New Approaches to Treat Vivax Malaria in Brazil

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Abstract

Background

Vivax malaria is a neglected disease. Recent reversal of the trend towards declining incidence has emphasized the importance of sustainable control strategies to move towards elimination. Although a more holistic approach will be needed to eliminate this poverty related disease, there is an irrefutable and urgent need for new treatments. Optimizing current therapeutic regimens is the first step to meet this demand.

This thesis was conceived as a treatment optimization development plan to provide information about new approach to treat vivax malaria using drugs normally used for *P. falciparum* treatment, namely Artemisinin-based Combination Therapy (ACT), in combination with a short course of a hypnozoiticidal drug. The intervention proposed was ACT with a concomitant short course of primaquine (total dose 3–4.2 mg/kg).

Methods

In this thesis, the safety, efficacy and effectiveness of these regimens has been thoroughly investigated in four pieces of work, (i) a systematic review to evaluate primaquine at alternative dosing schedules for preventing recurrence in people with plasmodium vivax malaria (chapter II); (ii) a retrospective open cohort to evaluate vivax recurrence in Brazil (chapter III); (iii) A randomized clinical trial (RCT) to evaluate the safety and efficacy of ACTs to treat malaria vivax in Brazil (chapter IV); and (iv) the evaluation of the pharmacokinetics/ pharmacodynamics of chloroquine and Artemisinin based Combination Therapies with primaquine within the RCT (chapter V).

Main results

1) Systematic review to evaluate primaquine at alternative dosing schedules for preventing recurrence in people with Plasmodium vivax malaria results:

The systematic review shows that are no difference in *P. vivax* recurrences at 6 months when using the same total dose of primaquine (0.5 mg/kg/day to 210 mg) over 7 days as compared to 14 days (RR 0.96, 95% CI 0.66 to 1.39)

2) Retrospective open cohort to evaluate vivax recurrence in Brazil results:

In Brazil, age ≤ 3 years, being male, literate, not-indigenous and having domestic working activities were identified as risk factors for recurrence. There was no difference in time to recurrence or recurrence frequency between patients treated with 14-day or 7–9 day primaquine regimens HR = 1.02 (0.96–1.09) and RR = 0.97 (0.90–1.04), respectively. The use of chloroquine alone was associated with a RR=1.43 (1.29–1.58, $p < 0.0001$) increased risk of *P. vivax* recurrence compared to patients who used chloroquine combined with short-course primaquine. The time to recurrence was longer in recipients of both primaquine and ACTs compared to patients treated with chloroquine alone or with concomitant primaquine, HR = 2.2 (1.62–2.99, $p < 0.0001$), HR = 1.27 (0.97–1.66, $p = 0.08$), respectively.

3) *Randomized clinical trial (RCT) to evaluate the safety and efficacy of ACTs to treat malaria vivax in Brazil results:*

The cure rate of all three treatment arms was greater than 90% at 28 and 42 days. Cure rates were below 90% in all three treatment groups at day 63, although the 95% confidence interval included 90% for all three treatments. Most of the adverse events were mild in all treatment arms. Significant drops in haemoglobin were rare.

4) Pharmacokinetics/ pharmacodynamics of chloroquine and Artemisinin based Combination Therapies with primaquine results:

Most recurrences in the ASMQ (67%), CQ (80%) and AL (85%) groups were considered related relapses. 8/9 (88.9%) of the patients with impaired CYP2D6 activity relapsed with related parasite compared to 18/25 (72%) with normal activity (RR= 1.23, 0.88;1.72, p=0.40). There were no associations between the measured PK parameters and recurrence. Patients with longer chloroquine half-lives had more pruritus (RR=1.09, 1.03;1.14, p=0.001).

Conclusion

The data presented here have increased the body of evidence to support the recommendation that short course primaquine regimens can be adopted into World Health Organization treatment guideline. However, although shorter course primaquine may improve adherence and the effectiveness of vivax treatment worldwide, there is still room for further improvements in treatment. One potential approach is to replace chloroquine by ACTs.

This thesis further investigated the efficacy of ACTs with concomitant use of primaquine short course in a randomized clinical trial in Brazil. This study demonstrated that their efficacy meet World Health Organization criteria. Through this study and the subsequent pharmacokinetic and pharmacodynamic evaluation, we endeavoured to addressed the relative lack of information about the safety of the concomitant use of ACT and daily primaquine. We conclude that all regimens were well tolerated.

Finally, the use of routine health information systems evaluated the effectiveness of these regimens and provided a basis for future studies of the deployment of new treatments nationwide in Brazil.

This work is dedicated to my grandparents *(in memoriam)*

Professora Edelena Albernaz de Melo Bastos (1923- 2017)

Comandante Paulo de Melo Bastos (1918-2019)

They taught me that solidarity, generosity and the common good are core values of the humankind.

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Thanks for the scientific support that I have got from the authors of the manuscripts, special thanks to Júlio.

Thanks for my son, Gabriel, who makes me a better person even if against my will.

Thanks for the capoeira that allowed me to finish a PhD in good mental health, *mens sana in corpore sano.*

Declarations

This work has not previously been accepted in substance for any degree, or been currently submitted in candidature for any degree.

I hereby give my consent for this thesis, if accepted, to be available for photocopying and for inter-library loan, and for the title and summary to be made available for outside organisations.

My role

The thesis is based on work done by myself jointly with others under the supervision of David Lalloo and Feiko O. ter Kuile. The manuscript authors' contributions made it clear that the work would not be possible without the help of the others, other sources are also acknowledged, and the bibliography appended. Herein, there is a brief description of my role in each chapter.

I conducted the bibliographic review background (Chapter I)

Chapter II is a protocol and the systematic review, "Primaquine at alternative dosing schedules for preventing relapse in people with plasmodium vivax malaria". I joined the Cochrane infectious disease group (CIDG) by invitation of Paul Garner, who had been elaborating the protocol with Patricia Graves and Rachael Milligan. I contributed in the refinement of the protocol, including the definition of outcomes and in the publication process. During the systematic review, Rachael and I evaluated all abstracts, debated on discordances, read all the selected manuscripts. We extracted the data and inserted in forms and Cochrane systems. Rachael guided me on CIDG procedures and Paul was a reliable supporter for clarifications. Rachel and I participated in the interpretation of the GRADE classifications and the results. With the other authors and CIDG editorial team, I reviewed the writing and participated in the publication process.

Chapter III is the study "Evaluation of Plasmodium vivax malaria recurrence in Brazil". This project had a grant from a larger funding scheme from the Brazilian Ministry of Health. I collaborated with colleagues from the Oswaldo Cruz Foundation, in the elaboration of the project and the funds were granted by Brazilian National Health Funds (FNS). In collaboration with the Paola Marchesini, and Ana Carolina Santelli, former heads of the Brazilian National Programme of Malaria Control, I led development of the study protocol. Maria Hermoso and myself did the ethical and administrative process to access the data. Feiko and David supported me when I was reviewing the record linkage software/ procedures and the data matching strategy with Antony Stevens. I performed the Quality Assurance (QA) of the record linkage process that was coded and conducted by Antony Stevens. I did the data cleaning and data management to lock the dataset. With support of David, Paola and Cor Jesus Fontes, and kind suggestions of Nick White I elaborated the statistical analytical plan and performed most of the analysis of the data, including the geospatial data analysis, however it would not have been possible without the supervision of the statistician Julio Castro, who did the PWP survival analysis. I participated in the interpretation of the results with Julio and my supervisors. I wrote the initial versions of the manuscript and coordinated the critical revisions from the other authors and the manuscript submission.

Chapter IV is an open label randomized clinical trial, "Efficacy and safety of Artemisinin-based Combination Therapy and chloroquine with concomitant primaquine to treat Plasmodium vivax malaria in Brazil". Tereza Cristina dos Santos and I wrote the project and funds were granted by the Brazilian National Council of Research/ Fiocruz Programme of Excellence in Clinical Research. I led the protocol development of the study with Paola Marchesini, and Ana Carolina Santelli from the Ministry of Health (MoH). As the study coordinator, I did the feasibility assessment in the study sites of the principal investigators: Marcus Lacerda (FMT) and Dhelio Pereira (CEPEM). Colleagues of the Clinical Research Platform of Fiocruz and I did the administrative process, including the supply of study drugs, material to the study sites, and human resources (HR) management. With the monitor, Ivan Maia and the site coordinator, Rosilene Ruffatto, I trained the study teams in the protocol and Good Clinical Practice (GCP), provided support to the ethical procedures, registered the study in a primary WHO repository. With Mariana Simões, I supervised QA procedures, participated in monitoring visits, and approved the sponsor's standard operational procedures (SOPs). Dhelio and I evaluated the adverse events and participated in the report to the regulatory agency and manufacturers. Leandro Amparo, and I elaborated the clinical record forms (CRFs). I supervised the assembling of the e-CRF, as the definition of the e-CRF software and the Good Manufacturing Practices (GMP) data storage. I developed the statistical analytical plan with support from David Lalloo and Julio Castro. Julio did the analysis, but David and I interpreted the results. I wrote an ICH final clinical report to the funders, and wrote the initial versions of the manuscript. I coordinated the critical revisions by other authors and the manuscript submission.

The fifth chapter is about the pharmacokinetics/ pharmacodynamics of chloroquine and Artemisinin based Combination Therapies with primaquine. This study started with a collaboration with colleagues from the Oswaldo Cruz Foundation, to write a project to raise the funds that were granted by FNS to perform pharmacokinetic studies. This is an ancillary study of the clinical trial above. With Laís Bastos Fonseca, Douglas Pinto and Gabriel Silveira from the SEFAR pharmacokinetic laboratory, we defined the validation of the pharmacokinetic (PK) whole blood sampling and storage procedure. During monitoring visits and with the help of the study sites coordinators, I trained the clinical study teams in the PK sampling procedures. Colleagues of the Clinical Research Platform of Fiocruz and I provided logistic support to sample transportation and support to the ethical procedures. I registered the study in a primary WHO repository. In LSTM with Ghaith Aljayyoussi, I debated the PK data analyses and tentative modeling. I made the arrangements to conduct the PCR analysis with Cristiana Brito and we discussed the results with her team including Taís de Sousa. David and Feiko and I elaborated the statistical analytical plan. With supervision of Julio Castro, I did the data cleaning, data management to lock the dataset, and performed the final analysis of the data. I interpreted the results with support from David, Feiko, Cristina and , Julio. I wrote the initial versions of the manuscript and coordinated the critical revisions by other authors and the manuscript submission.

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Chapter I

Bibliographic review

In need of new treatments

The world has experienced unprecedented advances in health technology during the last few decades. Unfortunately, the benefits of these innovations in health have not been evenly distributed among all populations. Progress on the development and deployment of tools to fight illnesses that afflict low income populations has not occurred at the same pace as research and development (R&D) to treat the diseases that affect the wealthiest (2001, World Health Organization.).

