The probability of a sequential *Plasmodium vivax* infection following asymptomatic *Plasmodium falciparum* and *P. vivax* infections in Myanmar, Vietnam, Cambodia, and Laos

Lorenz von Seidlein¹,²*, Pimnara Peerawaranun¹, Mavuto Mukaka¹,², Francois H. Nosten²,³, Thuy-Nhien Nguyen⁴, Tran Tinh Hien⁴, Rupam Tripura¹,²,⁵, Thomas J. Peto¹,², Tiengkham Pongvongs⁶,⁷, Koukeo Phommasone⁸,⁹, Mayfong Mayxay⁸,¹⁰, Mallika Imwong¹,¹¹, James Watson¹,², Sasithon Pukrittayakamee¹,¹², Nicholas P. J. Day¹,² and Arjen M. Dondorp¹,²

Abstract

**Background:** Adding 8-aminoquinoline to the treatment of falciparum, in addition to vivax malaria, in locations where infections with both species are prevalent could prevent vivax reactivation. The potential risk of haemolysis under a universal radical cure policy using 8-aminoquinoline needs to be weighed against the benefit of preventing repeated vivax episodes. Estimating the frequency of sequential *Plasmodium vivax* infections following either falciparum or vivax malaria episodes is needed for such an assessment.

**Methods:** Quarterly surveillance data collected during a mass drug administration trial in the Greater Mekong Subregion in 2013–17 was used to estimate the probability of asymptomatic sequential infections by the same and different *Plasmodium* species. Asymptomatic *Plasmodium* infections were detected by high-volume ultrasensitive qPCR. Quarterly surveys of asymptomatic *Plasmodium* prevalence were used to estimate the probability of a *P. vivax* infection following *Plasmodium falciparum* and *P. vivax* infections.

**Results:** 16,959 valid sequential paired test results were available for analysis. Of these, 534 (3%) had an initial *P. falciparum* monoinfection, 1169 (7%) a *P. vivax* monoinfection, 217 (1%) had mixed (*P. falciparum* + *P. vivax*) infections, and 15,039 (89%) had no *Plasmodium* detected in the initial survey. Participants who had no evidence of a *Plasmodium* infection had a 4% probability to be found infected with *P. vivax* during the subsequent survey. Following an asymptomatic *P. falciparum* monoinfection participants had a 9% probability of having a subsequent *P. vivax* infection (RR 2.4; 95% CI 1.8 to 3.2). Following an asymptomatic *P. vivax* monoinfection, the participants had a 45% probability of having a subsequent *P. vivax* infection. The radical cure of 12 asymptomatic *P. falciparum* monoinfections would have prevented one subsequent *P. vivax* infection, whereas treatment of 2 *P. vivax* monoinfections may suffice to prevent one *P. vivax* relapse.

**Conclusion:** Universal radical cure could play a role in the elimination of vivax malaria. The decision whether to implement universal radical cure for *P. falciparum* as well as for *P. vivax* depends on the prevalence of *P. falciparum* and...
Background

Novel approaches to the curative treatment and prevention of vivax malaria are urgently needed to achieve the elimination of malaria. Currently reductions in vivax malaria prevalence and incidence are lagging behind the more successful falciparum malaria elimination efforts [1]. Unlike Plasmodium falciparum, Plasmodium vivax infections relapse weeks to months after the initial attack [2]. Repeated relapses cause considerable morbidity, misery, and loss of income in vivax endemic areas [3]. Relapsing infections are also a persistent source of gametocytes, fuelling P. vivax transmission [4]. The triggers for hypnozoite activation are not completely understood, but acute febrile illness and by-products of haemolysis have been proposed [5–7].

