MONITORING ANTIMALARIAL DRUG EFFICACY IN THE GREATER MEKONG SUBREGION: AN OVERVIEW OF *IN VIVO* RESULTS FROM 2008 TO 2010

Abstract. *In vivo* Therapeutic Efficacy Studies (TES) have been routinely conducted in the Greater Mekong Subregion (GMS) for decades. Results from the last 10 years have contributed to update national antimalarial drug policies, to identify hotspots of multi-drug resistance and from 2008 onwards, to stimulate ambitious multi-country programs and innovative research projects to contain and eliminate artemisinin resistant *Plasmodium falciparum* strains in the subregion.

This paper describes the results of TES of first-line antimalarials in six countries of the GMS from 2008-2010 using the WHO *in vivo* standard protocol. A total of 91 studies were conducted at 32 sentinel sites testing dihydroartemisinin-piperaquine (DHA-PIP), artesunate+mefloquine (A+M), and artemether-lumefantrine (AL) against *P. falciparum* malaria, as well as chloroquine and DHA-PIP against *P. vivax*. Overall, artemisinin-based combination therapies (ACTs) remained efficacious against falciparum malaria with some exceptions. The 42-day adequate clinical and parasitological response (ACPR) for DHA-PIP dropped significantly to 73% (95% CI 53-87) in 2010 in the same hotspot area of western Cambodia known to harbor artemisinin resistant *P. falciparum* strains. Because *P. falciparum* sensitivity to artemisinin is a major concern, especially on the Cambodia-Thailand border, attempts were also made to strengthen the monitoring of parasite clearance time elsewhere in the region and globally. The proportion of patients still blood-smear positive on Day 3 above 10% is considered a proxy indicator to strongly suspect the appearance of falciparum resistance to artesunate. This has led to substantial extra measures to confirm the suspicion and eventually set up interventions to eliminate artemisinin resistant parasites. Notably, increasing proportions (>10%) of Day 3 positives among falciparum malaria patients treated with DHA-PIP have been observed in western Cambodia, Myanmar, Viet Nam and China from 2008. Percent Day 3 parasitemia associated with A+M has increased along the Thailand-Myanmar border to surpass 10% at several sites, adding to the known pool of sites with 'suspected' artemisinin resistance in the GMS.

Chloroquine remains highly effective against *P. vivax* except for northeastern and north-central Cambodia. TES results from this subregional-wide monitoring of antimalarial efficacy have influenced the changes of 1st line drugs against both *P. falciparum* and *P. vivax* in Cambodia, against *P. falciparum* in selected areas in Thailand, and pinpointed hotspot areas elsewhere that should be closely monitored in order to take action in a timely manner.

INTRODUCTION

The Greater Mekong Subregion (GMS), composed of Cambodia, China (Yunnan Province), Lao PDR, Myanmar, Thailand and Viet Nam, is known for multi-drug resistant malaria with the Cambodia-Thailand border, in particular, as the epicenter of antimalarial resistance (Singhasivanon *et al,* 1999). Since 1999, the Roll Back Malaria Mekong project has taken several steps to advance multi-country collaboration aimed at *in vivo* monitoring by Therapeutic Efficacy Studies (TES) of first line antimalarial drugs used in the Subregion. In January 2007, a WHO informal consultation in Cambodia extensively reviewed available data and acknowledged the decreased efficacy of artemisinin-based combination therapies (ACTs) against *P. falciparum* on the Cambodia-Thailand border (WHO, 2007a). Recommendations were made to better document drug resistance in the GMS through intensifying TES as part of routine activities of National Malaria Control Programmes (NMCP) and through in-depth research studies by recognized academic institutions (WHO, 2009c). The primary objective of the TES was to evaluate the therapeutic efficacy of the first- and second-line antimalarials as recommended by national programs. Specifically we evaluated the efficacy of ACTs against *P. falciparum,* and chloroquine and DHA-PIP against *P. vivax.* The secondary objective was to detect foci of artemisinin resistance by assessing the proportion of patients with delayed parasite clearance time, especially the presence of parasites on blood smear 3 days after treatment initiation (so-called "Day 3 parasitemia") considered as a proxy indicator of suspected *P. falciparum* resistance to artemisinins.

To coordinate these collaborative efforts, WHO was tasked to revitalize the "*Mekong Malaria Therapeutic Efficacy Study Network"* encompassing the six GMS countries. These countries have all been using ACTs or artemisinin monotherapies for uncomplicated falciparum malaria cases for over a decade. Through WHO's leadership, the *in vivo* Network, which includes collaboration between national malaria program managers and academic and government researchers, endorsed a single TES protocol across the Subregion. As a result, this standardized approach has led to progressive improvements in data quality, provided needed evidence for policy makers to update drug policies, and allowed for comparison of data across countries especially at border areas.

In this article, we describe the 91 studies conducted under the Subregional TES Network at 32 sentinel sites across the GMS, from 2008 to 2010 (Some sites began their studies in 2010 but did not complete patient recruitment until 2011). The findings and implications for antimalarial drug policy changes and artemisinin resistance containment operations are discussed.

MATERIALS AND METHODS

Two successive workshops were held by the WHO Mekong Malaria Programme (MMP),

one in Phuket, Thailand in 2007 and the other in Mandalay, Myanmar in 2009 and were attended by key personnel responsible for TES in the six GMS countries. Study sites were identified and all principal investigators (PI) familiarized with the WHO standard TES protocol (WHO, 2007b; WHO, 2009d) with a follow-up schedule of either 28 days or 42 days. In addition to the general WHO guidelines, the countries agreed on standard outcomes and methodology for data management and analysis, drugs to be monitored, sentinel sites, informed consent-form template (in English), and common supervision including training and reporting mechanisms. Each country had the informed consent translated into local language(s). Subsequently, at the national level, PIs and WHO staff finalized the individual country proposals, processed for approval by the national ethics committee, then by the WHO Ethics Review Committee (ERC) before the requested funds were officially released. All studies were registered with the Australia-New Zealand Clinical Trial Registration website (ANZCTR).

Supervisory and monitoring visits on site were conducted at least once a year by WHO MMP staff and several times a year by WHO national staff in conjunction with TES training workshops and microscopy refresher courses.

Study design

The aim of these one-arm TES was to evaluate the clinical and parasitological responses of uncomplicated *P. falciparum* and *P. vivax* infections to antimalarial drugs given under direct observed therapy. Patients were enrolled according to pre-determined criteria (see Study participants below), treated on site with the first-line drug according to the corresponding national treatment guidelines, and monitored through clinical and laboratory examinations during 28 or 42 days depending on the drug given (see Anti-malaria drugs and follow-up procedures below). The same drug was intended to be tested every other year at each sentinel site, but there were some exceptions for more or less frequent studies.

Sentinel sites

The TES were conducted from 2008 to 2010 at 32 sentinel sites across the six GMS countries: Cambodia (4), China (3), Lao PDR (3), Myanmar (8), Thailand (9), and Viet Nam (5) (Figs 1 and 2). The sentinel sites were chosen on the basis of high malaria burden, risk of drug resistance development as well as availability of local health facilities and staff for case finding, treatment and follow-up. Most of them are located along international borders.