The concept of neglected diseases emerged around the beginning of this century (MSF, 2001). Initially, they were called neglected because people suffering them could not afford the cost of the medicines, and investment in R&D for new treatments was neglected. Later renamed Neglected Tropical Diseases (NTD), the concept was rapidly accepted by many, including the World Health Organization (WHO) and the social responsibility agenda of the pharmaceutical industry (World Health Organization., 2012). The considerable progress has been undeniable (World Health Organization., 2017), but also highlighted the drawbacks of the concept: new diseases will emerge and some diseases will be left behind. Vivax malaria is one those left behind.

Vivax malaria is not listed as one of the seventeen Neglected Tropical Diseases by WHO (World Health Organization., 2016) However, there has been recent international agreement that the importance of vivax malaria has been neglected (Carlton et al., 2011, Baird, 2007, Mendis et al., 2001, World Health Organization., 2015a), and there is an

irrefutable need for better treatments (Price et al., 2011) with higher acceptability and a better safety profile. Furthermore, as for any other infectious disease, resistant parasites will always emerge requiring new treatment approaches. Finally, as the public health agenda moves from control to eradication of malaria (malERA, 2017b) the treatment emphasis might transition from curing individuals to stopping transmission.

The ideal and desirable features of any new treatment is described in a strategic document named Target Product Profile (TPP). While it is not a regulatory requirement, the US food and Drug Administration (FDA) issued a guidance on the subject (FDA, 2017), and it is widely used by pharmaceuticals industries, or by Public-Private Product Partnerships (PPP), such as Medicines for Malaria Venture (MMV) (Burrows et al., 2017). The TPP drives agreement among the stakeholders, whether industry, academic or the community. Whilst the screening and design of new molecules is important, most TPPs suggests incremental innovations may also have a place. Indeed, the malaria TPP starts with optimizing current treatments (Burrows et al., 2017).

The logic behind optimizing current treatments is that the R&D of a new chemical entity may take around 15 years (Burrows et al., 2017) and we cannot wait longer. Some successful stories of drug developments to treat neglected diseases followed the simple rule of "first picking the low hanging fruits". One example is the development of combination therapies. The use of combination therapy is broadly indicated in infectious diseases guidelines. It is a tool to increase treatments' potency and prevent resistance emerging. The introduction of the Artemisinin-based Combination Therapies (ACTs) in malaria was a landmark change in *P. falciparum* treatment (White and Olliaro, 1996, Nosten et al., 1998). The emergence of multidrug-resistant falciparum persuaded the

WHO to include ACTs in the malaria treatment guidelines in 2006 (World Health Organization. Roll Back Malaria Dept., 2006), and this *P. falciparum* treatment recommendation (World health Organization., 2015c) has been adopted in most countries. Currently, even as decreased artemisinin efficacy against *P. falciparum* is correlated with the Kelch13 molecular marker (Ariey et al., 2014), the evaluation of safety and efficacy of new ACT combinations still ongoing. As in HIV and tuberculosis treatment, triple or quadruple drug combinations may be the next generation of antimalarial treatments (Shanks et al., 2015, U.S. National Institutes of Health, 2017) (NCT02453308).

When considering optimizing current treatments, or incremental innovations, among many questions, we ought to ask ourselves: Can low toxicity old drugs be repurposed (Watkins and Sibley, 2011)? Can new regimens, formulations or presentations delay the effectiveness decay (malERA, 2017a)? Can the current treatment regimens be shortened (more patient friendly) without compromising efficacy (Milligan R, 2017)?

This thesis was conceived as treatment optimization development plan, similar to a drug development plan, (ICH, 1997) to provide information about a new approach to treat vivax malaria using *P. falciparum* treatment (ACT) in combination with a short course of a hypnozoiticidal drug. The intervention proposed was ACT with concomitant short course of primaquine (7–9 days: total dose 3–4.2 mg/kg).

Herein, the primaquine regimen evaluated was the one that Brazilian National Malaria Control Program recommends for radical vivax malaria cure, a seven days primaquine treatment (total dose from 3 mg/kg to 4.2 mg/kg) (Ministério da Saúde do Brasil., 2010),

a total dose that retain efficacy in some parts of the world (John et al., 2012). This guideline of seven days concurrent primaquine helps to address the need to improve adherence treatment (World Health Organization., 2015a).

In this thesis, the efficacy and effectiveness of this primaquine regimen in preventing relapses (Abdon et al., 2001a) has been thoroughly investigated in three pieces of work, a Cochrane review (chapter II) (Milligan R, 2017), a cohort study (chapter III) (Daher et al., 2019) and a clinical trial (chapter IV and V) (Daher et al., 2018a). The potential for short course primaquine has recently been demonstrated by preliminary results from the largest clinical trial ever in primaquine short course regimens suggesting that a high-dose primaquine course (7 mg/kg total dose given over 7 days) is non-inferior to the same dose given over 14 days (Taylor, 2018). The impact on adherence still needs to be further investigated (Pereira et al., 2011, Osorio-de-Castro et al., 2015), arguably, short courses improve completeness of treatment, particularly in oligo symptomatic, asymptotic/ chronic patients (Chen et al., 2016).

Vivax treatment using ACT has been recommended by WHO since 2010 (World Health Organization., 2010a), as it has equivalent *P. vivax* schizonticidal activity to chloroquine (Gogtay et al., 2013) and where chloroquine-resistant *P. vivax* exists, WHO recommends replacing this drug with ACTs. There are compelling arguments to look for a simpler and unified treatment for both malarias. A single radical treatment for both malarias is useful in co-endemic areas with unreliable species diagnosis (Douglas et al., 2010). It is also the treatment of choice for mixed infections. It can also prevent hypnozoite activation causing *P. vivax* relapse following *P. falciparum* infections (White, 2011). The logistical efficiencies of stock and supply management are an additional advantage for policy

makers. Higher levels of ACT procurement are an incentive to maintain ACT production at industrial scale should falciparum treatment needs reduce. Finally, the availability of more than one efficacious vivax treatment helps with public health resistance management, as it may avoid selective pressure over a single therapeutic regimen. In this scenario, the ACT could be chosen as first line treatment because of the pill burden and daily intakes, food restrictions, potential suppressive activity in sporozoites (Clyde et al., 1976), drug interactions and safety profile.

Despite these advantages, an ACT and primaquine combination to treat malaria is far from an ideal new treatment safety profile. It is not appropriate for those who are pregnant, children aged <6 months, or G6DP deficient populations. These restrictions impose serious limitations on the deployment of ACT with primaquine as an elimination tool in many parts of the world, specially, in *P. falciparum* infected patients that do not need full primaquine dose to achieve radical cure.

In order to provide additional evidence about the proposed intervention, a series of studies were conducted and published. The studies published during the period of 2016-19 were: (i) a systematic review to evaluate primaquine at alternative dosing schedules for preventing recurrence in people with plasmodium vivax malaria (chapter II); (ii) a retrospective open cohort to evaluate vivax recurrence in Brazil (chapter III); (iii) A randomized clinical trial (RCT) to evaluate the safety and efficacy of ACTs to treat malaria vivax in Brazil (chapter IV); and (iv) The evaluation of pharmacodynamics/ pharmacokinetics relationship in the RCT (chapter V).

This thesis produced some evidence to help to answer these questions about the optimization of the vivax treatment. Providentially, a new single dose anti-hypnozoite drug, tafenoquine was first registered in the FDA in 2018 (FDA, 2018). Unfortunately, the low hanging fruit approach might still be needed for a while. Tafenoquine is from the same drug class than primaquine. Its long half-life causes many concerns and its wide deployment requires a yet to be available quantitative point of care G6PD deficiency diagnosis and intense pharmacovigilance. New classes of drugs are in the R&D pipeline although none are anti-relapse molecules (Burrows et al., 2017). The pharmaceutical industry's inertia has been replaced with new ideas from PPPs, academics and communities and new unprecedented advances in health technology are expected. The antimalarial history is moving fast.

History of malaria treatment

The history of the first anti-malarial dates back to the seventeenth century, nevertheless it resembles the chronicle of R&D for most current medicines. It reflects great commercial and political interests, propriety rights (Greenwood, 1995), disputes on innovations and public health concerns. It makes an interesting case study for the economic sciences on how colonial empires strategies on chemistry (Crawford, 2014) and industrial pharmaceutical practices nowadays look-alike. Nevertheless, the lessons learned can provide fascinating insights on the need to shift the R&D focus from one that is market-driven to one that is public health driven.

This history began in South America. *Cinchona* is a tree native to the tropical Andean forests of western South America. The method for administering *Cinchona* or *Quina's* bark in an infusion to treat malaria was described by Jesuit pharmacists, in the *Schedula Romana.* It is the first time an efficacious malaria treatment was recorded in the western medical literature and dates back to 1630. The history of the plants characterization and its uses is full of disputes, but considering the medical knowledge of the Andean native population which included performing trepanation as early as 400 BC during the Inca Empire (Andrushko and Verano, 2008), it is not surprising that there is evidence that local healers knew the medicinal proprieties of *Cinchona* bark before the Jesuits (Crawford, 2014). The history unfolds to reinforce the narrative of the colonizer, as the tree is named after the wife of the Spanish viceroy in Lima, Countess of Chinchón.

It was not until nineteenth century that the Peruvian government made the sales of *Cinchona's* seeds forbidden. The aim was to protect a growing lucrative market that begun in 1820, when the Parisian chemistries, Pelletier and Caventou, isolated the alkaloid with high antimalarial activity. Quinine was named after the indigenous name of the tree, Quina (Oliveira and Szczerbowski, 2009). The efforts of the Peruvian government were in vain. The English and Germans smuggled the seeds and established plantations in India and Siri Lanka, respectively. Quinine facilitated the English imperial enterprise in Africa and south east Asia (Greenwood, 2002), but their plants had low concentrations of quinine. Soon after, the Dutch government sponsored the research on the best species of *Cinchona* and broke the Loja Spanish monopoly of high quality trees. By the time of the World War I, the Netherlands had already taken over the market from South America for quinine, amassing enormous profits (Oliveira and Szczerbowski,

2009). Any resemblance to pharmaceutical propriety protection nowadays is not mere coincidence.

The need for cheaper and faster quinine production was an important accelerator of the chemical industry as we know today, BASF *(Badische Anilin-& Soda-Fabrik),* AGFA (*Aktiengesellschaft für Anilinfabrikation*) and Bayer & Hoechst are amongst the industries that applied the knowledge from the synthesis of the initial compound into dyes to the developing textile industry. The perceived shortcomings of quinine before the WWII reignited the interest in the synthetic antimalarials (Greenwood, 1995). During WWII, allies took part in the R&D race, as the plantations were in German and Japanese occupied territories. Only in 1947, could synthetic quinine be partially produced (Oliveira and Szczerbowski, 2009), but by then chloroquine had already been synthetized. Resochin was discovered in 1934 by Germans researchers, however it was the North Americans who named it as chloroquine during WWII. Years later they acknowledged the molecule was the same (Greenwood, 2002). The antimalarial R&D efforts during the WWII are remarkable, even considering the limited knowledge of malaria life cycle in man and the ethical standards required nowadays (Sweeney, 2000).