The observation that people living in co-endemic regions have an increased rate of vivax malaria following a falciparum malaria episode compared to those who did not have a recent falciparum malaria episode suggests that in co-endemic regions a falciparum infection is a risk factor for vivax relapse [7, 8]. The risk of vivax malaria following falciparum malaria has been estimated as low as zero in several locations and as high as 65% in Papua-New Guinea [9, 10]. The lack of efficacy of the schizontocidal treatment against recurrent vivax infections and the timing of relapses has been interpreted as evidence that vivax recurrences following falciparum malaria are due to reactivation of P. vivax hypnozoites [11]. However, the available molecular tools are not able to discriminate whether a P. vivax infection is a relapse or a new infection [12]. In co-endemic regions, it has been proposed that “universal radical cure” be given for both P. vivax and P. falciparum infections [9].

The only class of drugs that can eliminate hypnozoites and hence prevent vivax relapse are the 8-aminoquinoline primaquine and tafenoquine [13, 14]. The small but real risk of haemolysis in glucose-6-phosphate dehydrogenase (G6PD) deficient individuals after the administration of 8-aminoquinoline regimens is a major barrier to the uptake of radical curative regimens and slows the elimination of vivax malaria. With the increasing availability of robust and accurate point of care tests for G6PD deficiency, health care providers are increasingly able to prescribe 8-aminoquinoline to clear vivax infections without putting the patient at risk. There is a broad consensus on the benefits of adding a course of 8-aminoquinoline to the schizontocidal treatment of vivax malaria. Detecting and treating asymptomatic P. vivax carriers is more challenging. In co-endemic regions P. falciparum infections could serve as a marker for earlier P. vivax infections. In such a scenario, the inclusion of 8-aminoquinoline in the treatment P. falciparum infections in addition to vivax malaria (universal radical cure), could benefit the P. vivax infected patient and accelerate the elimination of P. vivax. The relative benefits of such proactive treatment depend to a large part on the probability of an episode of P. vivax parasitaemia following a P. falciparum infection. To get a better understanding of such potential benefits this study explores the probabilities of sequential Plasmodium infections using data from a trial of mass drug administrations (MDAs) in villagers living in four countries of the Greater Mekong Subregion (GMS).

Methods

The data for the current study were collected during a cluster randomized trial conducted between 2013 and 2017 in Myanmar, Vietnam, Cambodia, and Laos [15]. The aim of the trial was to assess the effectiveness, safety, tolerability, and acceptability of mass administrations of three rounds of dihydroartemisinin–piperaquine (DHA–PPQ) with a single low dose primaquine (SLD PQ). The MDAs were conducted at months 0, 1, 2 in intervention villages. The MDA intervention was allocated by restricted randomization within pairs of villages matched for geographical proximity and parasite prevalence. Of the 4423 people residing during the MDAs in the 8 intervention villages 3790 (86%) completed at least one round (3 doses) of anti-malarials. In addition, there were 294 new-comers registered until month 12. The 4310 residents in 8 control villages at month 0 plus 733 new-comers who joined later were invited to participate in cross-over MDAs after 12 months (M12, M13, M14) with the exception of the residents in two control villages in Myanmar who were offered MDAs at M9, M10, M11. The surveillance data analysed in the current study are from the first 12 months in the control and intervention villages in Myanmar, Vietnam, Cambodia, and Laos.
and 9 months in the control villages in Myanmar. The
month 12 data from the control arm in Myanmar are not
included in the analysis as cross-over MDA took place
at month 9 because of accessibility concerns during the
rainy season.

**Surveillance**
At M0, directly preceding the MDA in intervention vil-
lages and subsequently every 3 months, all residents of
the study villages aged 6 months or older were invited
to participate in cross-sectional prevalence surveys,
including temporary inhabitants and migrant workers
arriving after the MDA was completed. The presence or
absence of each participant in the village during the pre-
vious period was assessed during the quarterly surveys.
Venous blood (3 mL) was collected from all individuals
aged ≥ 5 years, and 500 µL from children aged ≥ 6 months
to 5 years. Participants with fever ≥ 37.5 °C were tested
for malaria by rapid diagnostic tests (RDT) and malaria
positive cases were treated according to national
guidelines.