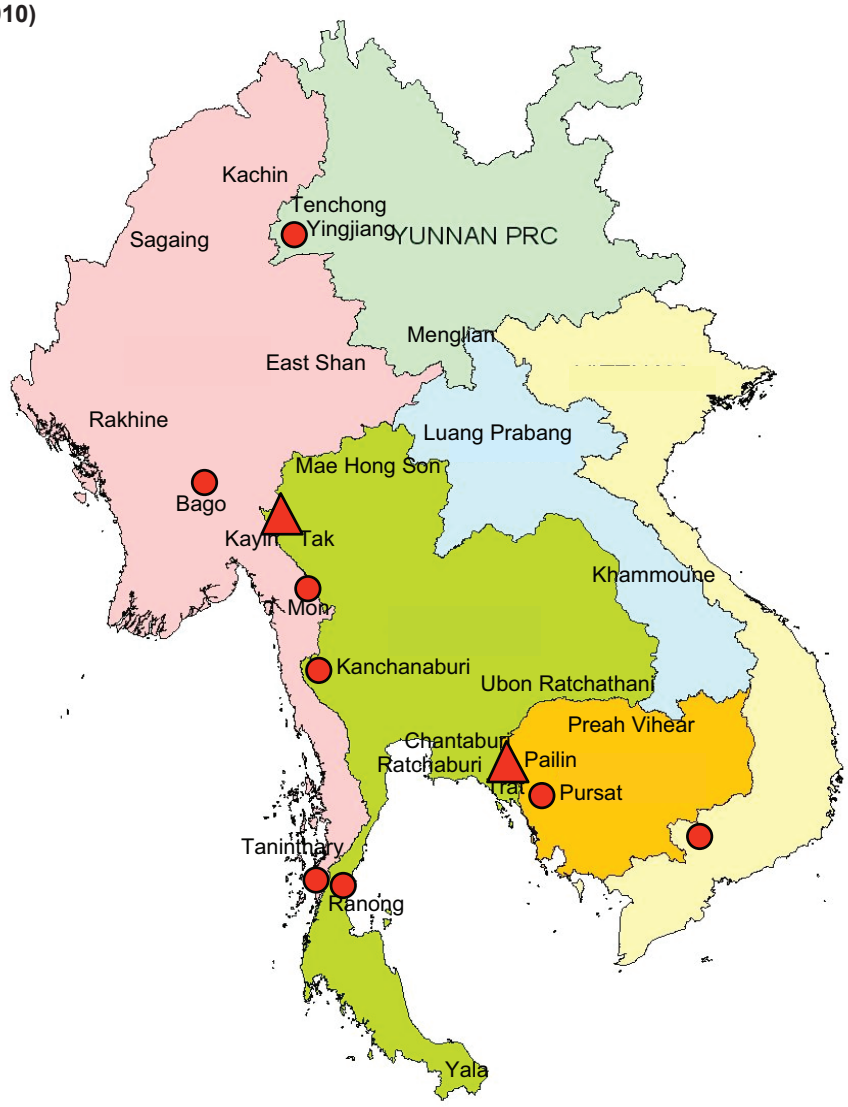
Study participants

Inclusion and exclusion criteria as per WHO standard protocol are presented in Box 1 (WHO, 2009a). Briefly we enrolled patients who were >2 years old with mono-infection of *P. falciparum* or *P. vivax.* Patients were enrolled at a remote village/commune health station, a district health center or a government malaria clinic. Often, project staff also conducted active

Fig 1–Location of sentinel sites for therapeutic efficacy studies (TES) in the Greater Mekong Sub-region (GMS), 2008-2010.

Sentinel sites (2008-2010)

1. Cambodia
   - 1.1 Pailin
   - 1.2 Pursat
   - 1.3 Preah Vihear
   - 1.4 Rattanakiri
2. Yunnan, China
   - 2.1 Menglian
   - 2.2 Yingjiang
   - 2.3 Tengchong
3. Lao PDR
   - 3.1 Luang Prabang
   - 3.2 Khammoune
   - 3.3 Attapeu
4. Myanmar
   - 4.1 Kachin
   - 4.2 Eastern Shan
   - 4.3 Rakhine
   - 4.4 Sagaing
   - 4.5 Kayin
   - 4.6 Bago
   - 4.7 Mon
   - 4.8 Tanintharyi
5. Thailand
   - 5.1 Mae Hong Son
   - 5.2 Tak
   - 5.3 Kanchanaburi
   - 5.4 Ratchaburi
   - 5.5 Ubon Ratchathani
   - 5.6 Chanthaburi
   - 5.7 Ranong
   - 5.8 Yala
   - 5.9 Trat (previous site)
6. Viet Nam
   - 6.1 Quang Tri
   - 6.2 Ninh Thuan
   - 6.3 Gia Lai
   - 6.4 Dak Nong
   - 6.5 Binh Phuoc



● Areas of suspected resistance to artemisinins (where more than 10% of enrolled patients still have parasites 72-hours after onset of treatment)

▲ Areas of confirmed resistance to artemisinins

Fig 2–Location of “hotspots” in the GMS where artemisinin resistance has been either “suspected” or “confirmed” from 2008 to 2010.

case surveys in surrounding villages when passive case detection at a local health center gave insufficient yield for the required sample size.

Due to the generally low malaria incidence in the Subregion (except in Myanmar and some locations in Cambodia), Day 0 parasite density cut-offs for inclusion of falciparum cases have been modified to include ≥100 parasites/µl blood, and patients with <500 parasites/µl blood must be febrile at enrolment. Similarly, the upper limit of parasitemia for inclusion has been raised to 250,000 parasites/µl blood, and the patients must still be without any other signs of severe malaria. Previously in the Subregion, Day 0 parasitemia of up to 150,000/µl was allowed due to difficulty in finding eligible cases.

Box 1

Inclusion and exclusion criteria used by the Mekong TES Network during 2008-2010.

Inclusion criteria

- age between 2 to 60 years old;
- mono-infection with *P. falciparum* or *P. vivax* detected by microscopy;
- *P. falciparum* parasitemia 500-250,000 asexual forms/µl; (see discussion pertaining to modified entry criteria)
- *P. vivax* parasitemia ≥ 250 asexuals/µl;
- presence of axillary temperature ≥ 37.5ºC or history of fever during the past 24 hour;
- ability to swallow oral medication;
- ability and willingness to comply with the study protocol for the duration of the study and to comply with the study visit schedule; and
- informed consent from the patient or from a parent or guardian in the case of children.

Exclusion criteria

- presence of general danger signs in children aged under 5 years or signs of severe falciparum malaria;
- mixed or mono-infection with another *Plasmodium* species detected by microscopy;
- presence of severe malnutrition (defined as a child whose growth standard is below –3 z-score, has symmetrical edema involving at least the feet or has a mid-upper arm circumference < 110 mm);
- presence of febrile conditions due to diseases other than malaria (*eg* measles, acute lower respiratory tract infection, severe diarrhea with dehydration) or other known underlying chronic or severe diseases (*eg* cardiac, renal and hepatic diseases, HIV/AIDS);
- regular medication which may interfere with antimalarial pharmacokinetics;
- history of hypersensitivity reactions or contraindications to any of the medicine(s) being tested or used as alternative treatment(s);
- unmarried female age 12-18 years old; and
- positive pregnancy test or breastfeeding.

Sample size

Assuming a treatment failure rate of 10% with a study precision of 10% and the desired confidence level of 95%, the minimum sample size was calculated to be 50 patients per study (*ie* per an individual drug at a specific site for a specific year). In practice, we targeted at enrolling 60 or more patients in order to account for withdrawals and loss-to-follow-ups.

Anti-malarial drugs and TES follow-up procedures

***P. falciparum* treatment.** GMS countries rely on various first-line ACT regimens. China and Viet Nam have used artesunate monotherapy for decades but currently promote DHA-PIP. Myanmar began using either DHA-PIP, A+M, or AL from 2002 depending on donor source. AL alone has been promoted in Lao PDR since 2002. Cambodia has recently switched from A+M to DHA-PIP countrywide except in Pailin where Atovaquone-Proguanil (Malarone®, Glaxo SmithKline, UK) was adopted in mid-2012. Loose tablets of artesunate and mefloquine given together have been the practice for Thailand, in selected locations since 1995, and countrywide since 2005. One exception is for Chantaburi and Trat Provinces on the southeastern border with Cambodia, where Atovaquone-Proguanil has been used since 2009 (see also Chapter 7) as a part of the artemisinin resistance containment operation.

***P. vivax* treatment.** Chloroquine was tested in all countries except Lao PDR because of its low incidence of vivax malaria. Cambodia added a TES of DHA-PIP for *P. vivax* in 2010. Under the *P. vivax* section of the protocol, primaquine was expected to be given after Day 28 as an anti-relapse drug, with either a 14-day or 8-week regimen depending on the G6PD status of the patient.

All drugs used in the studies met the international pharmacopoeia standards. Drugs used in the TES in Cambodia, China, Myanmar and Lao PDR were provided directly by WHO after QA testing in recognized reference laboratories. The National Institute of Drug Quality Control of Viet Nam (NIDQC) in Hanoi (a WHO Drug Quality Collaborating Center) provided QA lot testing for artesunate and DHA-PIP used in the TES in Viet Nam and Thailand. Certificates of passed batch analysis were obtained for all lots.

After initial treatment on Day 0, all patients were asked to return to receive subsequent doses (until Day 2 for ACTs and chloroquine, and Day 28 for primaquine), and for clinical assessment and blood film examination per protocol on Days 1, 2, 3, 7, 14, 21and 28 (or including Days 35 and 42 for those receiving A+M and DHA-PIP). China, Myanmar and Viet Nam initially could not implement the 42-Day follow-up scheme of DHA-PIP therapy for logistic reasons. Patients were also instructed to return on any other days if they experienced fever or other clinical symptoms suggestive of malaria or other diseases. Patients showing recurrent *P. falciparum* infection on follow-up received rescue treatment as per national guidelines (artemether+ACT in China;

iv artemether+mefloquine in Cambodia; quinine sulfate+ doxycycline in Lao PDR, Thailand and Viet Nam; and artesunate+doxycycline in Myanmar).

During all follow-up visits, each patient was routinely asked about any prior or newly developed symptoms. When clinically indicated, patients were further evaluated and treated appropriately. All adverse events were clearly documented on a special case report form.