Chloroquine was one of the most important and successful drugs deployed against malaria. Along with vector control (DDT) it was one of the main tools of the WHO malaria eradication campaign launched in 1955 (Wellems and Plowe, 2001). The campaign aims were deemed unachievable in 1969, but chloroquine use kept on increasing over the decades up to hundreds of tons (sufficient to provide hundreds of millions of treatments annually) by the 80's (McBride, 2010), >190 ton/year in Africa alone. In 1985 the global production of chloroquine reached 1300 tones/year

(Organization, 1990). This drug pressure was a tremendous driving force to the selection of chloroquine resistant parasites that replaced the chloroquine sensitive *P. falciparum* (Wellems and Plowe, 2001). Fortunately, this was not the case for *P. vivax.* The slow emergence of resistance of *P. vivax* may be due to the fact that standard chloroquine plus primaquine treatment has always been a combination therapy (World Health Organization., 2015a).

The 8-amino-quinolone class were the first promising synthetic antimalarials during the 20's (Baird and Rieckmann, 2003). Although the initial compounds (pamaquine or plasmoquine, quinacrine and mepacrine) were rejected due to safety profile concerns, primaquine, a close structural analog is the current drug of choice to treat the latent hepatic forms of *P. ovale* and *P. vivax*. The US WWII antimalarial R&D made many attempts to discover a new molecule or therapeutic approaches that would prevent relapse, but only after revisiting the initial studies performed in India with plasmoquine, the strategy made a U-turn and lead to the development of a new series of 8-aminoquinolines (Sweeney, 2000).

Primaquine, an 8-aminoquinoline, was first made available to US troops in the 50's (Baird and Rieckmann, 2003). The clinical trials were conducted with soldiers returning from the Korean war, including a comparison with pamaquine (Alving et al., 1953). Primaquine affects mainly the hepatic forms, but it is active against blood stages of vivax (Pukrittayakamee et al., 1994), and gametocytes in falciparum infections (Recht J, 2014). Concomitant use with chloroquine also has synergic effects on primaquine blood levels (Pukrittayakamee et al., 2014).

For most of the world, the first-line treatment recommendation for vivax malaria of chloroquine and primaquine has not changed since the 50's (Mendis et al., 2001) . In 2019, it is still the best vivax treatment option. It would not be a problem if this treatment was a safe single dose tablet, but the 14 days' treatment course is toxic to the carriers of the most common human genetic deficiency (G6PD deficiency), is contraindicated in pregnant women and is difficult to take. There are also concerns about chloroquine resistance emergence (Price et al., 2014), the main partner drug for primaquine. Treatment for chloroquine-resistant *P. vivax,* includes Artemisinin-based Combination Therapies (World Health Organization., 2010a). In Brazil, *P. vivax* accounted for 88% of the malaria burden (193,917 cases) in 2017 (Secretaria de Vigilância em Saúde, 2019). This means that other therapeutic options need to be ready available in case of the emergence of chloroquine-resistant vivax malaria.

Artemisia annua is a Chinese medicinal plant known for more than 2000 years with its first medical record dating back to 168 B.C. In a later 340 A.D. record it already recommended as a treatment for fever and chills. The antimalarial component, artemisinin was isolated by Chinese scientists in 1972. The first use in humans were published in 1979 (Global Partnership to Roll Back Malaria.). This speed of this outstanding initiative was a Chinese effort to help the Vietnamese army during the war against US (Premji, 2009). Today artemisinin-based drugs are the main backbone of all *P. falciparum* treatments worldwide. They are remarkably safe and well tolerated (Nosten and White, 2007). Its use has been demonstrated to be safe in pregnancy, including the first trimester (Mosha et al., 2014).

The safety profile of ACT is often defined by their partner drug (Nosten and White, 2007), and although they have been used for the last 20 years, there is a lack of data on the safety, pharmacodynamics and pharmacokinetic of these regimens with concomitant use of primaquine for the radical cure of vivax (World Health Organization., 2015a).

Mefloquine was developed in a partnership with the industry (Hoffman-La Roche), WHO and the US army (Global Partnership to Roll Back Malaria.), first as a preventive agent and soon after as a treatment (Trenholme et al., 1975). The single dose treatment was a breakthrough, but it did not last long. Clinical treatment failures due to resistance rapidly appeared in Asia when the drug was made widely available (Global Partnership to Roll Back Malaria.). The evidence that the combination of mefloquine with artesunate restored its efficacy and averted the further development of resistance changed the paradigm of malaria treatment (Nosten et al., 2000) to the current ACT.

In order to improve adherence and prevent resistance (due to single drug use), a fixed dose combination of artesunate and mefloquine was developed. It was an innovative approach to drug development that result in a south-south technology transfer and local production in middle income countries, Brazil and India. This was the very first product in the world resulting from this new drug development model proposed by DNDi (Wells et al., 2013). The safety profile of the mefloquine single dose was a concern, especially regarding early vomiting in the paediatric population, the group most in need. The long half-live of the drug allowed splitting the total dose over three days (once a day during three days) and improving tolerability in this population to the level of other ACTs (Sirima et al., 2016).

The history of artemether lumefantrine fixed dose combination (AL) is also unique. During the 1980s and 1990s, the Chinese army evaluated the use of both drugs in combination. From 1994 onwards, this work was undertaken in collaboration with Novartis, in the first joint development programme of a western pharmaceutical company and the Chinese government. The low efficacy of the four doses regimen was an initial concern, but a new six dose (twice a day during three days) regimen evaluated in Thailand proved to have cure rates > 90% and was well tolerated. In 2008, MMV developed the AL dispersible tablets for pediatric populations (Premji, 2009).

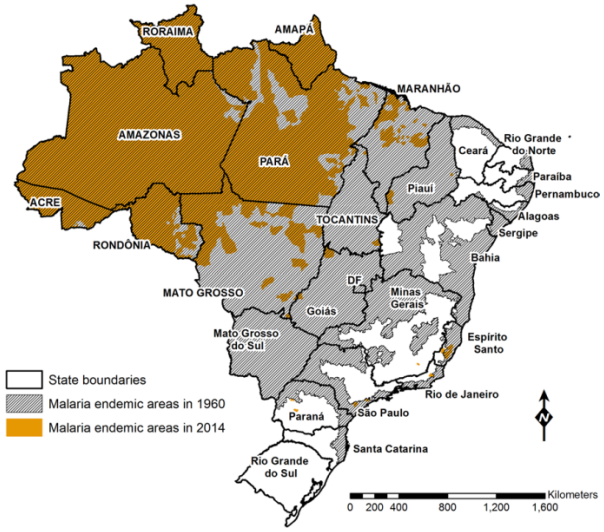
This thesis evaluated two fixed combination treatments: artesunate plus mefloquine and artemether plus lumefantrine; both are the first line *P. falciparum* treatments in Brazil (Ministério da Saúde do Brasil., 2010).

Epidemiology of malaria in Brazil

As early as 1905, malaria control actions were implemented in Brazil. Initial successful innovative interventions were put in place such as the first example of indoors anti-adult mosquito control (Ferreira and Castro, 2016). During the early 40's, the eradication of *Anopheles gambiae* in Brazil (Deane, 1988) led to nationwide anti-malarial campaigns. At that point, 2/3 of the 80 million Brazilian population lived in risk areas, leading to 6-8 million cases and 80,000 malaria related deaths/year (de Pina-Costa et al., 2014). Vector control using DDT and chloroquine use led to the lowest incidence ever, 36,900 cases in 1961 (Ferreira and Castro, 2016). The Malaria Eradication Campaign and further diagnosis, treatment and control measures promoted the virtual elimination of malaria from many areas of Brazil (

Figure 1).

Figure 1 Malaria endemic areas in 1960 and 2014 (Ferreira and Castro, 2016)



Not all innovative strategies were successful, though. The Pinnoti's strategy comprising deploying chloroquine in the cooking salt to control malaria in the most isolated areas, raised concerns about drug stability, effectiveness of low dose drug, drug resistance emergence and, last but not least, food taste (Silva and Hochman, 2011, Clyde, 1966).

The Amazon region was a geopolitical and economic frontier in the 70's and 80's, deforestation was sponsored by the federal government to promote logging, agriculture and livestock. Infrastructure projects including roads, dams, and hydroelectric plants, but also gold mining encouraged the massive influx of non-immune populations, especially to two states in the Amazon basin, Rondônia and Pará. In 1985, these two states accounted for 73 % of all malaria cases in the country (Cruz Marques, 1987).

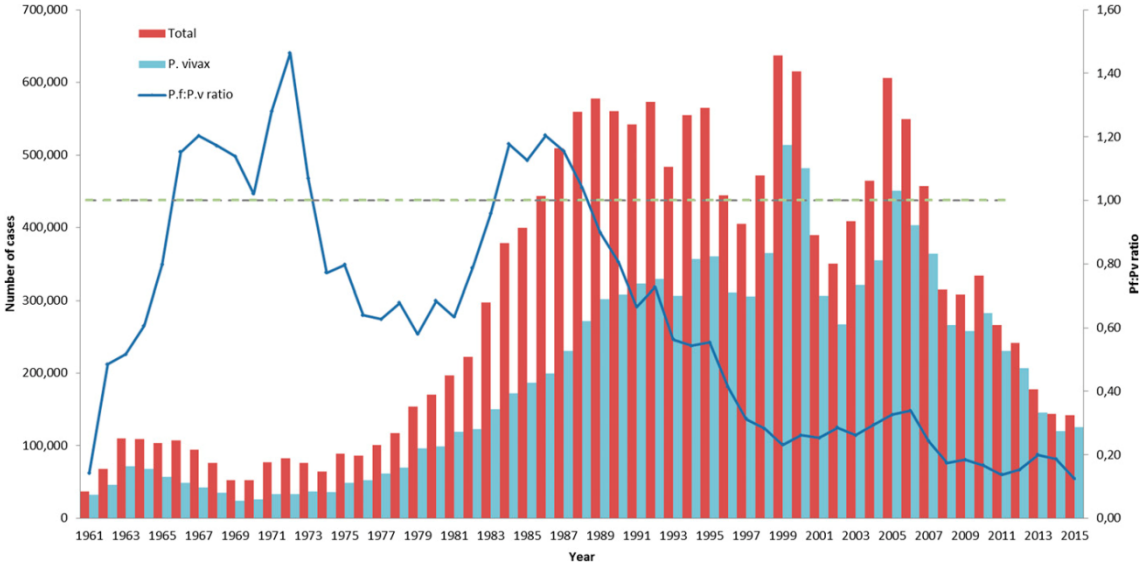
In 1988, an equitable Brazilian National Health System was created and health care access improved. Renewed efforts from the public health sector started in 90's with massive national and international investments, resulting in the reduction of malaria related deaths from 7/1000 to 1.8/1000 populations, and averting 1.9 million new cases of malaria and 231,000 deaths between 1988 and 1995 (Loiola et al., 2002, Akhavan et al., 1999). However, the lack of the sustainability of the funding and centralized action in the federal government undermined these efforts, there were more than half million cases per year during the nineties and the record peak malaria incidence was 637,470 cases in 1999 (Siqueira et al., 2016b).