**Laboratory**
The blood samples were stored in a cool box in the field
and then transported within 12 h to the local laboratory
and processed by separation of plasma, buffy coat, and
packed red blood cells, which were frozen and stored at
−80 °C. The frozen samples from Myanmar, Cambodia,
and Lao PDR were transported monthly on dry ice to the
Department of Molecular Tropical Medicine and Genet-
ics in Bangkok, Thailand for DNA extraction, and high-
volume ultrasensitive quantitative Polymerase Chain
Reaction (uPCR). The samples from the Vietnam sites
were shipped to the Oxford University Clinical Research
Unit in Ho Chi Minh City, Vietnam for DNA extraction,
and uPCR. Detailed description and evaluation of the
uPCR methods have been reported previously [16].

**Statistical analysis**
The conditional probability of a *P. vivax* infection in a
current survey was calculated given the *Plasmodium*
infection status 3 months earlier (the previous sur-
vey), which could be a *P. falciparum*, a *P. vivax*, a mixed
or no infection. Thus, the data point of each partici-
 pant included in this analysis had the same exposure
period. Only the status of infection 3 months earlier
was included in this analysis. The risk of *P. vivax* infec-
tions following *P. falciparum* or *P. vivax* infections was
assessed using risk ratios. The risk ratios were calculated
as the ratio of the conditional probabilities of *P. vivax* fol-
lowing *P. falciparum* or *P. vivax* infections in the preced-
ing survey to the conditional probability of having a *P.
vivax* infection when there was no *Plasmodium* species
detected previously. Since participants could contribute
more than one episode of malaria species infection, we
used the Generalized Estimating Equation (GEE) model
to account for repeated observations in the same study
participant. A GEE model with log binomial link function
was fitted to the outcome (present of subsequent *P. vivax*
infection) conditional on the preceding infection status
(*P. falciparum*, *P. vivax*, mixed or no infection). The
conditional probabilities, risk ratio and their 95% confidence
interval were obtained. The risk difference (RD) was cal-
culated as the difference between the assumed cure rate
of primaquine minus the observed conditional probability
of having no subsequent *P. vivax* infection when *P. fal-
ciparum* was detected at the time of the survey 3 months
earlier. The risk differences that account for clustering
were calculated from the conditional probabilities. The
95% confidence intervals were calculated by first obtain-
ing the standard error of the difference in probabilities.
The standard errors were calculated by squaring each of
the standard errors of the probabilities which were then
summed up and the square root taken. Then the 95%
confidence interval for the risk differences was calculated
in the usual way of risk difference plus or minus 1.96
multiplied by the standard error.

Next, the number of *P. falciparum* infected individu-
als needed to treat (NNT) with 8-aminoquinoline in
order to prevent one *P. vivax* infection where NNT = 1/
(risk difference) were estimated. The estimates make the
assumption that the radical cure using an appropriate
dose of primaquine has a 99% cure rate, i.e. nearly all sub-
sequent *P. vivax* infections could have been prevented if
the participants been treated appropriately. We also esti-
mated the number of *P. vivax* infected people needed to
treat (NNT) with 8-aminoquinoline in order to prevent
sequential same species *P. vivax* infections. The 95% con-
fidence intervals for NNT were calculated by obtaining
the inverse of the lower and upper limits of the 95% con-
fidence intervals for the risk difference and reversed their
order [17]. The standard error of the risk difference was
assumed to be the same in the observed data and in the
hypothetical data (in which cure rate was assumed to be
99%). The analysis was performed in Stata 15.0.