Laboratory procedures

Parasite enumeration. Giemsa-stained thick and thin blood films were examined at the magnification of 1,000x to identify the parasite species and to determine the parasite density (Parasite density = number of asexual parasites in 200 (1,000x) microscopic fields/number of white blood cells multiplied by an average white blood cell density of 8,000 per µl. When the number of asexual parasites was less than 10 per 200 white blood cells in follow-up smears, counting continued for at least 500 white blood cells). A blood slide was declared negative when no asexual parasites were detected against 1,000 white blood cells counted across the thick smear. The presence of gametocytes was recorded, but was not accounted for in the analysis. In the case of suspicious malaria species, the thin film was examined. Patients found to have mixed species infections were excluded.

Two qualified microscopists (Level 2 or above) read all slides independently and parasite densities were calculated using the average of the two counts. Blood smears with significant discordant results (difference between the two microscopists in species diagnosis and/or with >50% difference in parasite densities) were re-checked by a third, independent microscopist blinded to the initial readings, and parasite density calculated by getting the average of the two closest counts. As part of the quality control procedures for China, Cambodia, Thailand and Viet Nam, the third in-country (Level 1 or Level 2) microscopist independently re-read selected slides of their respective countries. Additionally, WHO certified Level 1 expert microscopist provided external cross-checking as the final validation of slides from China, Myanmar and Thailand.

Differentiating *P. falciparum* recrudescence from re-infection. In order to differentiate re-crudescence, which can be due to drug resistance, from a newly acquired infection, finger-prick blood samples collected as dry blood spots on filter paper were processed for *msp1*, *msp2* and *glurp* genotyping using standardized PCR techniques (WHO, 2008b). PCR was carried out in-country except for Lao PDR. Mahidol-Oxford Research Unit (MORU) in Bangkok, which serves as the regional reference lab validating country PCR results as needed, performed the genotyping of samples from Lao PDR (Mekong Molecular Surveillance Network). All results presented here are PCR-corrected, meaning that only recrudescent cases were accounted for in the ACPR calculation.

Chloroquine blood levels. To determine if the blood concentration of chloroquine was at an

adequate level in patients with vivax infection showing recurrence, 100 µl blood was collected by finger-prick on Days 0, 7, 28 and day of recurrence on filter paper. However, due to technical inconsistencies in blood sampling and storage, pharmacokinetic assay results of some countries were difficult to interpret (no results are included in this paper).

Classification of therapeutic outcomes and assessment of study results

Treatment outcomes were assessed on the basis of parasitological and clinical findings according to the latest WHO guidelines (WHO, 2009a) (Box 2). In brief, the patients were classified as having early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or an adequate clinical and parasitological response (ACPR). ACPR is the absence of parasitemia on Day 28 (or Day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

Patients initially enrolled for *P. falciparum* study who were later diagnosed with vivax malaria during the follow-up or on slide validation were not included in the (*P. falciparum*) ACPR analysis and vice versa for those initially enrolled as *P. vivax* cases. Patients who withdrew or were lost to follow-up anytime between D0 to D3 were excluded from the analysis.

In *P. falciparum*, the proportion of patients with PCR-confirmed recrudescence was used to estimate the therapeutic failure. Patients were further removed from the analysis if PCR results indicated that the failure was due to reinfection or if PCR results were inconclusive. Therefore, a PCR-corrected ACPR could be higher than the uncorrected ACPR.

Box 2

Classification of therapeutic outcomes

The study outcomes were classified on the basis of an assessment of the parasitological and clinical outcome of antimalarial treatment according to the latest WHO guidelines (WHO 2009a). All patients were classified as having early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or an adequate clinical and parasitological response (ACPR) based on the following determination:

- ETF: presence of danger signs or severe malaria on Days 1, 2 or 3 with evidence of parasitemia; parasitemia on Day 2 higher than on D0, irrespective of axillary temperature; parasitemia on D3 with axillary temperature ≥37.5ºC; and parasitemia on D3 ≥25% of count on D0.
- LCF: danger signs or severe malaria in the presence of parasitemia on any day between Day 4 and Day 28 (or till Day 42) in patients who did not previously meet any of the criteria of early treatment failure; presence of parasitemia on any day between Day 4 and Day 28 (Day 42) with axillary temperature ≥37.5ºC (or history of fever) in

patients who did not previously meet any of the criteria of early treatment failure.

- LPF: presence of parasitemia on any day between Day 7 and Day 28 (Day 42) with axillary temperature <37.5ºC in patients who did not previously meet any of the criteria of early treatment failure or late clinical failure.
- ACPR: absence of parasitemia on Day 28 (or Day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria of early treatment failure, late clinical failure or late parasitological failure.

Parasite clearance in ACT-treated *P. falciparum* patients

In the absence of molecular markers for artemisinin resistance or any established *in vitro* artemisinin resistance threshold, the monitoring of ACT efficacy should be supplemented with parasite clearance as measured by "Day 3 parasitemia." Day 3 parasitemia is defined as the percentage of *P. falciparum* patients who remain blood smear positive for asexual blood stage parasites on Day 3 (ideally 72 hours) following the initial dose of ACTs (WHO, 2012c). Day 3 parasitemia is the best available parasitological marker of artemisinin sensitivity although its interpretation is complex and influenced by multiple factors, some unknown. Based on the WHO's 'working definition' of artemisinin resistance, ≥ 10% Day 3 parasitemia is considered an early warning signal of 'suspected' artemisinin resistance at a given location. That information can be collected practically along with TES conducted in routine monitoring and is also of use to facilitate classification of tiers to plan artemisinin resistance containment operations (WHO, 2011b).

Data management and analysis

After study completion, data were independently double-entered by the individual country team into Microsoft Excel spread-sheets according to standard procedures and stored under confidential control of the country's Principal Investigator. Data were analyzed by two methods: the Kaplan-Meier intent-to-treat method and per-protocol method analysis (WHO, 2009b).

The primary outcomes representing therapeutic efficacy endpoints were the 28-day (for AL) or preferably 42-day (for A+M and DHA-PIP) PCR-corrected cure rates of each study using Kaplan Meir-survival analysis. Secondary outcomes were the percentages of *P. falciparum* patients whose Day 3 blood smears remained positive for asexual falciparum parasites. As a supplement, the positive and negative predictive values for treatment failure of Day 3 parasitemia, in a few selected sites, are also calculated.

Data analysis was conducted using Intercooled Stata 8.0 software (Stata Corporation, College Station, TX, SN: 198049310). Therapeutic outcome estimates as represented by

Table 1

Patient characteristics by malaria species and country.

Country	Cambodia *n*=540	China *n*=127	Lao PDR *n*=62	Myanmar *n*=1,099	Thailand *n*=698	Viet Nam *n*=306
P. falciparum						
Males (%)	361 (66.9)	81 (63.8)	48 (77.4)	798 (72.6)	493 (70.6)	211 (69.0)
Adults: >15 yrs (%)	355 (65.7)	100 (78.8)	35 (56.5)	860 (78.3)	629 (90.1)	208 (68.0)
Day 0 fever ≥37.5 C (%)	501 (92.8)	115 (90.6)	60 (96.8)	767 (69.8)	633 (90.7)	292 (95.4)
Day 0 parasite count (μl^{-1})						
Geometric mean	15,328	5,060	6,595	8,267	14,464	11,892
(range)	(506-233,379)	(80-127,600)	(317-136,597)	(100-124,030)	(119-243,750)	(643-187,686)
	n=388	*n*=76		*n*=552	*n*=220	*n*=111
P. vivax						
Males (%)	279 (71.9)	50 (65.8)		411 (74.4)	158 (71.8)	62 (55.9)
Adults (%)	257 (66.2)	56 (73.7)		413 (74.8)	176 (80.0)	49 (44.1)
Day 0 fever ≥37.5 C (%)	367 (94.6)	46 (60.5)		410 (74.3)	189 (85.9)	107 (96.4)
Day 0 parasite count (μl^{-1})						
Geometric mean	4,149	4,381		4,319	4,341	4,313
(range)	(259-73,214)	(225-46,227)		(261-166,471)	(120-49,672)	(117-66,852)

ACPRs, prevalence of Day 3 parasitemia (%) and predictive values (positive and negative) of Day 3 parasitemia are reported with 95% confidence intervals (binomial exact). Differences of ACPRs and % Day 3 parasitemia across years were compared using Pearson chi-square test.

RESULTS

Patient characteristics (Table 1)

The majority of the patients were male (>70%) and adults 16-70 years old. Lao PDR had the largest proportion of children ≤15 years old (44%).

Day 0 geometric mean parasite densities for *P. falciparum* ranged from 5,060 in China to 15,328/µl blood in Cambodia, and for *P. vivax* from 4,149 to 4,381/µl in Cambodia and China, respectively. Most patients were febrile (axillary temperature ≥37.5ºC) at the time of enrolment (>80% in both the *P. falciparum* and *P. vivax* groups).