The subsequent Plan of Intensified Control of Malaria in Amazon Region (PIACM) focused on decentralization of malaria control actions to state and municipality levels, high political commitment, strengthening of patient centered care with early diagnosis and treatment and vector control, all of which started to reverse the 90's epidemiological trends (Siqueira et al., 2016b).

At that point, the first-line regimens for uncomplicated falciparum malaria recommended by the Ministry of Health of Brazil were quinine plus doxycycline for seven days or a single dose of mefloquine. In 2006 Artemisinin-based Combination Therapies (ACTs) were introduced and an evaluation with 26,000 patients demonstrated *P. falciparum* malaria incidence rates decreased, with lower hospital admission rates, and a reduced proportion of *P. falciparum*/ *P. vivax* infections in the Jurua Valley (Santelli et al., 2012). The impact of ACT deployment on the *P. falciparum*/ *P. vivax* ratio was also demonstrated in a national malaria surveillance system (SIVEP-malaria) (Oliveira-Ferreira et al., 2010).

P. vivax malaria has become the predominant species since 1990, as it is more refractory than *P. falciparum* to the current control strategies (Ferreira and Castro, 2016). In 1988, both falciparum and vivax had the same incidence. In the following year, *P. falciparum* was the cause of 44,3% of malaria cases. In 2009, *P. vivax* represented 84% of all cases and *P. falciparum* only 16% (Oliveira-Ferreira et al., 2010). In 2014, the same falciparum/vivax ratio was sustained (**Figure 2**).

Figure 2. Malaria incidence in Brazil.

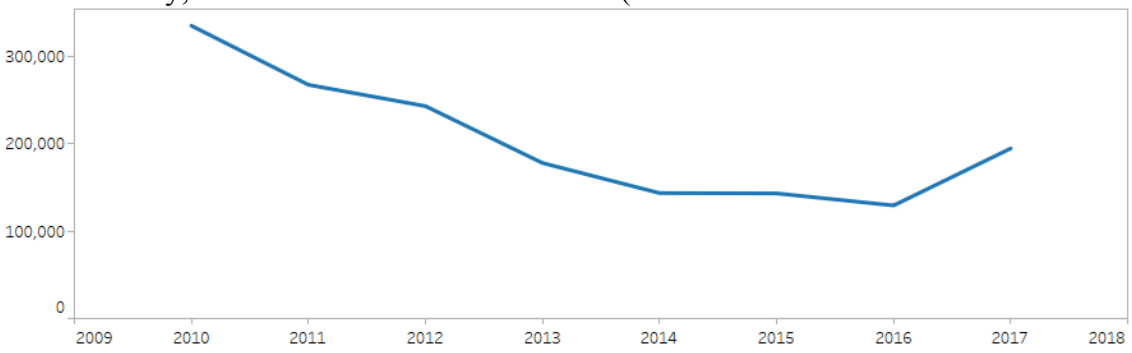


Absolute number of malaria cases in Brazil in the 1960–2015 period is shown as total cases (red bars) and due to *Plasmodium vivax* (pale blue bars) corresponding to the left y axis. The *Plasmodium falciparum:P. vivax* ratio is shown as a blue line (right y axis, Pf:Pv ratio) (Siqueira et al., 2016b).

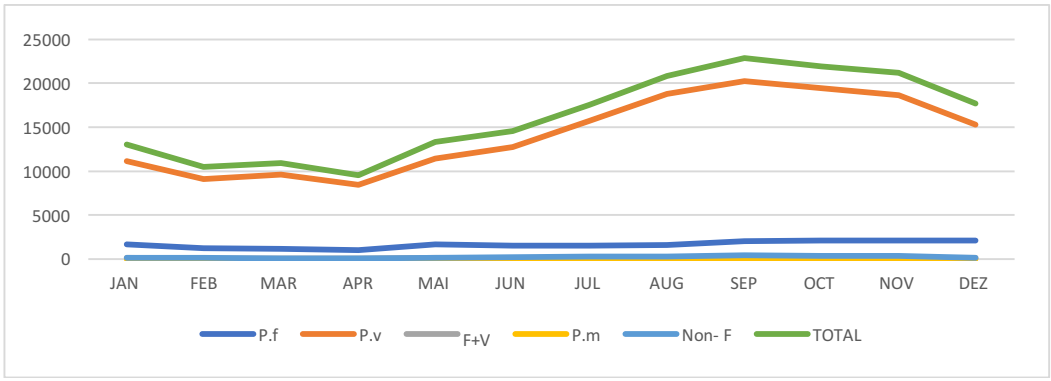
In 2014, 143.552 malaria cases were reported (Secretaria de Vigilância em Saúde, 2015). It represents a 19% decline in comparison to 2013, which is an incidence decrease of the same magnitude as that occurring globally over the same period (World Health Organization., 2015d). In 2014, the total number of cases of malaria was the lowest in the last 35 years and it was in line with the goal of 75% malaria incidence reduction by 2015 (World Health Organization., 2005). *P. vivax* was the most frequent cause of hospitalizations (65%) due to malaria in the Brazilian Amazon region (total of 203). In

the same year, 13 deaths were due to confirmed *P. vivax,* out of the 38 malaria deaths in Brazil (Secretaria de Vigilância em Saúde, 2015).

Unfortunately, this trend has not been sustained (



), and 193,917 malaria cases were reported in the Malaria National Surveillance System (SIVEP) in 2017. *P. vivax* was still the most prevalent species, 88% of all cases. (**Figure 4** Confirmed case of malaria in Brazil in 2017 by species



) (Secretaria de Vigilância em Saúde, 2019).

Figure 3 Confirmed case of malaria in Brazil from 2009 to 2017 (Pan American Health Organization., 2019)

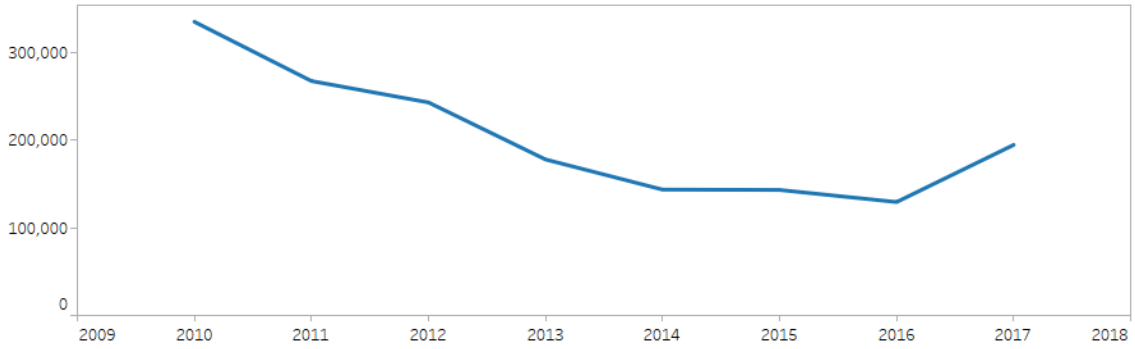
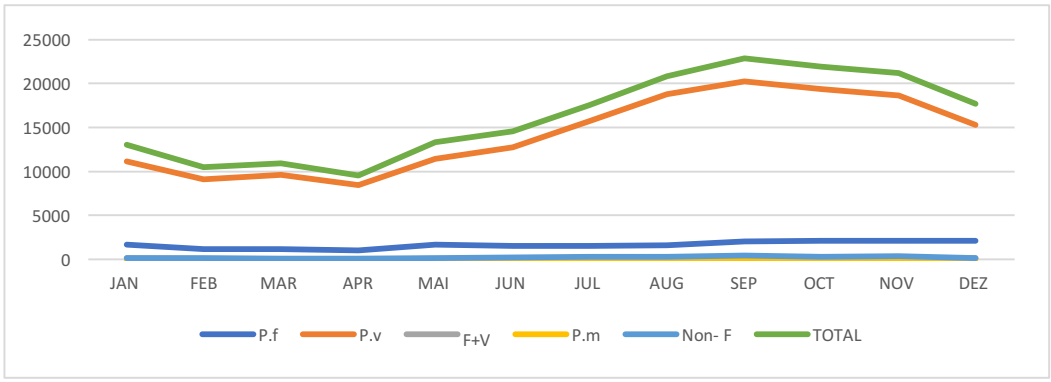
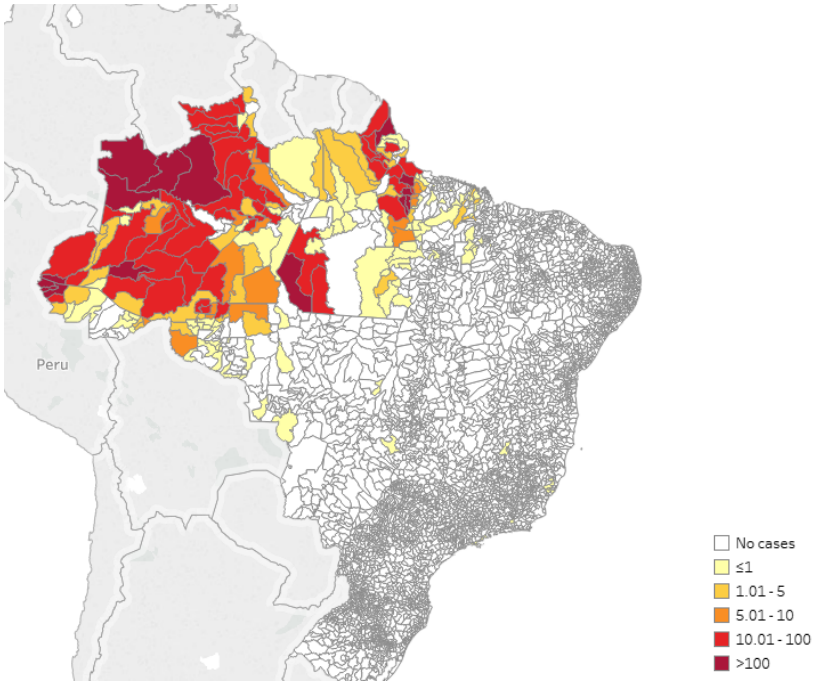


Figure 4 Confirmed case of malaria in Brazil in 2017 by species



This recent failure in malaria control may be related to the fusion of the National Malaria Control Programme with the chikungunya, zika and dengue control programme, and a possible loss of political commitment in fighting malaria. As the total incidence decreases, the care cost per patient rises, posing a political dilemma in resources constrained health systems. The political crisis in Venezuela has also contributed to the increased incidence rate in the borders (**Figure 5**).