**Results**
Of the 9760 residents living in the 16 villages during the
12-month study period, 6235 residents (1372 from Myan-
mar, 2004 from Vietnam, 1267 from Cambodia, and 1592
from Laos) contributed 16,959 valid sequential paired
test results included in this analysis. Of these, 534 (3%)
had a *P. falciparum* monoinfection, 1169 (7%) a *P. vivax*
monoinfection, 217 (1%) had mixed (*P. falciparum + P.
vivax*) infections, and 15,039 (89%) had no *Plasmodium*
infection in the initial survey.
As shown in Table 1, of the 534 participants who had an initial monoinfection with *P. falciparum*, 47 had a subsequent *P. vivax* infection detected at next survey (9%; 95% Confidence Interval: 7% to 12%). Of 1169 participants who had an initial mono *P. vivax* infection, 584 had a subsequent *P. vivax* infection detected at the next survey (45%; 95% CI 42% to 48%). Of the 217 participants with mixed *P. vivax* and *P. falciparum* infection 104 were found to have a subsequent *P. vivax* infection at the next survey (47%; 95% CI 40 to 54%). Out of the 15,039 participants who were initially found to be uninfected 515 had subsequently *P. vivax* infections (4%; 95% CI 3 to 4%).

The risk of subsequent *P. vivax* infections following *P. falciparum* monoinfections was about two-fold increased (Risk Ratio 2.4, 95% CI 1.8 to 3.2) compared to the risk in uninfected participants. The risk of subsequent *P. vivax* infections following *P. vivax* in monoinfections was about 12 times increased (RR 12.2, 95% CI 11.0 to 13.6) compared to uninfected participants. When *P. falciparum* parasites were detected in participants with either mono- or mixed-infections during the preceding survey, the risk of subsequent *P. vivax* infection was almost 5 times increased (RR 4.9, 95% CI 4.1 to 5.9) compared to uninfected participants.

Table 2 summarizes the number of individuals needed to be treated with 8-aminoquinoline in order to prevent one *P. vivax* infection. Assuming that radical cure will prevent 99% of subsequent *P. vivax* infections (relapse), treatment of 12 individuals with asymptomatic *P. falciparum* mono-infections with an appropriate 8-aminoquino- noline regimen will prevent one *P. vivax* infection (NNT 12, 95% CI 9 to 22) while treatment of 2 *P. vivax* mono-infected individuals will prevent one *P. falciparum* mono-infected cases to be treated with 8-aminoquinoline to prevent one *P. vivax* infection varied between study sites (Fig. 1). In Laos, the country with the highest baseline *P. falciparum* prevalence (7%), 12 (95% CI 7 to 33) *P. falciparum* infections would need to be treated with an 8-aminoquinoline to prevent one *P. vivax* infection and in Cambodia, with a baseline *P. falciparum* prevalence of 2%, 37 (95% CI 8 to ∞) *P. falciparum* cases would need to be treated.

**Table 1** Conditional probabilities of subsequent *P. vivax* infections adjusted for correlation among qPCR test result from same individual

| Plasmodium parasites detected in the preceding survey | n/N | Probability of a subsequent *P. vivax* infection (95% CI) | RD (95% CI) | RR (95% CI) |
|------------------------------------------------------|-----|----------------------------------------------------------|-------------|-------------|
| *P. falciparum* (monoinfection)                      | 47/534 | 0.090 (0.068, 0.119)                                      | 0.054 (0.029, 0.080)   | 2.4 (1.8, 3.2) |
| *P. falciparum* + *P. vivax* (mixed infection)       | 104/217 | 0.466 (0.402, 0.540)                                      | 0.430 (0.361, 0.498)   | 12.3 (10.3, 14.6) |
| *P. vivax* (monoinfection)                           | 584/1169 | 0.447 (0.416, 0.480)                                      | 0.411 (0.379, 0.442)   | 12.2 (11.0, 13.6) |
| Negative                                             | 515/15,039 | 0.036 (0.033, 0.039)                                      | Reference             | Reference     |