In Lao PDR in 2009, there were too few patients with *P. falciparum* but more patients matching entry criteria were enrolled in 2010 and data collection continued for almost a year into 2011 in Khammoune and Luang Prabang. The sample size, however, remained small despite active case finding effort in surrounding villages, which were experiencing very low transmission. In China, sentinel sites were located close to the Myanmar border and at two of the three sites

Table 2

PCR-corrected therapeutic efficacy results for *P. falciparum* by drug, country/site and year of study, 2008-2010.

Drug/Country	Site	Year								
		2008			2009			2010		
		n (Total analysed)	28-day ACPR (95% CI)	42-day ACPR (95% CI)	*n* (Total analysed)	28-day ACPR (95% CI)	42-day ACPR (95% CI)	*n* (Total analysed)	28-day ACPR (95% CI)	42-day ACPR (95% CI)
DHA-PIP										
Cambodia	Pailin	49	98% (89.1-99.9)	100% (91.6-100)	38	92% (78.6-98.3)	92% (78.1-98.3)	29	76% (56.5-89.7)	73% (52.8-87.3)
	Pursat	76	100% (95.2-100)	100% (95.3-100)				54	92.6% (82.1-97 9)	88.7% (77-95.7)
	Preah Vihear				59	100% (93.9-100)	100% (93.9-100)			
	Rattanakiri				54	100% (93.5-100)	100% (93.4-100)	60	100% (94-100)	100% (93.9-100)
China	Tenchong							19	100% (83.2-100)	
	Yingjiang							22	95.5% (77.2-99.9)	
	Menglian				71	100% (94.9-100)				
Myanmar	Kawthaung				80	97.4% (90.8-99.7)		57	94.7% (85.4-98.9)	
	Rakhine							80	100% (95.5-100)	
	Kachin							57	98.2% (90.6-99.9)	
	Eastern Shan							51	100% (93-100)	
	Bago				72	100% (95-100)				
	Mon							75	98.7% (92.8-99.9)	
Viet Nam	Binh Phuoc				46	97.8% (90.9-99.9)				
	Dak Nong				37	100% (90.5-100)				
	Gia Lai		48	100% (93.9-100)				60	95% (87.1-99)	94.8% (85.6-98.9)
	Quang Tri		66	100% (94.6-100)				15	100% (78.2-100)	100% (78.2-100)
A+M										
Cambodia	Preah Vihear				46	100% (92.3-100)	97.9% (88.5-99.9)			
Thailand	Mae Hong Son	38	100% (90.7-100)	100% (90.7-100)	27	96.40%	92.3% (74.9-99.1)			
	Tak	62	98.4%	96.8% (88.8-99.6)	53	89.1%	90.4% (78.9-96.8)	39	92.5%	92.3% (79.1-98.4)

Table 2 (Continued).

Drug/Country	Site	Year								
		2008			2009			2010		
		n (Total analysed)	28-day ACPR (95% CI)	42-day ACPR (95% CI)	*n* (Total analysed)	28-day ACPR (95% CI)	42-day ACPR (95% CI)	*n* (Total analysed)	28-day ACPR (95% CI)	42-day ACPR (95% CI)
	Kanchanaburi				53	94.6%	92.5% (81.8-97.9)			
	Ratchaburi	50	100% (92.9-100)	100% (92.9-100)				44	100% (91.9-100)	100% (91.9-100)
	Ranong	35	100%	97.15 (85.1-99.9)	47	90%	87.2% (74.3-95.2)	47	93.6%	91.5% (79.6-97.6)
	Yala	47	100% (92.5-100)	100% (92.5-100)				52	98.1%	98.1% (89.7-99.9)
	Ubon Ratchathani	45	(100% 92.1-100)	(100% 92.1-100)						
AL										
Cambodia	Rattanakiri							62	94.9% (85.9-98.9)	88.7% (78.9-95.3)
Lao PDR	Khammoune				24	95.8% (78.9-99.9)		26	100% (86.3-100)	
	Luang Prabang							12	91.7% (61.5-99.8)	
Myanmar	Kawthaung				80	93.8% (86-97.9)		84	94% (86.7-98)	
	Rakhine							70	100% (94.6-100)	
	Kachin							59	100% (93.9-100)	
	Sagaing				73	98.6% (92.6-99.9)				
	Eastern Shan							50	100% (92.7-100)	
	Bago				86	98.8% (93.7-99.9)				
	Kayin							66	97% (89.2-99.6)	

almost all patients contracted malaria in Myanmar. China also experienced difficulty in reaching the requested sample size especially in 2009 and 2010.

Therapeutic efficacy of ACTs against *P. falciparum* (Table 2)

Efficacy of DHA-PIP (Fig 3a). Over the 3-year study period, DHA-PIP remained highly efficacious (42-day ACPR>98%) against *P. falciparum* in Rattanakiri and Preah Vihear Provinces of Cambodia (Rithea *et al*, 2013). However, in Pailin, the 42-day ACPR decreased significantly (p=0.001) from 100% (95%CI 91.6-100, n=49) in 2008, to 92% (95% CI 78.1-98.3, n=38) in

2009, and 73% (95% CI 52.8-87.3, *n*=29) in 2010, although the 2010 sample size was rather small. In Pursat, just southeast of Pailin, 42-day ACPR also significantly declined from 100% (95% CI 95.3-100, *n*=76) in 2008 to 89% (95%CI 77.0-95.7, *n*=54) in 2010 (*p*=0.003).

In Myanmar, TES were carried out at six sites across the countries during 2009-2010. All showed 28-day ACPRs to be consistently >96% indicating that the drug remained highly efficacious. In 2010, results of small samples (<30 at each site) from China showed >95% ACPR in Tengchong and Yingjiang. Similarly, in Viet Nam, the 28-day ACPRs for all 4 sites were 100% or approaching 100%.

Two ETFs were recorded in Gia Lai, Viet Nam in 2010. All other cases who failed DHA-PIP were LPF or LCF.

Efficacy of A+M (Fig 3b). A+M therapeutic efficacies are available for Cambodia (1 site) and Thailand (7 sites) and all are based on 42-day follow-up.

The ACPR was 100% in Preah Vihear (north-central Cambodia) in 2009. In Thailand, the 2008 ACPR at each of the six sentinel sites was >96%. The ACPRs remained consistently >98% in Ratchaburi and Yala through 2010. However, there was a consistent decline for 2008 compared to 2010 at 3 of the 4 sites along the Thai-Myanmar border, *ie* Mae Hong Son: 100% to 92% (95% CI 75.0-99.1, *n*=27), Tak: 97% (95% CI 88.8-100, *n*=62) to 92% (95% CI 79.1-98.4, *n*=39), and Ranong: 97% (95% CI 85.1-99.9, *n*=35) to 87.2% (95% CI 74.3-95.2, *n*=47). Overall, the 2010 ACPR for these three border sites was slightly above 90% (pooled data). The other site along the same border, Kanchanaburi, only had a single data point with an ACPR of 92.5% (95% CI 81.8-97.9, *n*=53) in 2009. Preliminary 2011 data indicated a further drop at this site to <90% ACPR (WHO, 2012 b).

Efficacy of AL (Fig 3c). The 28-day ACPR was 95% (95% CI 85.9-98.9) for Rattanakiri (north eastern Cambodia) in 2010.

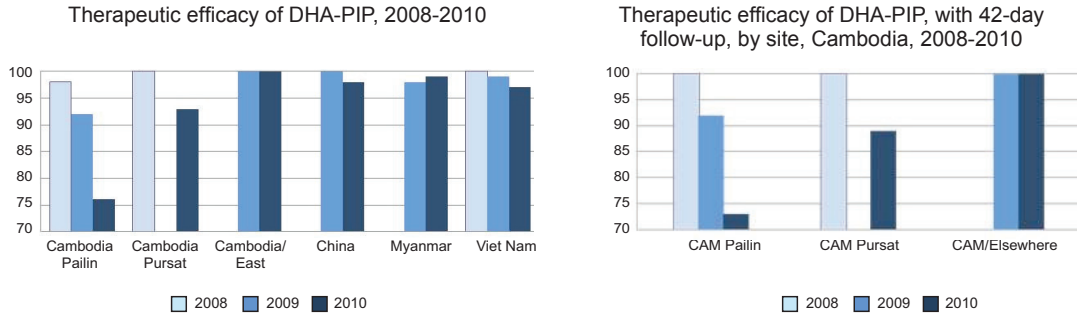
In Myanmar, seven TES for AL (28-day follow-up) were conducted during 2009-2010. ACPRs at two sites in 2009 and four sites in 2010 ranged from 94% to 100%. Lower ACPRs were noted for Kawthuang bordering the southwestern border of Thailand in both years; it was 94% (95% CI 86.0-97.9) in 2009 and 94% (95% CI 86.7-98) in 2010. One case in Bago developed ETF in 2009.