Figure 5. 2017 malaria incidence map of Brazil, from Pan American Health Organization (Pan American Health Organization., 2019)



Worldwide data for the period 2015–2017 highlight that no significant progress in reducing global malaria cases in this timeframe anywhere in the world, except in South East Asia (World Health Organization., 2018).

In Brazil, the alarming growing malaria burden in 2017 (Secretaria de Vigilância em Saúde, 2019), with *P. vivax* accounting for 88% of the cases, means that other therapeutic options need to be ready available in case of emergence of chloroquine-resistant vivax malaria.

Vivax resistance in Brazil

From a practical public health perspective, it is important to detect lack of efficacy of any current therapeutic regimen due to resistance. A national control programme should consider changing the first line treatment, if its cure rate is below 90% in a study conducted according to WHO methods for surveillance of antimalarial drug efficacy (World Health Organization., 2009). The study presented here (Daher et al., 2018a) adopted this approach as it is the most meaningful result to guide public health policy change, however it is not the most sensitive test for surveillance of chloroquine-resistant vivax.

Surveillance for the emergence of chloroquine-resistant vivax is of paramount importance, but poses several methodological challenges when interpreting results. Ideally, in order to avoid bias due to drug-drug interactions, the evaluation of any anti-infective resistance must be conducted using a single drug treatment. This methodological approach increases the sensitivity of the test, but on the other hand it loses its external validity, meaning these results cannot be extrapolated to real life, as a single drug treatment is not recommended to treat malaria.

Plasmodium vivax chloroquine resistance *in vivo* is defined as failure to produce parasitological clearance during 28 days in the presence of the sum of CQ and its main active metabolite, desethylchloroquine (DCQ) above therapeutic levels (>100 ng/ml) in whole blood (Baird et al., 1997). Although this threshold to define chloroquine resistance has been widely accepted, this may need further investigation (World Health Organization., 2009) and updates.

The estimate of the minimal effective concentration (MEC) and time to recurrence were based on clinical data, including drug plasma levels from 1945. In 1997, this threshold (>100 ng/ml) was updated based on whole blood samples of 101 Indonesian patients using a high-performance liquid chromatography method described in 1983 (Patchen et al., 1983, Baird et al., 1997). There are three possible concerns that should be considered when interpreting CQ- resistance based on this definition: (i) analytical methods of the 40s. (ii) plasma CQ levels versus whole blood DCQ+CQ levels, and (iii) time to recurrence. As the ratio of plasma and whole blood CQ concentrations range from 5 to 10, the estimated MEC is in the range of 75 to 150 ng/ml whole blood. Likewise, the time to recurrence was based on clinical data from non-immune single exposed patients and it may be prolonged to 35 days in this population depending on the pattern of relapse in the geographic area of infection. However, 28 days follow up may be a short time and it might not reflect time to relapse of people harboring multiple broods of hypnozoites accumulated over years of exposures. Finally, this definition does not allow differentiation between the degree of resistance, i.e., parasites surviving the maximum drug exposure (failure to clear parasitaemia), recrudescence after initial clearance and those relapsing from hypnozoites penetrating waning levels of drugs in 28 days would all be considered resistant, although the clinical approach and public health relevance of these findings are entirely distinct (Baird, 2004).

Other classifications have been adopted to define chloroquine resistant *vivax* irrespective of the drug blood level. The WorldWide Antimalarial Resistance Network (WWARN) Vivax Survey contains data from 237 clinical trials and 26 case reports since 1960 (2019). This group considers category 1 evidence of CQ resistance to be an incidence of

recurrence greater than 10% by day 28 (with a lower 95%CI >5%). This group reported that in Brazil since 1960 (***Figure 6***), one trial had reported category 1 CQ resistance (de Santana Filho et al., 2007), one trial had reported category 2 CQ resistance (at least 5% recurrences by day 28 and lower 95%CI >5%) (Marques et al., 2014) and two case reports had described CQ resistance (Garavelli and Corti, 1992, Alecrim et al., 1999). Over the same period, 13 studies showed vivax CQ susceptibility (Margarete do Socorro et al., 2015, Pinto et al., 2003, Machado et al., 2003, Silva et al., 2003, Abdon et al., 2001b, Da et al., 1989, Llanos-Cuentas et al., 2014, Villalobos-Salcedo et al., 2000, Orjuela-Sánchez et al., 2009, Negreiros et al., 2016, Pereira et al., 2016a, Duarte et al., 2001, Pedro et al., 2012),

Figure 6. WorldWide Antimalarial Resistance Network (WWARN) Vivax Surveyor, Brazil from 1960 to 2019

Red= Category 1 evidence; Orange= Category 2 evidence; Green= Case reports; Blue= Uncategorised: Studies that do not fall into any of the above categories, or include imported cases from mixed regions are coloured in blue.

It is important to note that the quality of malaria trials has been consistently improving in the last 10 years in Brazil; during this period, no trial using the nationally recommended

treatment guideline showed evidence of resistance (Margarete do Socorro et al., 2015, Negreiros et al., 2016), including the study described herein (Daher et al., 2018a).

An important consideration is the role of the role of primaquine. However, when primaquine administration was withheld till 28 days or later (Siqueira et al., 2016a, Ladeia-Andrade et al., 2019), pharmacokinetics (PK) evaluation show CQ, DCQ drug levels above 100ng/ml in patients who fail, characterizing CQ-resistance. In the area with the highest malaria incidence rates in Brazil, Acre, the failure rate (2.2%) of the sequential CQ-PQ regimen (Ladeia-Andrade et al., 2019) did not provide clinical evidence of resistance, but drug levels of 6 out of 8 patients who had parasitological failure did suggest CQ-resistant parasites. Similarly, in Bolivia, a neighbour country of Acre, using CQ monotherapy, 6/10 CQ-resistant failures were detected by the criteria of failure with drug levels above 100 ng/ml (Añez et al., 2015). These results underestimated the efficacy of the regimen, as they did not take in account the synergic effect of chloroquine and primaquine (Alving et al., 1955, Commons et al., 2018, Pukrittayakamee et al., 1994), however, as malaria control is relaxed in Brazil and neighbour countries, these results are a matter of great concern.

As the political situation and public health systems has deteriorated in recent years in South America, the need for therapeutic options ready to be deployed in case of the emergence of chloroquine resistance is an unfortunate reality.

Malaria is a poverty related disease

The failure to progress over the last few years should not discourage the fight against malaria, but conversely it highlights the need of ongoing political commitment and continuous innovations and improvements in the current strategies.

Technological innovations have crucial roles to play (Tse et al., 2019). The deployment of tafenoquine, a major change in vivax malaria treatment, is keenly awaited. Nevertheless, its large-scale deployment is restricted currently by the lack of a point of care test of G6PD. Meanwhile every little help to reduce malaria incidence is welcome. Incremental improvement in the Brazilian malaria treatment are still ongoing. Current studies demonstrated that the Brazilian guidelines to treat recurrences (7 days primaquine in same total dose than the 14 days' regimen) has acceptable cure rates (Daher et al., 2018b) (Pereira et al., 2016b).

Incremental treatment improvement is the main aim of this thesis, but malaria control and future elimination is much broader, and there is a clear need of an integrated approach. This should comprise vector control, diagnosis and early treatment, a better use of routine data, and social issues as the underfunding of the local health systems, education in health, civil society engagement and environmental management. As we understand malaria as a poverty related disease (Silva Santelli, 2016), we may move the focus from a disease centered perspective to a community and patient centered one; this means a fundamental shift from a flawed neglected disease concept to widening it to neglected populations (Ehrenberg and Ault, 2005). A more holistic approach of malaria control, ought to pursue a state of complete physical, mental and social well-being, meaning the health of the

population (1946). The experience of many countries where malaria has been eliminated shows that advances in better living conditions are of utmost importance (Rieckmann, 2006), and there is not a single magic bullet to eliminate this tenacious parasites (malERA, 2017b).

Chapter II

Introduction Chapter II

The need to prevent *P. vivax* relapses in North American soldiers returning from the Korean war led the FDA to approve primaquine in 1952. The regimen approved was 15mg once a day for 14 days. This regimen was based on efficacy results against Korean strains (Arnold et al., 1954, Coatney et al., 1953, Alving et al., 1953, Jones et al., 1953), rather than the overall efficacy against all strains of *P. vivax*. This regimen was also designed to tackle the needs of military personal and did not necessarily address the effectiveness requirements of the endemic area population, nor patient centered approach were considered. Choice of the dose was also heavily influenced by the need to avoid hemolytic anemia in African Americans who might not have adequate medical supervision (Hill et al., 2006).

The Korean war has still not formally ended and almost 70 years later, the assembling of evidence on the efficacy of vivax treatment seems to follow the same pace as the peace talks. This chapter presents some evidence to fill this knowledge gap, in particular exploring the most suitable dose regimen of primaquine for civil populations in endemic areas.

It has long been suggested that it is the total dose of primaquine, rather than the duration of treatment, that determines efficacy (Clyde and McCarthy, 1977b). Shorter course primaquine may improve adherence and the effectiveness of vivax treatment worldwide. This chapter explores alternative primaquine patient-friendly regimens, as the Brazilian treatment guidelines that includes a daily total dose from 3 mg/kg to 4.2 mg/kg for 7-9 days (Ministério da Saúde do Brasil., 2010). Although there are studies that have

evaluated doses as high as 1 mg/kg/day (Chu and White, 2016) for 7 days to address the susceptibility of the South East Asia vivax strains, the shortest duration possible may not be limited only by a higher frequency of adverse events associated with high daily doses, but it also may be limited by an apparent lack of efficacy when the treatment duration is shorter than five days (Carmona-Fonseca and Maestre, 2009).

The main challenges in assessing the efficacy of vivax treatments efficacy has been, and remains, the inability to differentiate reinfection and relapse. Initial studies addressed this issue by removing any risk of reinfection, moving the patients (soldiers) to non-endemic areas after treatment or evaluating treatment response in experimental *P. vivax* inoculations in penitentiary population in US (Cooper et al., 1950, Coatney and Getz, 1962, Comfort, 2009). This is a major constrain on the conduction of vivax clinical trials nowadays, as the patients must be recruited in non-autochthonous transmission areas.

In order to overcome this, new molecular approaches, methodological tools and innovative initiatives are being developed. To overcome the lack of large trials, data from many small trials in non-endemic areas can be aggregated in pooled analysis of individual patient data, helped by the shift towards more open data and thriving clinical trial data repositories (Pisani et al., 2016, Commons et al., 2018).