RD risk difference, RR relative risk

**Table 2** Numbers of individuals needed to be treated with an 8-aminoquinoline to prevent one *P. vivax* infection, assuming 99% *P. vivax* radical cure rate adjusted for correlation among qPCR test result from same individual

| Plasmodium parasites detected in the preceding survey | Number of no subsequent *P. vivax* infection, n/N | Probability of no subsequent *P. vivax* infection (95% CI) | Probability no subsequent *P. vivax* with radical cure assumed | RD (95% CI) with 99% *P. vivax* radical cure | NNT (95% CI) |
|------------------------------------------------------|--------------------------------------------------|----------------------------------------------------------|----------------------------------------------------------------|------------------------------------------|-------------|
| *P. falciparum* (monoinfection)                      | 487/534                                          | 0.090 (0.068, 0.119)                                      | 0.99                                                           | 0.080 (0.045, 0.116)                     | 12 (9, 22) |
| *P. falciparum* + *P. vivax* (mixed infection)       | 113/217                                          | 0.466 (0.402, 0.540)                                      | 0.99                                                           | 0.456 (0.359, 0.553)                     | 2 (2, 3)   |
| *P. vivax* (monoinfection)                           | 585/1169                                          | 0.447 (0.416, 0.480)                                      | 0.99                                                           | 0.437 (0.392, 0.482)                     | 2 (2, 3)   |
| Negative                                             | 14,524/15,039                                    | 0.036 (0.033, 0.039)                                      | 0.99                                                           | 0.026 (0.022, 0.031)                     | 38 (33, 46) |

RD risk difference, NNT number needed to treat
prevent one subsequent *P. vivax* infection but this number varied by location.

A recent systematic review examined the risk of clinical vivax episodes following clinical falciparum malaria [9]. The investigators thought the risk of clinical vivax malaria episodes following falciparum malaria was mainly determined by the terminal half-life of the antimalarial drug used to treat the falciparum malaria episode and the periodicity of the *P. vivax* relapse pattern. In regions with short relapse periodicity including the GMS the risk was higher than in regions with longer intervals between relapses, i.e. regions further removed from the equator. By day 63 after a presentation with clinical falciparum malaria, independent of the type of schizontocidal drug administered for the falciparum episode, at least 15% of study participants had *P. vivax* parasitaemia in co-endemic countries.

One of the principal differences of the current study to earlier work is the use of asymptomatic infections to estimate probabilities and not clinical malaria episodes. There are good reasons to treat and clear asymptomatic infections in the interest of the infected individual [18] as well as to reduce and ultimately interrupt transmission, but asymptomatic infections may have different epidemiological characteristics and are likely to have different probabilities for subsequent vivax relapse than clinical malaria episodes. Second the current study detected infections in quarterly intervals. Events occurring after *P. falciparum* infection but ending before the next quarterly survey were missed by the current analysis. Using the quarterly surveys and in the absence of appropriate genotyping, we were unable to distinguish vivax re-infections or relapses from persistent infections. A recent analysis of data from the study site in Vietnam showed that asymptomatic *P. vivax* as well as *P. falciparum* infections persisted frequently for months in the absence of a curative treatment [19]. The number needed to treat (NNT) is an epidemiological measure used in communicating the effectiveness of a health-care intervention. The NNTs presented here do not include the reduction in vivax malaria transmission resulting from the implementation of the universal radical cure. The overall benefits of universal radical cure are therefore likely to be even larger than suggested by the NNTs.
Conclusion

Rational decision making whether to implement universal radical cure should consider benefits relative to safety risks. Considering the tangible and intangible costs of vivax malaria infections and the prospect of interrupting transmission, even treating 37 individuals with falciparum malaria to prevent one P. vivax episode, the highest number-needed-to-treat observed, seems justified. However, in the administration of 8-aminoquinoline safety concerns have a high priority. The introduction of robust and accurate tests which allow the quantitative estimation G6PD activity will make the administration of 8-aminoquinoline safer and the licensing of tafenoquine which can be administered as a single dose is likely to increase adherence to the radical cure. Good reasons to implement universal radical cure are accumulating. Whether the potential benefits outweigh the risks remains a judgement call for policymakers and needs to be based on local circumstances specifically the malaria and G6PD deficiency prevalence and the local capacity to diagnose G6PD deficiency correctly.