In Lao PDR, low malaria endemicity delayed study completion. Enrolment had to be extended and results included data from the 2011 malaria season. Based on small sample sizes (Ns<30 per site per year), the 28-day ACPRs were 92% (95% CI 61.5-99.8, *n*=12) in Luang Prabang in 2011 and 98% (95% CI 81.9-100, *n*=49) in Khammoune in 2010 and 2011.

Parasite clearance as indicated by Day 3-positive parasitemia (%) (Table 3)

For DHA-PIP, the most striking finding came from Pailin, western Cambodia, in the area

a



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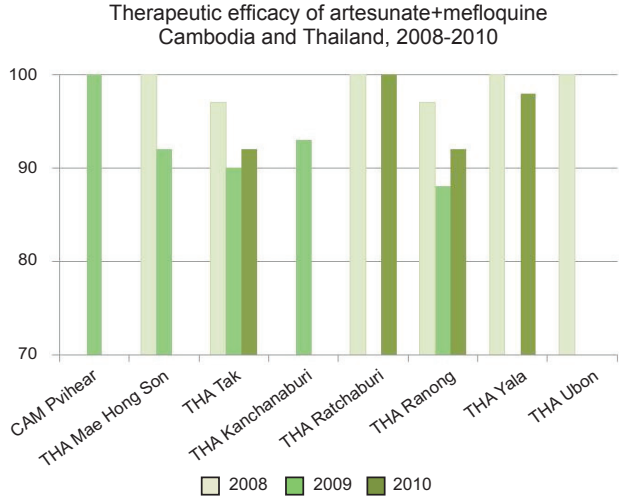

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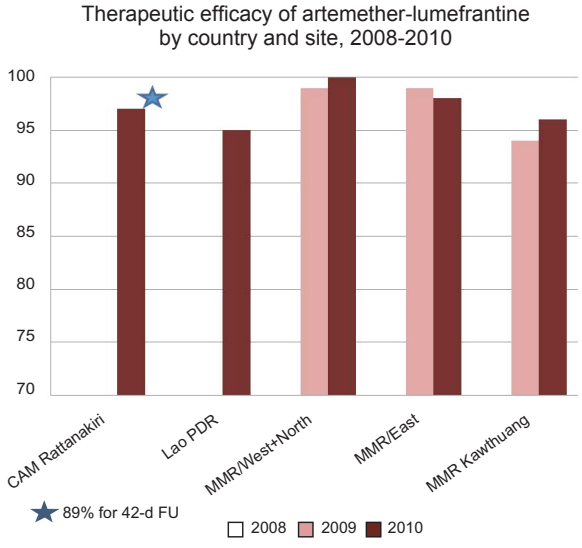

Fig 3–a-c Bar graphs depicting the therapeutic efficacy of DHA-PIP, A+M and AL by sentinel site and/or country, 2008-2010. (28-day follow-up, otherwise indicated).

where artemisinin resistance emerged. There, Day 3 parasitemia rose from 25.5% (95%CI 15.6-41.0, *n*=53) in 2008 to 45% (95% CI 26.4-64.3, *n*=29) in 2010 (Fig 4a). In Myanmar, Day 3 parasitemia began to increase to about 20% at two sites near the Thai border (Kawthaung and Mon State) during 2009-2010. Also at two sites in Viet Nam and one in China, the proportions of Day 3 parasitemia went up slightly above 10% during 2009-2010. In Viet Nam, one-year data from Binh Phuoc (southern Viet Nam) in 2009 showed 15% (95% CI 7.2-26.9, *n*=46). In Gia Lai (also south-central Viet Nam) there was a significant increase from 0% (95% CI 0-6.1, *n*=48) in 2008 to 11% (95% CI 4.6-21.6, *n*= 60) in 2010 (*p*=0.008).

In Thailand, an increase in the percentage of Day 3 parasitemia associated with A+M was observed along the Myanmar border (Fig 4b). In 2008 it was 0% in both Tak and Ranong Provinces, but went up significantly to 9.1% (95% CI 2.5-21.7, *n*=39, *p*=0.009) and 5.4% (95% CI 1.1-14.9, *n*=47, *p*=0.005), respectively, in 2010. A single data point from Kanchanaburi, also located on the Myanmar border south of Tak and north of Ranong, showed 25% (95% CI 13.8-38.3, *n*=53) in 2009. In western Cambodia, Day 3 parasitemia >10% associated with A+M therapy had been documented since before 2008. The 2009 data from Preah Vihear (north-central Cambodia) showed 0% Day 3 parasitemia indicating that such a delayed parasite clearance may so far be confined to the western part of the country.

At the sites where AL data were available, Day 3 parasitemia was much less common (Fig 4c). In Lao PDR, all patients cleared parasites by Day 3. In Myanmar, the Day 3 parasitemia for all sites were <10% and in Rattanakiri (northeastern Cambodia), it was 3.6% in 2010. However, it should be noted that in western Cambodia, where AL was not tested during 2008-2010, Day 3 parasitemia has exceeded 10% in studies done between 2002 to 2004 (Denis *et al*, 2006b).

Inclusion of patients with initially high-density parasitemia (above 150,000/µl blood) (Table 4)

Inclusion of patients with Day 0 parasitemia above 100,000/µl was allowed in the TES conducted in the Mekong countries. As malaria incidence dropped further, more cases with high parasitemia were enrolled. The effects of including patients with Day 0 parasitemia >150,000/µl up to 250,000/µl were explored. The inclusion only minimally changed the ACPR estimates and the differences in % Day 3 positive parasitemia were negligible. Table 4 shows comparison of the results with and without the inclusion for selected sites in Thailand and Cambodia, countries that were most affected.

Efficacy of chloroquine and DHA-PIP against *P. vivax* (Fig 5)

In Cambodia, while chloroquine showed >95% ACPR for *P. vivax* in the western border, ACPRs were documented at less than 90% in 2008-2009 in the northeastern province of Rattanakiri (83%, 95% CI 68.6-92.2) and Preah Vihear (88%, 95% CI 77.4-95.2) (Rithea *et al*, 2013).

Table 3a

Day 3 parasitemia in DHA-PIP treated *P. falciparum* cases per site/country and year of study.

	DHA-PIP										
Country	CAMBODIA								CHINA		
Site	Pailin			Veal Veng, Pursat		Rattanakiri		Preah Vihear	Menglian	Yingjiang	Tengchong
Year	2008	2009	2010	2008	2010	2009	2010	2009	2009	2010	2010
Total analysis: 42day	49	38	29	76	54	54	60	59	71	22	19
Day 3 % parasitemia	25.5%	33.0%	45.0%	7.8%	7.4%	0	0	5.1%	0	13.6%	5.3%
(95% CI)	(15.6-41)	(19.1-50.2)	(26.4-64.3)	(2.8-15.6)	(2.1-17.9)	(0.0.-6.6)	(0.0-5.9)	(1.1-14.1)	(0.0-5.1)	(2.9-34.9)	(0.1-26)
p-value		0.261			0.984		NA	-	-	-	-

Site	Kawthaung		Bago	Rakhine	Mon	Kachin	East Shan	Quang Tri		Gia Lai		Binh Phuoc	Dak Nong
Year	2009	2011	2009	2010				2008	2010	2008	2010	2009	2009
Total analysis: 42day	79	57	72	80	75	57	51	66	15	48	60	46	37
Day 3 % parasitemia	19.0%	22.8%	4.2%	0	22.5%	3.5%	2.0%	0	0	0	11.3%	15.2%	0
(95% CI)	(11-29)	(12.7-35.8)	(0.9-11.7)	(0.0-4.5)	(13.9-33)	(0.4-12.1)	(0.1-10.4)	(0.0-5.4)	(0-21.8)	(0-6.1)	(4.6-21.6)	(7.2-26.9)	(0.0-9.5)
p-value	0.587		-	-	-	-	-	NA		0.008		-	-

Table 3b

Day 3 parasitemia in Artemether-Lumefantrine (AL) treated *P. falciparum* cases per site/country and year of study.

AL													
Country	MYANMAR								LAO PDR			CAMBODIA
Year	2009	2010	2009		2010				2009	2010	2011	2010
Site	Kawthaung		Bago	Sagaing	Kayin	Rakhine	Kachin	East Shan	Khammoune	Kahmmoune	Luang Prabang	Rattanakiri
Total analysis: 28day	80	84	86	73	66	70	59	50	24	26	12	56
Day 3 % parasitemia	6.3%	8.3%	10.2%	0	4.5%	0	1.7%	2%	0	0	0	3.2%
(95% CI)	(2-13.9)	(3.4-16.4)	(4.8-18.5)	(0.0-4.7)	(0.9-12.7)	(0.0-5.1)	(0.0-8.9)	(0.1-10.6)	(0.0-14.20	(0.0-13.7)	(0.0-26.5)	(0.4-12.3)
p-value	0.609		-	-	-	-	-	-	NA		-	-

Table 3c

Day 3 parasitemia in A+M treated *P. falciparum* cases per site/country and year of study.

