Chloroquine remains effective for treating *Plasmodium vivax* malaria in Pursat Province, Western Cambodia

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Running head: Chloroquine efficacy for *P. vivax* malaria

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Chloroquine (CQ) is used to treat *Plasmodium vivax* malaria in areas where CQ resistance has not been reported. The use of artemisinin (ART)-based combination therapies (ACTs) to treat CQ-sensitive *P. vivax* infections is effective and convenient, but may promote the emergence and worsening of ART-resistance in sympatric *P. falciparum* populations. Here we show that CQ effectively treats *P. vivax* malaria in Pursat Province, Western Cambodia, where ART-resistant *P. falciparum* is highly prevalent and spreading.

Half of Cambodia’s population is at risk of acquiring malaria (1). In response to the emergence of ART-resistant *P. falciparum* in multiple provinces (2, 3)(4), Cambodia has developed a strategic plan to combat malaria (5). Its goals are to eliminate ART-resistant *P. falciparum* by 2015, *P. falciparum* malaria deaths by 2020, and all forms of malaria (including *P. vivax*) by 2025. This approach recognizes that as control measures become more effective, *P. vivax* will cause an increasingly greater proportion of malaria cases. This is because *P. vivax* hypnozoites survive blood-stage antimalarials, are not routinely treated with primaquine due to the 14% prevalence of G6PD deficiency in Cambodia (6), and thus can cause relapsing malaria (7).

CQ is the frontline treatment for *P. vivax* malaria in areas where resistance has not been reported, while ACTs are the recommended treatments in areas where resistance is prevalent (8) – especially where ACTs are used as frontline treatments for *P. falciparum* malaria (9). CQ-resistant *P. vivax* malaria was first described in Papua New Guinea in 1989 (10) and has since been documented in parts of Southeast Asia and the Americas (11-14). Only two studies have measured the efficacy of CQ in treating *P. vivax* malaria in Cambodia: Poravuth et al. (15)
reported a 98% cure rate in Pailin Province (Western Cambodia) in 2007-2008; and Leang et al. (16) reported 96%, 88%, and 83% cure rates in Pailin, Preah Vihear (Northern Cambodia), and Ratanakiri (Eastern Cambodia) in 2009. In response to the declining efficacy of CQ in Preah Vihear and Ratanakiri, Cambodia changed its frontline treatment for *P. vivax* malaria from CQ to dihydroartemisinin-piperaquine (DHA-PPQ) in all 24 provinces in 2011. Here we investigate (2, 3, 16) the efficacy of CQ for treating acute *P. vivax* malaria in Pursat (Western Cambodia), where ART-resistant *P. falciparum* is highly prevalent (2, 3)(4).

We based our study on the standardized World Health Organization (WHO) protocol for assessing the therapeutic response to chloroquine (17). From August 2012 to January 2013, we screened 1929 individuals seeking treatment for fever at Sampov Meas Referral Hospital in Pursat. Of these, 1518 patients had a negative rapid diagnostic test (RTD, First Response, pLDH/HRP2 Combo, Premier Medical Corp. Ltd., India) for malaria parasitemia. Giemsa-stained smears of RDT-positive blood samples showed that 178 patients had *P. falciparum* infection, 42 had mixed *P. falciparum* and *P. vivax* infections, and 191 had *P. vivax* malaria (mono-infection, any parasite density, and axillary temperature ≥37.5°C or history of fever in the previous 2 d).

Of these 191 patients, 87 met additional inclusion criteria: age >2 years, hematocrit ≥25%, and willingness to complete treatment in hospital and receive follow-up visits at home. Patients were excluded if they were pregnant or used any antimalarial in the previous 2 months. No patient had signs of severe malaria, malnutrition, concomitant or chronic disease, or reported hypersensitivity to CQ. Adult patients or the parents of children gave written informed consent.
The study was approved by the Cambodian National Ethics Committee for Health Research and the NIAID Institutional Review Board (ClinicalTrials.gov identifier, NCT00663546).

Patient characteristics on admission are presented in Table 1. Most patients were male (79.3%), febrile (≥37.5°C, 86.2%), and reported multiple symptoms: headache (86.2%), body aches (75.9%), malaise (73.6%), vomiting (19.5%), shivering (99.0%), chills (99.0%), and sweats (85.0%). A minority (13.8%) of patients reported experiencing a classic paroxysm. The median hematocrit was 39% (IQR 35-43). Just prior to drug administration, the median asexual parasite density was 5784/µl (IQR 2600–11902) and the median gametocyte density was 349/µl (IQR 112–1131). Only 6 (7%) patients had a parasite density below the WHO-recommended cut-off of 250/µl as an inclusion criterion (17). On admission, asexual parasite density correlated significantly with axillary temperature (r=0.4739, P<0.0001, Spearman correlation test, GraphPad Software, La Jolla, CA) and gametocyte density (r=0.3945, P=0.0002). Neither asexual parasite density (r=0.1916, P=0.0754) nor gametocyte density (r=0.1536, P=0.1555) correlated with age.

