment Unit, Global Malaria Programme, World Health Organization, Geneva, Switzerland, E-mail: ringwaldp@who.int. Nicholas J. White, Wellcome Trust–Mahosot Hospital–Oxford University Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Laos, Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7LJ, United Kingdom; and Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, E-mail: nickw@tropmedres.ac. Paul N. Newton, Wellcome Trust–Mahosot Hospital–Oxford University Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Laos, Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Nuffield Department of Medicine, University of Oxford, Oxford OX3 7LJ, United Kingdom, E-mail: paul@tropmedres.ac.

REFERENCES

1. Noedl H, Se Y, Schaecher K, Smith BL, Socheat D, Fukuda MM, 2008. Artemisinin Resistance in Cambodia 1 (ARC1) Study Consortium. Evidence of artemisinin-resistant malaria in western Cambodia. *N Engl J Med 359:* 2619–2620.
2. Rogers WO, Sem R, Tero T, Chim P, Lim P, Muth S, Socheat D, Ariey F, Wongsrichanalai C, 2009. Failure of artesunate-mefloquine combination therapy for uncomplicated *Plasmodium falciparum* malaria in southern Cambodia. *Malar J 12:* 10.
3. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, Tarning J, Lwin KM, Ariey F, Hanpithakpong W, Lee SJ, Ringwald P,

Silamut K, Imwong M, Chotivanich K, Lim P, Herdman T, An SS, Yeung S, Singhasivanon P, Day NP, Lindegardh N, Socheat D, White NJ, 2009. Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med 361:* 455–467.

4. Luxemburger C, Brockman A, Silamut K, Nosten F, van Vugt M, Gimenez F, Chongsuphajaisiddhi T, White NJ, 1998. Two patients with falciparum malaria and poor *in vivo* responses to artesunate. *Trans R Soc Trop Med Hyg 92:* 668–669.
5. Carrara VI, Zwang J, Ashley EA, Price RN, Stepniewska K, Barends M, Brockman A, Anderson T, McGready R, Phaiphun L, Proux S, van Vugt M, Hutagalung R, Lwin KM, Phyo AP, Preechapornkul P, Imwong M, Pukrittayakamee S, Singhasivanon P, White NJ, Nosten F, 2009. Changes in the treatment responses to artesunate-mefloquine on the northwestern border of Thailand during 13 years of continuous deployment. *PLoS ONE 4:* e4551.
6. Na-Bangchang K, Ruengweerayut R, Mahamad P, Ruengweerayut K, Chaijaroenkul W, 2010. Declining in efficacy of a three-day combination regimen of mefloquine-artesunate in a multi-drug resistance area along the Thai-Myanmar border. *Malar J 9:* 273.
7. Stepniewska K, Ashley E, Lee SJ, Anstey N, Barnes KI, Binh TQ, D'Alessandro U, Day NP, de Vries PJ, Dorsey G, Guthmann JP, Mayxay M, Newton PN, Olliaro P, Osorio L, Price RN, Rowland M, Smithuis F, Taylor WR, Nosten F, White NJ, 2010. *In vivo* parasitological measures of artemisinin susceptibility. *J Infect Dis 201:* 570–579.
8. White NJ, 1997. Assessment of the pharmacodynamic properties of the antimalarial drugs *in-vivo*. *Antimicrob Agents Chemother 41:* 1413–1422.
9. White NJ, 2004. Antimalarial drug resistance. *J Clin Invest 113:* 1084–1092.
10. World Health Organization, 2006. *Guidelines for the Treatment of Malaria*. Geneva: World Health Organization.
11. Sengaloundeth S, Green MD, Fernández FM, Manolin O, Phommavong K, Insixiengmay V, Hampton CY, Nyadong L, Mildenhall DC, Hostetler D, Khounsaknalath L, Vongsack L, Phompida S, Vanisaveth V, Syhakhang L, Newton PN, 2009. A stratified random survey of the proportion of poor quality oral artesunate sold at medicine outlets in the Lao PDR: implications for therapeutic failure and drug resistance. *Malar J 8:* 172.
12. Mayxay M, Khanthavong M, Lindegårdh N, Keola S, Barends M, Pongvongsa T, Yapom R, Annerberg A, Phompida S, Phetsouvanh R, White NJ, Newton PN, 2004. Randomized comparison of chloroquine plus sulphadoxine-pyrimethamine *versus* artesunate plus mefloquine versus artemether-lumefantrine in the treatment of uncomplicated falciparum malaria in the Lao PDR (Laos). *Clin Infect Dis 39:* 1139–1147.
13. Mayxay M, Thongpraseuth V, Khanthavong M, Lindgårdh N, Barends M, Keola S, Pongvongsa T, Phompida S, Phetsouvanh R, White NJ, Newton PN, 2006. An open, randomized comparison of artesunate plus mefloquine versus dihydroartemisinin-piperaquine for the treatment of uncomplicated falciparum malaria in the Lao People's Democratic Republic (Laos). *Trop Med Int Health 11:* 1157–1185.
14. Mayxay M, Keomany S, Khanthavong M, Souvannasing P, Stepniewska K, Khomthilath T, Keola S, Pongvongsa T, Phompida S, Ubben D, Valecha N, White NJ, Newton PN, 2010. A phase III, randomized, non-inferiority trial to assess the efficacy and safety of dihydroartemisinin-piperaquine in comparison with artesunate-mefloquine in patients with uncomplicated *Plasmodium falciparum* malaria in southern Laos. *Am J Trop Med Hyg 83:* 1221–1229.
15. World Health Organization, 2000. Severe and complicated malaria. *Trans R Soc Trop Med Hyg 94 (Suppl 1):* 1–90.
16. Snounou G, Zhu X, Siripoon N, Jarra W, Thaithong S, Brown KN, Viriyakosol S, 1999. Biased distribution of msp1 and msp2 allelic variants in *Plasmodium falciparum* populations in Thailand. *Trans R Soc Trop Med Hyg 93:* 369–374.
17. World Health Organization, 2009. *Methods and Techniques for Clinical Trials on Antimalarial Drug Efficacy: Genotyping to Identify Parasite Populations*. Geneva: World Health Organization. Available at: http://www.who.int/malaria/resistance.
18. World Health Organization, 2009. *Method for Surveillance of Antimalarial Drug Efficacy*. Geneva: World Health Organization. Available at: http://www.who.int/malaria/resistance.
19. Landis JR, Koch GG, 1997. The measurement of observer agreement for categorical data. *Biometrics 33:* 159–174.
20. *Statistics of the Immigration at Lao-Cambodia Borders: Nong Nokkhiad and Dongkalor Checkpoints*. Khong District, Champasack Province. Unpublished Lao Immigration Office Report, 2010.