Methodological tools, such as systematic reviews, can gather published information and help draw conclusions about the overall evidence available. Previous systematic and critical literature reviews have shown evidence of the efficacy of short course primaquine (0.5 mg/kg/day) (Carmona Fonseca, 2015, John et al., 2012, Zuluaga-Idarraga et al., 2015). However, distinct patterns of time to relapse associated with different geographic

areas (Battle et al., 2014), different study designs, including follow up times and comparators, and the susceptibility of the vivax strains worldwide adds complexity in drawing overall conclusions. For instance, in East Asia and Oceania, the recommended doses of primaquine is 0.5 mg/kg/day rather than standard 0.25 mg/kg/day for 14 days, , where tropical frequent-relapse strains are prevalent and there is allegedly "more resistant to primaquine" (Organization, 2015). This might be another knowledge gap as during the primaquine initial drug trials, the 30 mg/day for 14 days treatment arm was dropped due to safety concerns (Jones et al., 1953) and 30 mg/day for seven days primaquine was demonstrated to be effective against Chesson strain vivax (Clyde and McCarthy, 1977a) in non comparative trials. Herein we conduct a new meta-analysis that provides a more precise estimate of the effects on the efficacy and safety of alternative primaquine regimens for radical cure of *P. vivax* malaria compared to the WHO recommended 14 days of primaquine (0.25 or 0.5 mg/kg/day).

Protocol:

Primaquine at alternative dosing schedules for preventing relapse in people with Plasmodium vivax malaria. ***Cochrane Database of*** **Systematic Reviews. 2017**

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Primaquine at alternative dosing schedules for preventing relapse in people with Plasmodium vivax malaria (Protocol)

Milligan R, Daher A, Graves PM

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[Intervention Protocol]

Primaquine at alternative dosing schedules for preventing relapse in people with *Plasmodium vivax* malaria

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ABSTRACT

This is a protocol for a Cochrane Review (Intervention). The objectives are as follows:

To assess the efficacy and safety of alternative primaquine regimens for radical cure of*P. vivax malaria* compared to the standard 14 days of primaquine 0.25 mg/kg/day.

BACKGROUND

Malaria is a potentially life-threatening disease caused by the *Plasmodium* parasite, which is transmitted by the bite of an infected female *Anopheles* mosquito. There are five species of Plasmodium malaria parasites that can cause malaria disease in humans, of these, *Plasmodium vivax* and *Plasmodium falciparum* are generally recognized as the most significant threat to human health (WHO 2016a). In 2015, there were an estimated 212 million cases of malaria worldwide, with 429,000 attributable deaths (WHO 2016b). By 2030, the World Health Organization (WHO) aims to reduce malaria case load and mortality by at least 90% (WHO 2016a).

Historically, *P. vivax* infection was thought to be a milder form of malaria with minimal morbidity, with the greater focus for research on *P. falciparum*, because of the high number of deaths it causes (Bassat 2016). In recent years the morbidity and mortality of *P. vivax* have been shown to have been underestimated, with evidence of direct fatality and contribution to mortality in patients who have other co-morbidities, such as malnutrition, human immunodeficiency virus (HIV), or co-existing infections (Baird 2013; Bhattacharjee 2013; Rizvi 2013; Singh 2013; Battle 2014; Douglas 2014; Kochar 2014; Arévalo-Herrera 2015; Baird 2015b). Repeated *P. vivax* infections through childhood and adulthood also affect personal well-being, development, and education and can thus negatively impact economic development, both for the individual and the community (Mendis 2001). *P. vivax* malaria in pregnancy is associated with maternal anaemia, spontaneous abortion, stillbirth, and low birthweight, with especially poor pregnancy outcomes for women with severe infection (McGready 2012; Rijken 2012; Brutus 2013).

Description of the condition

P. vivax infection caused an estimated 13.8 million cases of malaria in 2015 and is responsible for almost half of the global cases of malaria outside of Sub-Saharan Africa (WHO 2015c). The geographical distribution of*P. vivax* malaria is more widespread than any of the other forms of human malaria - around 35% of the world's population is thought to be at risk, with two-thirds of cases occurring in South-East Asia (WHO 2015a). Co-infection with *P. falciparum* is also common in many regions (Kumar 2007; Mueller 2009). As malaria control accelerates, the *P. vivax* proportion in co-endemic areas tends to rise compared to that of *P. falciparum*, which highlights the importance and challenge of this infection (John 2012).

P. vivax is also important as many countries progress towards malaria elimination, as it is increasingly recognized as a potential roadblock to eradication (Cibulskis 2015; Bassat 2016). Despite a reduction in the number of cases of *P. vivax* malaria over the past 20 years, it has several characteristics that enable it to evade control (Newby 2016). The early appearance of gametocytes in the blood, often prior to symptoms of malaria, increases the chance of onward transmission by mosquitoes (WHO 2015a). *P. vivax* differs to *P. falciparum* in that as well as having a blood stage schizontal infection, hypnozoites develop in the liver that can be dormant for weeks to months before developing into an infection (Llanos-Cuentas 2014). It is not known what triggers these relapses. There is difficultly in distinguishing between relapse, recrudescence (subpar treatment of the initial blood stage infection), and reinfection (new infection with *P. vivax*) (Betuela 2014). A study in Papua New Guinea suggested that relapses cause four-fifths of *P. vivax* infections, so are important in sustaining transmission (Robinson 2015). Parasites show high genetic diversity, even in countries that are at malaria elimination stage, where you would expect reduced transmission to result in reduced diversity (Koepfli 2015). *P. vivax* is likely underestimated worldwide as the dormant liver stage is not detected in routine surveys (Gething 2012). Submicroscopic infections (asymptomatic infection reservoirs) may also lead to underdiagnosis or misdiagnosis. A systematic review showed that across all study sites the polymerase chain reaction (PCR) prevalence of *P. vivax* was significantly higher than that identified by light microscopy (Cheng 2015). The effect that this may have on *P. vivax* malaria studies is unclear. There are different strains of *P. vivax*, which have varying relapse patterns, and this can further complicate matters (White 2016). The Chesson, or tropical, strain is commonly found in South East Asia, Oceania, and parts of the Indian subcontinent and has the shortest relapse interval of about three weeks (if untreated), while the temperate strain may relapse after months (John 2012).

Currently primaquine, an 8-aminoquinoline, is the only drug available on the market for treating the hypnozoite stage of infection (Ashley 2014). One of the main barriers in *P. vivax* treatment is the reluctance to use primaquine due to it causing haemolysis in patients with glucose-6-phosphate-dehydrogenase (G6PD) deficiency. G6PD deficiency is the commonest enzyme deficiency worldwide and affects red blood cells, by leading to their premature lysis (Nkhoma 2009). G6PD deficiency is common in countries where *P. vivax* malaria is endemic, with an estimated population prevalence of 8% (Howes 2012). Within G6PD deficiency there are differing phenotypes, meaning some people may be mildly sensitive to primaquine, while others may be very sensitive and experience life threatening haemolysis (Baird 2015a), which explains the varying responses to primaquine. In many countries where *P. vivax* is pre-dominant, locally available testing for G6PD is not available (Baird 2015b). A newer alternative, tafenoquine, another 8-aminoquinoline, has completed phase III trials and is on track for submission to the Food and Drug Administration (FDA) in 2017 (MMV 2016). Tafenoquine has shown promise in reducing relapses, but there are increased safety concerns in patients with undiagnosed G6PD deficiency compared to primaquine, due to its longer half-life (Rajapakse 2015).

Description of the intervention

People with *P. vivax* malaria require treatment with a blood stage antimalarial drug to treat the schizont infection, and a drug to treat the hypnozoite stage (radical cure). The WHO recommends treatment with either chloroquine or an artemisinin-based combination therapy (ACT) for the blood-stage infection, followed by treatment with 0.25 to 0.5 mg/kg primaquine for 14 days (WHO 2015b). ACTs and chloroquine have been shown to be effective and comparable in treating the blood stage infection of *P. vivax* malaria (Gogtay 2013). A previous Cochrane Review showed that primaquine regimes of five days or fewer had similar relapse rates to placebo or no primaquine. Of the comparisons included in the systematic review, a regime of 0.25 mg/kg (15 mg) a day of primaquine for 14 days had the lowest relapse rates of *P. vivax* infection (Galappaththy 2013). There were no trials at that time that compared higher doses of primaquine at 14 or seven days.

Primaquine was first made available to American soldiers in the 1950s (Baird 2004). Its mechanism and metabolism is not widely understood, but it has a broad spectrum of activity against the *Plasmodium* parasite. As well as preventing relapse of *P. vivax* malaria by targeting the latent and developing hypnozoites in the liver, it is also used in malaria prophylaxis (Baird 2003). It is absorbed from the gastrointestinal tract, has a half life of about four to nine hours, and crosses the placenta in pregnancy (Baird 2004). New advancements in studying *P. vivax* in humanized mice may lead to a greater understanding of the mechanism of action of primaquine (Mikolajczak 2015).

Adverse effects of primaquine include production of methaemoglobin, an oxidated state of haemoglobin which cannot transport oxygen to tissues. Methaemoglobinaemia (an abnormal build-up of methaemoglobin) can result in cyanosis when levels exceed 10% of the usual haemoglobin level (Vale 2009). As described above, primaquine causes haemolysis in people with G6PD deficiency, which leads to severe intravascular

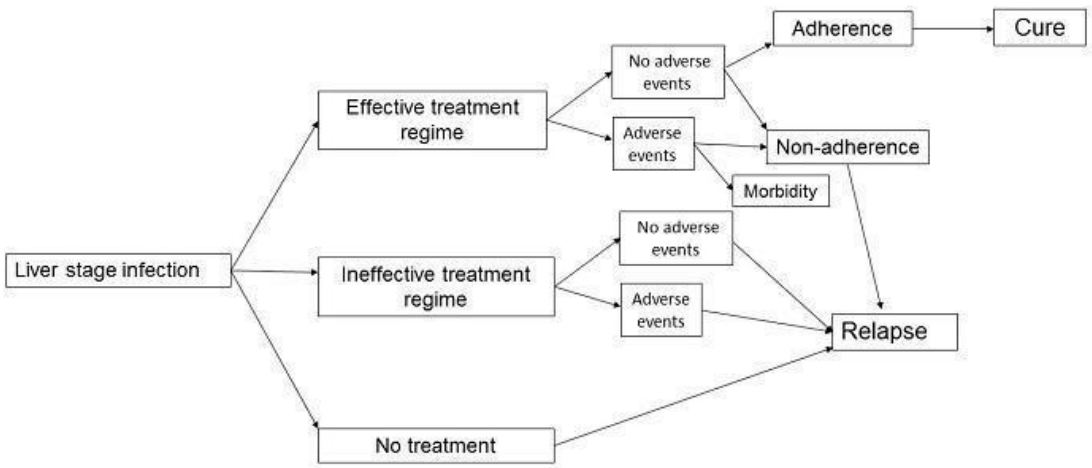
haemolysis and anaemia (Ashley 2014). When taken on an empty stomach it can cause abdominal pain and gastrointestinal upset (Vale 2009). Primaquine cannot be given in pregnancy or early infancy as the G6PD status of the baby is unknown and there would be risk of haemolysis and possible termination if the foetus was G6PD-deficient. There is currently debate about whether the levels of primaquine in breast milk would be sufficient to cause haemolysis in a G6PD-deficient baby, but it is not recommended at this time.