All patients were hospitalized and treated orally with CQ phosphate (250 mg salt/tablet, Ranbaxy Pharmaceuticals, FL) 10 mg/kg at 0 and 24 h, and 5 mg/kg at 48 h. We specifically withheld primaquine therapy to avoid inducing hemolytic anemias in patients with G6PD deficiency. Axillary temperatures were measured and parasite densities estimated as described (2) every 24 h until asexual parasitemia was undetectable (i.e., no asexual parasites observed per 500 leukocytes) on one blood smear instead of two consecutive blood smears as
recommended by the WHO (17). Asexual parasite densities dropped significantly at 24 h (median 5,784/µl to 32/µl, 99.4% reduction, P<0.0001, Wilcoxon matched-pairs signed rank test) (Figure 1A). Gametocyte densities also dropped at 24 h (median 349/µl to 16/µl, 96.4% reduction, P<0.0001) (Figure 1B). The proportions of patients with asexual parasitemia at 0, 24, 48, and 72 h were 100, 79.3, 4.60, and 0%, with gametocytemia were 85.0, 58.6, 11.5, and 2.30%, and with fever (≥37.5°C) were 86.2, 21.8, and 0%. Nearly all (90.8%) patients, however, were given paracetamol for fever.

Patients were followed up on days 7, 14, 21, and 28. At each visit, axillary temperature was measured and blood smears examined for recurrent malaria parasitemia. Data from 3 patients who were lost to follow-up were not analyzed, but data from 8 patients who missed 1, 2, or 3 follow-up visits were analyzed since all were seen on day 28 and none reported fever since discharge. Among 84 patients with adequate follow-up, 1 had asymptomatic *P. vivax* parasitemia (60/µl) on day 28, 1 had asymptomatic *P. falciparum* parasitemia (402/µl) on day 28, and 1 had *P. falciparum* malaria (16149/µl, 40.1°C) on day 21. Thus, the cumulative risk of recurrent *P. vivax* parasitemia at 28 d was 1.2% (1/84). Whether this recurrent parasitemia episode was due to relapse, recrudescence, or reinfection has not been determined.

In Pursat in 2012, our use of RDTs and expert microscopy accurately detected *P. vivax* mono-infections, which were treated effectively with CQ. The lingering presence of CQ at levels above the minimally effective concentration likely prevented the detection of relapse infections, which have a high probability of occurring within 28 d. In other provinces where ART-resistant
*P. falciparum* is prevalent, programs that accurately diagnose and monitor the clearance of *P.* vivax mono-infections may help to minimize the use of ACTs. Since the proportion of patients with detectable parasitemia at 72 h was 0%, an increase in this proportion may be a useful phenotypic marker for emerging CQ resistance in Pursat. “Day 3 positivity” rates, which estimate this proportion, have previously been associated with CQ resistance in Preah Vihear (3.3%) and Ratanakiri (4.3%), and with CQ sensitivity in Pailin (0%) (16). Baseline data for each province could be useful to future surveillance and elimination programs in Cambodia. Nearly all patients had undetectable gametocytemia at 72 h, suggesting that CQ also remains effective in reducing *P. vivax* transmission in Pursat.

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**FIG 1.** Asexual parasite densities (A) and gametocyte densities (B) following chloroquine treatment at 0, 24, and 48 h. Median values are indicated. In (A) there are 18, 65, and 4 zero values at 24, 48, and 72 h which are not shown on the logarithmic scale. In (B) there are 36, 59, and 2 zero values at 24, 48, and 72 h which are also not shown.
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Temperature (°C)</th>
<th>Hematocrit (%)</th>
<th>Asexual parasite density (/μl)</th>
<th>Gametocyte density (/μl)</th>
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<tr>
<td></td>
<td>79.3%</td>
<td>Median 26</td>
<td>Median 53</td>
<td>Median 38.0</td>
<td>Median 39</td>
<td>Median 5784</td>
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<td></td>
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<td>IQR 18-32</td>
<td>IQR 46-59</td>
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<td>IQR 2600-11902</td>
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<td>Range 4-68</td>
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<td>Range 0-5553</td>
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REFERENCES


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