A+M															
Country	THAILAND														CAMBODIA
Site	Tak			Ranong			Mae Hong Son		Yala		Ratchaburi		Ubon Rat-chathani	Kancha-naburi	Preah Vihear
Year	2008	2009	2010	2008	2009	2010	2008	2009	2008	2010	2008	2010	2008	2009	2009
Total analysis: 42day	62	53	39	35	47	47	38	27	47	52	50	44	45	53	46
Day 3 % parasitemia	0	14.3%	9.1%	0	17%	5.4%	4.8%	9.1%	0	0	0	4.5%	0	24.5%	0
(95% CI)	(0.0-5.5)	(6.4-26.2)	(2.5-21.7)	(0-7.5)	(7.6-30.8)	(1.1-14.9)	(0.6-16.2)	(1.9-24.3)	(0.0-7.5)	(0.0-6.8)	(0.0-7.1)	(0.6-16.2)	(0.0-7.9)	(13.8-38.3)	(0.0-7.3)
p-value	0.009			0.005			0.521		NA		0.128		-	-	-

Table 4

Comparison of Day 3 and ACPR outcome in relation to initial parasitemia in selected sites.

Excluding patients with Day 0 parasitemia >150,000												
Country (Drug)	Cambodia (DHA-PIP)					Thailand (A+M)						
Site	Veal Veng, Pursat		Rattanakiri		Preah Vihear	Ranong			Ratchaburi		Ubon Ratchathani	Kanchanaburi
Year	2008	2010	2009	2010	2009	2008	2009	2010	2008	2010	2008	2009
Total analysis	75	48	52	58	57	32	47	47	49	44	45	53
Day 3 % parasitemia	5 (6.4%)	2 (2.4%)	0	0	3 (5%)	0	8 (17%)	3 (5.4%)	0	2 (4.5%)	0	13 (24.5%)
(95% CI)	(2.2-14.9)	(0.5-14.3)	(0.0-6.8)	(0.0-6.2)	(1.0-13.9)	(0-10.9)	(7.6-30.8)	(1.1-14.9)	(0.0-7.3)	(0.6-15.5)	(0.0-7.9)	(12.9-36.4)
p-value	0.704		NA		-		0.024		0.221		-	-
PCR-corrected ACPR	100%	88%	100%	100%	100%	96.9%	87.2%	91.5%	100%	100%	100%	92.3%
(95% CI)	(95-100)	(74.8-95.3)	(93.2-100)	(93.8-100)	(93.9-100)	(85.1-99.9)	(74.3-95.2)	(79.6-97.6)	(92.7-100)	(91.9-100)	(92.1-100)	(81.5-98)
p-value	0.003		NA		-		0.329		NA		-	-
Excluding patients with Day 0 parasitemia >250,000/cumm												
Country (Drug)	Cambodia (DHA-PIP)					Thailand (A+M)						
Site	Veal Veng, Pursat		Rattanakiri		Preah Vihear	Ranong			Ratchaburi		Ubon Ratchathani	Kanchanaburi
Year	2008	2010	2009	2010	2009	2008	2009	2010	2008	2010	2008	2009
Total analysis	76	54	54	60	59	35	47	47	50	44	45	53
Day 3 % parasitemia	6 (7.8%)	4 (7.4%)	0	0	3 (5%)	0	8 (17%)	3 (5.4%)	0	2 (4.5%)	0	13 (24.5%)
(95% CI)	(2.8-15.6)	(2.1-17.9)	(0.0-6.6)	(0.0-5.9)	(1.1-14.1)	(0-7.5)	(7.6-30.8)	(1.1-14.9)	(0.0-7.1)	(0.6-16.2)	(0.0-7.9)	(13.8-38.3)
p-value	0.989		NA		-		0.005		0.128		-	-
PCR-corrected ACPR	100%	88.7%	100%	100%	100%	97.1%	87.2%	91.5%	100%	100%	100%	92.5%
(95% CI)	(95.3-100)	(77-95.7)	(93.4-100)	(93.9-100)	(93.9-100)	(85.1-99.9)	(74.3-95.2)	(79.6-97.6)	(92.9-100)	(91.9-100)	(92.1-100)	(81.8-97.9)
p-value	0.003		NA		-		0.283		NA		-	-

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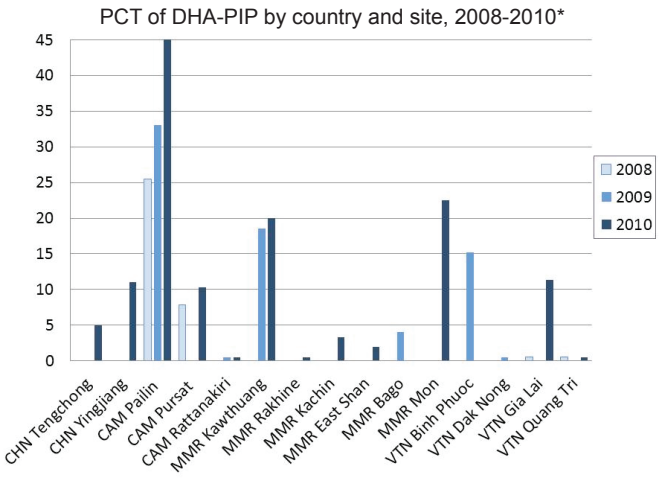

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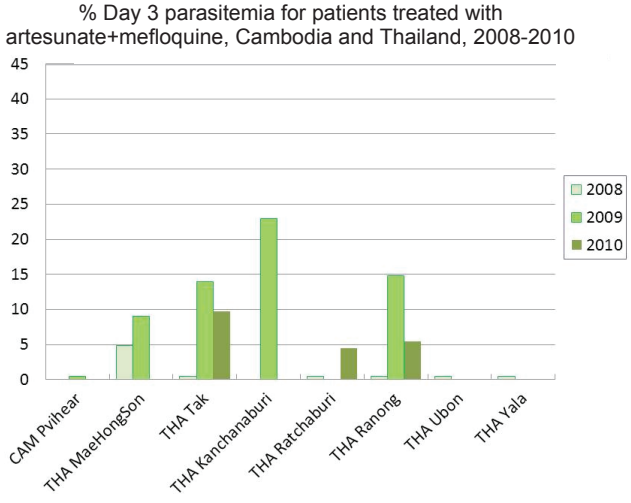

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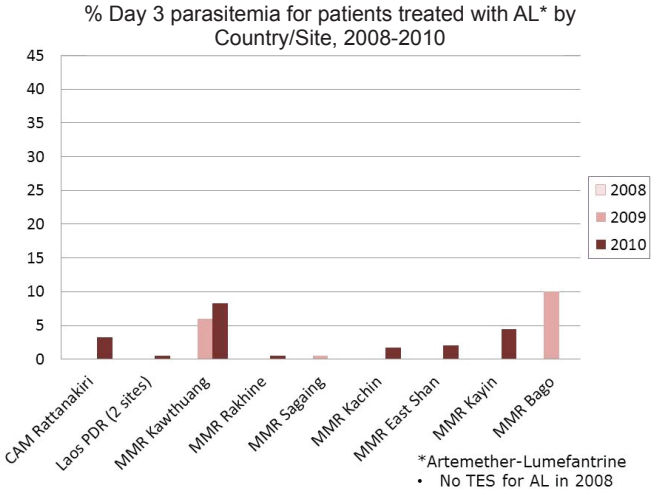

Fig 4–a-c Bar graphs depicting Day 3 parasitemia in TES patients treated with DHA-PIP, A+M and AL by sentinel site and/or country, 2008-2010.