Abbreviations

°C: degrees Celsius; µL: microlitre; 95% CI: 95% confidence interval; DHA: dihydroartemisinin; G6PD: glucose-6-phosphate dehydrogenase; GEE: Generalized Estimating Equation; GMS: Greater Mekong Subregion; M1, M2, M3, …: Month 1, Month 2, Month 3, …; MDA: mass drug administration; ml: millilitre; NNT: number needed to treat; PPQ: piperaquine; RD: risk difference; RR: risk ratio; SLDPQ: single low dose primaquine; uPCR: high-volume ultrasensitive quantitative polymerase chain reaction; Lao PDR: Lao People’s Democratic Republic; MORU: Mahidol-Oxford Research Unit; DP: dihydroartemisinin-piperaquine; Hb: haemoglobin.

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Authors’ contributions

KP wrote the first draft of this article. KP, LV, NPJD, AMD, PNN, NJJW, and MMa developed the study concept. KP, FS, and PP curated the data. KP, FV, LMa analysed the data. NPJD, AMD, NJJW acquired the funding for the study. LV, KV, PNN, MMa administered the project. KP, FV, MI, GH, TP, BA, TJP, CP, MD, NPJD, FC, AMD, PNN, NJJW, LVs, and MMa supervised the project. All authors read and approved the final manuscript.

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Data availability

The data are available upon request to the Mahidol Oxford Tropical Medicine Research Unit Data Access Committee (http://www.tropmedres.ac/data-sharing) for researchers and following the Mahidol Oxford Tropical Medicine Research Unit data access policy (http://www.tropmedres.ac/_asset/file/datassharing-policy-v1-1.pdf). Queries and applications for datasets should be directed to Rita Chanviriyavuth (rita@tropmedres.ac).

Ethics approval and consent to participate

The studies were approved by the Cambodian National Ethics Committee for Health Research (0029 NECHR, dated 04 Mar 2013) the Institute of Malariology, Parasitology and Entomology in Ho Chi Minh City (185/HDGD dated 15 May 2013), the Institute of Malariology, Parasitology and Entomology in Qui Nhon (dated 14 Oct 2013), the Lao National Ethics Committee for Health Research (Ref No 013-2015/NECHR), Government of the Lao PDR and the Oxford Tropical Research Ethics Committee (1015-13, dated 29 Apr 2013). Each participant or parent/guardian in the case on minors provided Individual, signed, informed consent or a fingerprint for illiterate participants countersigned by a literate witness. (ClinicalTrials.gov Identifier: NCT018727702).

Consent for publication

Not applicable as no personal data are included.

Competing interests

The authors declare that they have no competing interests.

Author details

1. Mahidol Oxford Tropical Medicine Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. 2. Centre for Tropical Medicine and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK. 3. MShoklo Malaria Research Unit, Mae Sot, Thailand. 4. Oxford University Clinical Research Unit, Wellcome Trust Major Overseas Programme, Ho Chi Minh City, Vietnam. 5. Center of Tropical Medicine and Travel Medicine, Department of Infectious Diseases, Amsterdam University Medical Centers, Meibergdreef, University of Amsterdam, Amsterdam, The Netherlands. 6. Savannakhet Provincial Health Department, Savannakhet, Savannakhet Province, Lao PDR. 7. Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. 8. Lao-Oxford-Mahosot Hospital-Wellcome Trust Research Unit (LOMWRU), Microbiology Laboratory, Mahosot Hospital, Vientiane, Lao PDR. 9. Amsterdam Institute for Global Health & Development, AHTC, Amsterdam, Netherlands. 10. Institute of Research and Education Development, University of Health Sciences, Vientiane, Lao PDR. 11. Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. 12. The Royal Society of Thailand, Dusit, Bangkok, Thailand.

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