How the intervention might work

The WHO advises that 0.25 mg to 0.5 mg/kg of primaquine for 14 days should be used for radical cure of *P. vivax* malaria in patients over six months old, excluding people with G6PD deficiency and patients who are pregnant or breastfeeding (WHO 2015b). There has been suggestion of failure of the 0.25 mg/kg/14-day dosing regimen of primaquine for the Chesson strain of *P. vivax*, which is what was behind the suggestion of the increased dosing of 0.5 mg/kg/day. In the last review, Galappaththy 2013, there were no trials found which compared higher doses of primaquine to the 14-day regime. The WHO recommends a weekly dose of 0.75 mg/kg for eight weeks for patients with G6PD deficiency but the evidence for this is low quality as there are few high-quality trials (WHO 2015b).

The 14-day course of primaquine, which can lead to adherence issues in patients, as well as safety concerns about haemolysis in places where G6PD testing is not available, means that shorter courses of primaquine are desirable. Failure to treat the hypnozoite stage of *P. vivax* malaria leads to repeated relapses, morbidity, and persistent infection. The logic framework for developing efficacious and safe treatment regimes for *P. vivax* is illustrated in Figure 1.

Figure 1. Logic framework: treatment outcome pathways in Plasmodium vivax liver hypnozoite infection



It has long been suggested that it may be the total dose of primaquine that is important in treatment of the hypnozoite stage rather than the length of the course (Schmidt 1977). If a higher dose of primaquine could be administered safely over a shorter period of time, this may improve adherence rates, thus reducing relapse rates and morbidity and mortality resulting from *P. vivax* infection. There are small trials from the 1970s that suggest that shorter, higher dose regimes were as efficacious as the 14-day courses (Clyde 1977), and there is also evidence of similar efficacy (Saint-Yves 1977). At the time of the last review (Galappaththy 2013) there were no recent large high-quality trials that had investigated the use of higher doses given over seven days. We plan to include any such trials in this Cochrane Review.

Why it is important to do this review

The use of primaquine for radical cure of *P. vivax* malaria continues to pose a therapeutic dilemma for healthcare providers in areas without adequate screening for G6PD status. Clinicians must either chose to give primaquine and risk haemolysis if the patient is G6PD-deficient, or withhold treatment and accept the complications of ongoing parasite infection and relapses. This is why when clinicians choose to treat with primaquine they prefer a lower dose over a more prolonged period - although this then risks difficulties with adherence and thus reduced efficacy.

From the previous systematic review on primaquine with chloroquine for radical cure (Galappaththy 2013), we know 14 days of 0.25 mg/kg or 15 mg/day (210 mg total dose) is better than shorter regimens of similar daily doses and placebo. A major problem with the radical cure of *P. vivax* is difficulty with the adherence of the 14-day course of primaquine, which has led to many countries shortening the regime. Peru was once such example, although a study revealed that patients often still discontinued the therapy after around three days, when they started to feel better (Grietens 2010). A study that compared directly observed therapy (DOT) for 14 days of primaquine, versus non-DOT primaquine found that the vivax relapse rate was significantly lower in the DOT group (Takeuchi 2010). These problems have led to a more urgent call for shorter treatment regimes. Various trials are investigating regimens that improves dosing and duration of treatment, to improve adherence and reduce the potential for incomplete treatment and development of resistance. As mentioned previously, the significance of the total cumulative primaquine dose given, rather than length of course, is one avenue of investigation. In areas where G6PD screening is present, using higher dosing regimes over shorter time periods, if at least similarly efficacious, could improve adherence and reduce morbidity associated with *P. vivax* parasitaemia.

WHO guidelines suggest a higher dosing regimen of primaquine for areas with the tropical strain of *P. vivax* (WHO 2015b), although the previous Cochrane Review, Galappaththy 2013, did not find any trials that assessed this. Therefore investigating the evidence base for this is important. The 2015 WHO guidelines also suggest an alternate dosing regimen of weekly primaquine, which may be safer in patients with G6PD. In the last Cochrane Review, only data from one trial assessed this, so it will be useful to see if there is any further evidence to substantiate this guidance.

In this Cochrane Review, we will exclude comparisons between blood stage drug (chloroquine/ACT) with and without primaquine as the rationale for primaquine use has been sufficiently demonstrated in a previous Cochrane Review (Galappaththy 2013). Similarly, we will not include comparisons that look at different blood stage drugs compared using the same dose of primaquine as an update to an existing Cochrane Review will address this (Gogtay 2013). However, we will stratify our results according to partner drug, as there is increasing evidence that primaquine is metabolized via the CYP2D6 pathway and efficacy may thus be affected if the blood stage antimalarial drug is a CYP2D6 inhibitor (Bennett 2013). This review will exclude comparisons of regimens that do not use the control of 14 days of primaquine at 0.25 mg/kg/day. Also, it will not include comparisons of primaquine regimens of 0.25 mg/kg daily for less than 14 days as Galappaththy 2013 has already assessed these shorter, same daily dose regimens.

Currently there is a lack of consensus among studies as to what the minimum time frame for follow-up of relapse in *P. vivax* malaria should be. The WHO guidance on clinical trials in malaria sets out standard follow-up for blood (or schizontal) stage infection as 28 days after treatment commencement, but has no clear definition on the follow-up period for radical cure in primaquine studies. It states that "follow up varies from three months to a year in the literature, and should be adapted to regional parasite characteristics" (WHO 2009). In a recent review, John 2012 described relapse of the tropical strain of *P. vivax* as typically three weeks, but this varies according to blood stage treatment: "three weeks following quinine therapy" and "six to eight weeks following chloroquine" (White 2011). With exposure to primaquine - even if radical cure is not achieved - relapses may be at longer intervals (Sutanto 2013). In the Cochrane Review (Galappaththy 2013), the period of follow-up was 30 days after starting primaquine treatment. Despite this, the definition of relapse used in the review was the presence of *P. vivax* parasites more than 28 to 30 days after the full course of primaquine in people living in a non-endemic area (Murphy 1993; Looareesuwan 1997). Because of the varying lengths of relapse time in *P. vivax* malaria, as well as the longer schizonticidal half-life in ACTs, 28 days from treatment commencement may not allow true assessment of radical cure. It also makes assessment of the weekly primaquine regime difficult, as the follow-up time is before the eight-week treatment course has finished. In this Cochrane Review we plan to assess parasitaemia at 3, 6, and 12 months follow-up, in keeping with WHO guidance. We intend to describe the length of follow-up across studies, and then group them into meaningful lengths of follow-up, depending on the regimen.

We intend to answer the following questions by comparing the new regimens to the standard regimen of 14 days of primaquine at 0.25 mg/kg (15 mg adult dose).

- Are higher doses (0.5 mg/kg or 30 mg primaquine/day for 14 days) more efficacious and safe compared to standard therapy (0.25 mg/kg/day for 14 days), in all areas, or only in areas where they are standard treatment (for tropical *P. vivax* strains in Asia, Pacific)?
- Are shorter, higher dose regimes (0.5 mg/kg or 30 mg primaquine/day for 7 days) as efficacious and safe compared to standard therapy (0.25 mg/kg/day for 14 days)?
- Are weekly dosing regimens (0.75 mg/kg or 45 mg/week for 8 weeks) as efficacious and safe compared to standard therapy (0.25 mg/kg/day for 14 days)?

OBJECTIVES

To assess the efficacy and safety of alternative primaquine regimens for radical cure of*P. vivax malaria* compared to the standard 14 days of primaquine 0.25 mg/kg/day.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials (RCT). We will exclude quasi-RCTs.

Types of participants

Adults and children with confirmed clinical and parasitological (light microscopy or polymerase chain reaction (PCR), or both) diagnosis of *P. vivax* malaria. We will include trials that have excluded people with glucose-6-phosphate-dehydrogenase (G6PD) deficiency, and trials that included populations that had not been screened for G6PD deficiency.

Types of interventions

Intervention

Any regimen of either chloroquine or an artemisinin-based combination therapy (ACT) plus primaquine with any of the following.

- Higher daily doses for 14 days.
- Shorter regimens with the same total dose.
- Using weekly dosing regimens.

Control

Standard regimen of 14 days of primaquine at 0.25 mg/kg (15 mg adult dose) plus either chloroquine or an ACT.
We will include trials that use chloroquine or ACT as the treatment for blood-borne infection, and we will stratify by the schizonticidal agent.

Types of outcome measures

Primary outcomes

- *P. vivax* parasitaemia detected (by light microscopy or polymerase chain reaction (PCR), or both) at 3 months, 6 months, and 12 months follow-up.

Secondary outcomes

- *P. vivax* parasitaemia detected (by light microscopy or polymerase chain reaction (PCR), or both) at one to three months follow-up.

Adverse effects

- Serious adverse effects (fatal, life-threatening, or requiring hospitalization).
- Adverse effects that result in discontinuation of treatment.
- Events known to occur with primaquine (cyanosis, leucopenia, methaemoglobinaemia, hypertension, cardiac arrhythmia, abdominal pain, nausea, vomiting, or haemolysis) or those due to a comparator drug used along with primaquine.
- Anaemia or change in haemoglobin status.
- Other adverse effects.

Search methods for identification of studies

We will attempt to identify all relevant studies regardless of language or publication status (published, unpublished, in press, or in progress).

Electronic searches

We will search the following databases: the Cochrane Infectious Diseases Group (CIDG) Specialized Register; the Cochrane Central Register of Controlled Trials (CENTRAL), published in the Cochrane Library; MEDLINE (PubMed); Embase (OVID); and LILACS (BIREME), using the search terms detailed in Appendix 1 (Lefebvre 2011). We will also search the World Health Organization (WHO) International Clinical Trials Registry Platform (http://www.who.int/ictrp/search/en/) and ClinicalTrials.gov (https://clinicaltrials.gov/), to identify ongoing trials, using "vivax" and "primaquine" as search terms.

Searching other resources

We will check the reference lists of all studies identified by the above methods for other potentially relevant studies. We will contact researchers working in the field and the World Health Organization (WHO) for unpublished and ongoing trials. We will also search the reference lists and included studies of the Cochrane review by Galappaththy 2013.

Data collection and analysis

Selection of studies

Two review authors will independently screen the titles and abstracts of the search results to identify potentially eligible trials, and will code the articles as either 'retrieve' or 'do not retrieve'. We will obtain the full-text reports of potentially eligible trials and will assess them for inclusion in the review using a predesigned eligibility form based on the inclusion criteria. We will resolve discrepancies through discussion or, if required, we will consult a third review author. Where necessary we will contact the trial authors for clarification of trial methods. We will list the excluded trials and the reasons for exclusion in a 'Characteristics of excluded studies' table. Where there are multiple reports relating to the same trial, we will include all reports. However, we will only extract data from the most up-to-date report that includes the specified outcome. We will detail the trial selection process in a PRISMA diagram.