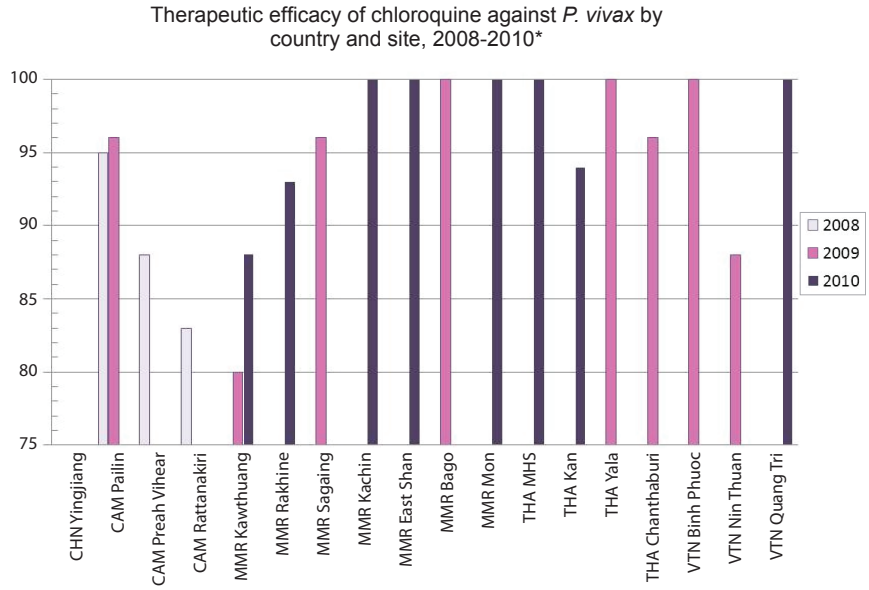

Fig 5–ACPR of chloroquine against *P. vivax* by country/site, 2008-2010.

The therapeutic efficacy of chloroquine in Myanmar remained above 90% at all sites (Sagaing Division, Rakhine, Mon, Kachin and Eastern Shan states) except Kawthaung (Thanintharyi Region) in the south bordering Thailand, where the Day 28 ACPR was 80% (95% CI 68.2-89.4) in 2009 and 88% (95% CI 77.8-94.7) in 2010.

In Thailand, chloroquine was assessed against *P. vivax* in Chanthaburi and Yala in 2009 and in Kanchanaburi and Mae Hong Son in 2010. The therapeutic efficacy remained above 90% at all of the four sites. All treatment failures were LPF and the study participants were asymptomatic. Comparing results from the 4 sites, patients from Chanthaburi showed the highest level of parasitemia on admission and the slowest parasite clearance, but there was no clear evidence of *P. vivax* resistance to chloroquine from this site (Congpuong *et al*, 2011), or any other site in Thailand.

In Viet Nam, chloroquine demonstrated generally high efficacy against vivax malaria. An ACPR of 100% was documented in Binh Phuoc in 2009 and in Quang Tri in 2010. However, in Ninh Thuan it was 88% (95% CI 77.2-94.5) in 2009. Data was available from only one site in China; the ACPR was 96% (95%CI 86.0-100, *n*=45) in Yingjiang in 2009. Due to low incidence of *P. vivax,* TES were not conducted in Lao PDR during 2008-2010.

DISCUSSION

In 2001, the WHO recommended ACTs as first-line drugs (WHO, 2001), and in 2006, urged

member states to "*cease the provision in both the public and private sectors of oral artemisinin monotherapy*" (WHO, 2010b). Compliance with these recommendations by the National Malaria Control Programs (NMCPs) in the six countries of the GMS has evolved slowly (Chapter 7).

As the result of documented high-level falciparum resistance to mefloquine in the border areas, the Thai NMCP decided to implement a two-day artesunate-mefloquine combination (A+M) regimen in 1995 in selected locations only (Wongsrichanalai *et al,* 2004) which in retrospect might have contributed to the increasing tolerance of falciparum to artemisinins (WHO, 2007b). The country adopted the two-day A+M regimen nationwide in 2005 and then modified the regimen to three-day in accordance with WHO recommendations in 2008 (Chapter 7).

In Cambodia, A+M has been used to manage falciparum infections since 2000, but the national program decided to shift to dihydroartemisinin-piperaquine (DHA-PIP) in selected western provinces in 2009 as the result of increasing failure rate of A+M on the border with Thailand. From 2010, DHA-PIP has been made the first-line therapy for the management both *P. falciparum* and *P. vivax* infections following evidence of increased A+M treatment failures in western border provinces and chloroquine treatment failures *in vivax* infections in the eastern part of Cambodia (Chapter 7). However, the actual implementation of the policy took place in 2012.

In Myanmar the initial policy to use A+M as the first line drug against *P. falciparum* was set in 2002, then DHA-PIP and AL were added in 2005, thus the country operated on multiple first-line antimalarial drugs depending on funding sources (Chapter 7).

After more than two decades of successful countrywide implementation of a 5 to 7-day artemisinin monotherapy regimen, national programs in Viet Nam and China decided to shift to an ACT, DHA-PIP in 2009, to manage uncomplicated falciparum infections (Thanh *et al*, 2010; Cawthorne and Schapira, 2010; Cawthorne *et al*, 2010). Viet Nam also used CV8 (a fixed combination tablet composed of DHA, PIP, trimethoprim and primaquine) nationwide up to the commune level from 2003 to 2008 (WHO, 2005a). In China, three other ACTs are currently part of the first-line drug policy namely: artesunate-amodiaquine in blister pack, co-formulated single dose naphthoquine-artemisinin and artemisinin-piperaquine (also co-formulated) mainly for trade and export purposes, but actually using only DHA-PIP against falciparum infections in the country (Chapter 7).

In 2001, the national program in Lao PDR decided to shift from chloroquine + sulfadoxine-pyrimethamine to artemether-lumefantrine (AL) to manage both falciparum and non-falciparum infections. Countrywide implementation of that policy started only in 2005 with Global Fund to Fight AIDS, TB, and Malaria (GFATM) support. Monitoring the therapeutic efficacy of AL started in 2003 at four sentinel sites across the country. To date, as per *in vivo* TES network results, which have been confirmed by similar studies conducted in Lao PDR, AL remains highly effica-

cious countrywide (Mayxay *et al*, 2012b).

With the scaling up use of ACTs as first line antimalarial drug in the region, the importance of carefully and systematically tracking the therapeutic efficacy of ACTs in each country and in the GMS has been taken seriously by programme managers with the technical support of WHO and development partners (WHO, 2007b). The Mekong *in vivo* therapeutic efficacy study Network jointly managed by NMCPs and WHO has contributed to provide policy makers with early warning signals of decreasing efficacy of ACTs across the GMS. This was acknowledged during a strategic technical consultation in Cambodia in 2007 (WHO, 2007a) which was the basis to further strengthen the capacity of the TES Network engaging country principal investigators to operate common good clinical and laboratory practices (WHO, 2010b; WHO, 2012b).

All GMS countries have gradually changed their first line national drug policies to follow WHO recommendations and are actually promoting free or at-cost ACTs from government-run health stations with progressive involvement of private providers. The scaled-up implementation of new and more expensive drug policies in remote places and as part of development projects attracting migrants remains a serious challenge which is discussed in Chapter 4 & 7. It includes the actual implementation of the ban of artemisinin monotherapies and various unknown cocktail regimens and sub-standard medicines still widely available in some locations (Newton *et al*, 2008; Nayar *et al*, 2012).

Prior to 2008, ACTs have remained highly efficacious in the GMS with the notable exception of western Cambodia and southeastern Thailand, where A+M efficacy (42-day follow-up) significantly dropped below 90% (Denis *et al*, 2006; Vijaykadga *et al*, 2006). Emerging falciparum resistance to selected ACTs on the Cambodia-Thailand border became a source of regional and global concern for policy makers and researchers triggering additional research and development partners' interest (WHO, 2007a).

Interpretation of results from *in vivo* TES of combination artemisinin derivatives with different partner drugs, with different but synergistic modes of action is not as straightforward as it was with other previous antimalarials used in malaria control (WHO, 2007a). The rising A+M therapeutic failure rate observed in selected locations in Thailand could be the consequence of the pre-existing mefloquine resistance in the country (Wongsrichanalai *et al,* 2004). Drug pressure on mefloquine has been considerable over the last decades especially in Thailand where mefloquine has been used as monotherapy or with sulfadoxine-pyrimethamine from 1986 at different regimens in different locations (15 or 25 mg/kg in one or 2-day regimens) and later in combination with artesunate (loose tablets) in selected locations and then countrywide from 2005 (Vijaykadga *et al*, 2006). Thus the progressive decline in A+M therapeutic efficacy is a major concern not only for Thailand, but in the whole Subregion and globally since falciparum response to artemisinins (which have been under pressure) is seriously declining in selected

locations (Wongsrichanalai and Meshnick, 2008; Rogers *et al*, 2009; WHO, 2010b). As a result, the Thai NMCP continues to monitor A+M and is actively searching for suitable safe alternatives to A+M in collaboration with national and international academic and research institutions.

Treatment failures observed from 2008 to 2010 with DHA-PIP in Pailin and Pursat have quickly reached unacceptable levels. This is in the same area of western Cambodia and eastern Thailand that resistance of *P. falciparum* to other antimalarials has previously emerged (Singhasivanon *et al*, 1999). The recent results are of serious concern and could be related to emerging falciparum resistance to piperaquine, a partner drug which shares chemical structure similarities with chloroquine and had been used as monotherapy previously (Yang *et al*, 1997; Davis *et al*, 2005). The long history of mefloquine and piperaquine being used as monotherapies in the region is well known (Yang *et al*, 2007) as well as the unregulated availability and use of DHA-PIP in some locations in Cambodia and the northern part of Myanmar bordering China for the past decades (Cawthorne and Schapira, 2010). That context raises concern on the expected long-term effectiveness of DHA-PIP as a first line drug in the GMS.

Determination of parasite clearance time as measured by percent Day 3 parasitemia (ideally 72-hours after initiation of treatment) is so far considered as a surrogate marker to identify suspected hotspots of *P. falciparum* resistance to artemisinins (Stepniewska *et al*, 2010). This data can be captured in sentinel sites from quality TES managed by national programs. Unfortunately, the number of sentinel sites per country is limited due to the number of skilled staff, funds, and mandatory protocol requirements necessary to implement TES standards. Following field experiences from NGOs in Cambodia, one suggested option to intensify artemisinin resistance monitoring was to limit individual follow-up of each patient only until Day 3 in additional sites (WHO, 2011a). However, even if considered far less complicated than the full TES study design, these “Day 3 simplified studies” still require a highly demanding protocol in terms of entry criteria, adherence to treatment, quality microscopy, quality drugs, accurate follow-up, adequate data management, and mandatory ethical prerequisites. To avoid multiplication of false alerts and further investigations to cross check results, WHO recommended that such studies should be conducted by research institutions in collaboration with field partners (WHO, 2012c).

To date there is no established relationship between prolonged parasite clearance and ACT therapeutic failures in *P. falciparum.* Percent Day 3 parasitemia has a low positive predictive value for 28-day (and likely so for 42-day) treatment failures with A+M in Thailand; however, the negative predictive value is consistently high, meaning that patients who cleared parasites before Day 3 are likely to be fully cured (Vijaykadga *et al*, 2012). Results from selected sites in recent TES confirm these conclusions. A negative predictive value of 99.4% and 92.3% in Tak and Pursat, respectively, but only 72% in Pailin, as may be expected from other resistance data, have been found.

Between 2001 and 2007, the proportion of patients with parasites on Day 3 associated with either AL or A+M has been progressively exceeding 10% in the western part of Cambodia, including the provinces of Pailin, Battambang, and Kampot. Across the border in Trat and Chanthaburi Provinces of Thailand, the proportion of slow-clearing patients under A+M therapy has increased in 2001 to exceed 10% in 2010. From TES, the parasite clearance time (PCT) reached 3.7 days in 2007 in Trat Province as compared to 2.0 days in 2003 (Vijaykadga, BVBD, 2006, personal communications). These findings, in addition to *in vivo* and *in vitro* studies conducted across the region, have mobilized the international community and highlighted the urgent need to better understand the situation, build additional evidence, and address the emerging artemisinin resistant threat first on the Cambodia-Thailand border and further in the region and globally (WHO, 2007a; 2008a; 2011b; Chapter 7)

The emerging foci of delayed parasite clearance associated with DHA-PIP in eastern Myanmar deserve attention and action. Almost 20% of patients treated with DHA-PIP remained positive on Day 3 in Kawthaung (Tanintharyi region bordering the province of Ranong in Thailand) and in Mon State. These observations from the TES led to a 7-day artesunate monotherapy study with additional pharmacokinetic investigations in 2011. Results demonstrated a high percentage of patients remaining parasite positive on Day 3 (27%, 95% CI 15.6-41) but no single patient with LTF was detected during the 28-day follow-up. Pharmacokinetic results showed an appropriate blood level of the drug and could not explain the delayed parasite clearance (WHO, 2012b). Thus the area remains classified as a 'suspected foci' for artemisinin resistance (Fig 2) as per WHO classification (WHO, 2011b).

The proportion of DHA-PIP treated patients remaining with parasites on Day 3 in some locations in Viet Nam also raises concerns that these area are becoming a suspected hotspot of reduced artemisinin sensitivity either *de novo* or extended from the Cambodia-Thailand border. Recent studies in Viet Nam showed that DHA-PIP remained highly efficacious from a therapeutic viewpoint but demonstrated longer parasite clearance times (Hien *et al*, 2012).

The proportion of patients treated with AL who remained positive on Day 3 was below 10% at all locations with the exception of Bago Region, Myanmar, where that proportion was only slightly above the threshold in 2009. Further investigations are needed to confirm those findings. However, AL appears to remain one of the most powerful ACTs in the GMS except in western Cambodian provinces (Denis *et al*, 2006a).

The actual implication of the delayed parasite clearance time (which is partly explained by the parasite's heritable trait) under ACTs in the Subregion and elsewhere remain to be fully understood. Meanwhile, the increasing number of patients still documented with falciparum parasites on Day 3 with DHA-PIP in Cambodia, Viet Nam and Myanmar, and with A+M in Thailand is of concern. These data underscore the importance of continued monitoring of ACTs by

national malaria programs alongside national, regional and global research institutions.

Small sample sizes, due to the declining number of malaria cases in the Subregion, might have contributed to less precise outcome estimates. In some countries, the study duration extended to two malaria seasons in an effort to meet the required sample size. Studies were planned to take place during the peak transmission season, but start dates were sometimes delayed by the ethical approval process, logistic hurdles, and fund transfer mechanisms. As a result, the study duration varied depending on the pace of patient recruitment at individual sites. Challenges in identifying an adequate number of patients also led to including a small number of cases with parasitemia levels above 100,000/µl and below 1,000/µl. However, as per TES results, inclusion of additional patients with these entry criteria did not significantly change ACPRs or the proportion of patients still positive at Day 3.

The Mekong *in vivo* TES Network has significantly contributed to addressing the knowledge gap by increasing the pool of valuable information in the GMS. Information obtained from studies conducted in a standardized way across Mekong countries have already proven beneficial to countries in the programmatic use of antimalarials and strategic planning such as the recent policy change decisions made in Cambodia and Thailand. With WHO technical assistance, complemented by strong financial support from the President's Malaria Initiative (PMI) and the GFATM, the Mekong Network has also created a leading partnership and commitment among the six countries in the GMS to work collaboratively. Funding support from national governments and direct participation by local public health personnel further enhanced the awareness of the program managers of the need to continue assessing the efficacy of first-line antimalarial medicines and to increase the capacity of national programs to perform quality research studies. Ultimately the multi-country TES results have helped to define geographical areas or 'tiers' in order to plan and advocate for specific artemisinin resistance containment interventions (WHO, 2011b).

The TES results of chloroquine indicated that vivax infections are properly managed by chloroquine in the GMS with the exception of eastern Cambodia and southern Myanmar where ACPRs were less than 90%. This triggered a policy shift to DHA-PIP in Cambodia. Subsequent results in 2010 from all sites in eastern and western Cambodia showed a 100% efficacy with DHA-PIP. The situation in Myanmar also deserves further investigations. Continuous TES monitoring of chloroquine for *P. vivax* is being planned in all countries except Lao PDR and Cambodia.

In conclusion, results presented here consolidate the latest situation of GMS countries in their ongoing efforts to track drug resistant malaria. Standardized *in vivo* TES have been implemented across multiple settings in the GMS. That approach makes possible a comprehensive picture of the resistance status of ACTs and allows comparison of therapeutic efficacy results of individual ACTs in various and even remote geographical locations.

ACTs have been widely used in the GMS since 1995 and in other areas from 2002 (Delacollette *et al*, 2009). ACTs are largely acknowledged as the cornerstone of modern-day malaria control efforts and globally considered as the most effective treatments to manage uncomplicated falciparum malaria. Overall in the GMS, the therapeutic efficacy of ACTs remains satisfactory and has contributed to accelerating substantial gains in reducing malaria incidence and malaria deaths in the subregion during the last decade (Chapter 2). However, TES results from some sentinel sites presented here are raising concern over possible extension of artemisinin resistance development. To date, only two locations in the world have been documented as 'confirmed' artemisinin resistance hotspots based on WHO working definition (WHO, 2011a,b). Both of them are in the GMS, namely the western border of Cambodia and Thailand, and in Tak Province of northwestern Thailand bordering Myanmar (Noedl *et al*, 2008; Dondorp *et al*, 2009; Phyo *et al*, 2012). These confirmations have triggered extra specific containment interventions in those locations (Chapter 7). Public health policy makers and malaria researchers should take the issue seriously in order to protect gains reached so far towards malaria elimination and to mitigate the extension of resistance beyond the Mekong Subregion.

Successful TES require good team work, a well-planned study scheme, attention to details, and stringent QA/QC processes to ensure accuracy of data to inform policy-makers and sustain funding support. Improvement has been noticeable as compared to previous studies in the Subregion and national programs have realized the importance of running high quality TES sentinel sites.

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