Data extraction and management

Two review authors will independently extract data from the included trials using a data extraction form, designed for this review, in keeping with Cochrane guidance (Higgins 2011).
For each included trial we plan to extract a minimum of the following data if available.

- Study design.
- Endemicity/population demographics.
- G6PD status of participants (known/unknown).
- CYP2D6 status (if available).
- Blood stage antimalarial drug choice.
- Dose/duration/timing of treatment arms.
- Supervised or non-supervised therapy.
- Duration of follow-up.
- Adverse events.
- Reported outcomes.

We will resolve any differences in data extraction through discussion and consult a third review author if there is any discrepancy. We will enter the extracted data into Review Manager 5 (RevMan 5) (RevMan 2014). We will contact the authors of primary trials in case of any doubts regarding missing data or methodological details of the trial. We will note the limitations in the included studies.
We will group comparisons as illustrated in Table 1.

Assessment of risk of bias in included studies

Two review authors will independently assess the risk of bias of each included trial using the Cochrane 'Risk of bias' assessment tool, and discuss any differences of opinion. In the case of missing or unclear information, we will contact the trial authors for clarification. We will summarize the results using Cochrane 'Risk of bias' tables (Higgins 2011).

Measures of treatment effect

For dichotomous data, we will compare interventions using risk ratios to measure treatment effect. Where trial authors present data as odds ratios, we will recalculate the effect. We will define statistical significance as $P < 0.05$ and for all results we will calculate 95% confidence intervals (CIs). For comparable trials, we will perform meta-analyses if there is sufficient data.

Unit of analysis issues

For this Cochrane Review, cluster-randomized designs would be inappropriate for evaluating the research questions. If trials that have used cluster-randomization meet our inclusion criteria we would expect the results to have been controlled for clustering. If they have not, we will contact the trial authors for an estimate of the intra-cluster correlation coefficient (ICC) value. We will analyse clustered data using the methods outlined in the *Cochrane Handbook for Systematic Reviews of Interventions* (Higgins 2011).
We will split trials that include more than two comparison groups and will analyse them as individual pair-wise comparisons. If there is a shared control group we will split the control group so that participants are only counted once in the overall meta-analysis.

Dealing with missing data

We will analyse missing data using available case analysis if we judge the trial to be at low risk of bias for incomplete outcome data. We will attempt to contact trial authors to obtain missing or unclear data. If the missing data render the result uninterpretable, we will exclude the data from meta-analyses and clearly state the reason for exclusion. If the missing data means that results are interpretable but likely to be at high risk of bias, we may use imputation methods to investigate the impact of the missing data. We will analyse extracted data on an intention-to-treat basis where there is no missing data.

Assessment of heterogeneity

We will inspect forest plots for overlapping CIs. We will also apply the Chi^2 test as a statistical test for the presence of heterogeneity, with a P value of 0.10 used to indicate statistical significance, and we will compute the I^2 statistic to quantify the percentage of the variability in effect estimates that is due to heterogeneity rather than sampling error (chance). We will investigate possible causes of heterogeneity by subgroup analysis. If substantive heterogeneity persists, which we define as an I^2 statistic value of greater than 50%, we will use a random-effects meta-analysis.

Assessment of reporting biases

We will examine the likelihood of reporting bias using funnel plots provided that there is a sufficient number of included trials.

Data synthesis

We will analyse the data using Review Manager 5 (RevMan 5) (RevMan 2014). We will assess the certainty of the evidence for each outcome measure using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach and we will construct 'Summary of findings' tables using GRADEpro Guideline Development Tool (GDT) (GRADEpro GDT 2014). We will stratify results according to blood stage partner drug. Length of follow-up will vary with regimes and between studies. We will describe regimes and follow-up periods and define sensible groupings for follow-up. If there is sufficient data, we will also perform subgroup analyses according to CYP2D6 status (when available), geographical region/endemicity, length of follow-up, and directly observed therapy (DOT) or non-DOT.

Subgroup analysis and investigation of heterogeneity

We will group the analysis by drug regimen. We will describe the interventions and outcomes in all included trials. We will conduct an inventory of length of follow-up against each drug regimen and then group *P. vivax* parasitaemia relapse by appropriate groupings for length of follow-up.

Sensitivity analysis

We will assess the risk of bias that contributed data to the meta-analyses for the prespecified outcomes with sensitivity analyses against concealment of allocation.

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* *Indicates the major publication for the study*

ADDITIONAL TABLES

Table 1. Data extraction: Grouping of comparisons to address the review objectives

Objective	Intervention	Control
Are higher doses more effective (0.5 mg/kg or 30 mg primaquine/day for 14 days), in all areas, or only in areas where they are standard treatment (Asia, Pacific)?	Blood-stage antimalarial drug with primaquine 0.5 mg/kg (30 mg) per day for 14 days (total dose 420 mg) Both intervention and control groups must have received the same treatment: either CQ or ACT for the blood-borne stage of infection	Blood-stage antimalarial drug with primaquine 0.25 mg/kg (15 mg) per day for 14 days (total dose 210 mg) Both intervention and control groups must have received the same treatment: either CQ or ACT for the blood-borne stage of infection
Are shorter, higher dose regimes of primaquine over 7 days as effective as treatment over 14 days (is the total dose rather than the length of treatment the important factor)?	Blood-stage antimalarial drug with primaquine 0.5 mg/kg (30 mg) per day for 7 days (total dose 210 mg) Both intervention and control groups must have received the same treatment: either CQ or ACT for the blood-borne stage of infection	
Are weekly dosing regimens (0.75 mg/kg or 45 mg/week for 8 weeks) as effective?	Blood-stage antimalarial drug with primaquine 0.75/kg (45 mg) per week for 8 weeks (total dose 360 mg)	

CQ = Chloroquine
ACT = Artemisinin-based combination therapy

APPENDICES

Appendix 1. Detailed search strategy

Search set	Search terms
1	primaquine [Title/Abstract]
2	"Primaquine"[Mesh]
3	1 or 2
4	"Plasmodium vivax " [Title/Abstract]
5	"Plasmodium vivax"[Mesh]
6	"vivax malaria " [Title/Abstract]
7	"Malaria, Vivax"[Mesh]
8	4 or 5 or 6
9	3 and 8

Search terms used in combination with the search strategy for retrieving trials developed by the Cochrane Collaboration (Lefebvre 2011).
This is the preliminary search strategy for MEDLINE (PubMed). We will adapt it for other electronic databases and we will report all search strategies in full in the final version of the review.

CONTRIBUTIONS OF AUTHORS

Patricia Graves (PMG), Paul Garner (CIDG Co-ordinating Editor) and Rachael Milligan (RM) contributed to the conception of the research question.

All protocol authors contributed to the protocol design and approved the final version.

DECLARATIONS OF INTEREST

RM has no known conflicts of interest.

AD has no known conflicts of interest.

PMG has no known conflicts of interest.

SOURCES OF SUPPORT

Internal sources

- Liverpool School of Tropical Medicine, UK.

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- Department for International Development, UK.
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Systematic Review:

Primaquine at alternative dosing schedules for preventing relapse in people with Plasmodium vivax malaria.

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Primaquine at alternative dosing schedules for preventing relapse in people with *Plasmodium vivax* malaria (Review)

Milligan R, Daher A, Graves PM

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[Intervention Review]

Primaquine at alternative dosing schedules for preventing relapse in people with *Plasmodium vivax* malaria

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ABSTRACT

Background

Malaria caused by *Plasmodium vivax* requires treatment of the blood-stage infection and treatment of the hypnozoites that develop in the liver. This is a challenge to effective case management of *P vivax* malaria, as well as being a more general substantial impediment to malaria control. The World Health Organization (WHO) recommends a 14-day drug course with primaquine, an 8-aminoquinoline, at 0.25 mg/kg/day in most of the world (standard course), or 0.5 mg/kg/day in East Asia and Oceania (high-standard course). This long treatment course can be difficult to complete, and primaquine can cause dangerous haemolysis in individuals with glucose-6-phosphate dehydrogenase (G6PD) deficiency, meaning that physicians may be reluctant to prescribe in areas where G6PD testing is not available. This Cochrane Review evaluated whether more patient-friendly alternative regimens are as efficacious as the standard regimen for radical cure of*P vivax* malaria.

Objectives

To assess the efficacy and safety of alternative primaquine regimens for radical cure of *P vivax* malaria compared to the standard or high-standard 14 days of primaquine (0.25 or 0.5 mg/kg/day), as well as comparison of these two WHO-recommended regimens.

Search methods

We searched the Cochrane Infectious Diseases Group (CIDG) Specialized Register; the Cochrane Central Register of Controlled Trials (CENTRAL); MEDLINE (PubMed); Embase (Ovid); and LILACS (BIREME) up to 17 December 2018. We also searched the WHO International Clinical Trials Registry Platform (ICTRP) and ClinicalTrials.gov, and checked the reference lists of all studies identified by the above methods.

Selection criteria

Randomized controlled trials (RCTs) of adults and children with *P vivax* malaria using any regimen of either chloroquine or an artemisinin-based combination therapy (ACT) plus primaquine with either higher daily doses for 14 days, shorter regimens with the same total dose, or using weekly dosing regimens; compared with the usual standard regimens recommended by the WHO (0.25 or 0.5 mg/kg/day for 14 days), or a comparison of these two WHO-recommended regimens.

Data collection and analysis

Two review authors independently assessed trial eligibility and quality, and extracted data. We calculated risk ratios (RRs) with 95% confidence intervals (CIs) for dichotomous data. We grouped efficacy data according to length of follow-up. We analysed safety data where this information was included.

Main results

High-standard 14-day course versus standard 14-day course

Two RCTs compared the high-standard 14-day regimen with the standard 14-day regimen. People with G6PD deficiency and pregnant or lactating women were excluded. We do not know if there is any difference in *P vivax* recurrences at 6 months with 0.5 mg/kg/day primaquine therapy for 14 days compared to 0.25 mg/kg/day primaquine therapy for 14 days (with chloroquine: RR 0.82, 95% CI 0.47 to 1.43, 639 participants, very low-certainty evidence; with chloroquine or an ACT: RR 1.11, 95% CI 0.17 to 7.09, 38 participants, very low-certainty evidence). No serious adverse events were reported. We do not know whether there is a difference in adverse events with the higher dosage (very low-certainty evidence).

0.5 mg/kg/day primaquine for 7 days versus standard 14-day course

Five RCTs compared 0.5 mg/kg/day primaquine for 7 days with the standard 14-day course. There may be little or no difference in *P vivax* recurrences at 6 to 7 months when using the same total dose (0.5 mg/kg/day to 210 mg) over 7 days as compared to 14 days (RR 0.96, 95% CI 0.66 to 1.39; 1211 participants; low-certainty evidence). No serious adverse events were reported. There may be little or no difference in the number of adverse events known to occur with primaquine between the primaquine shorter regimen as compared to the longer regimen (RR 1.06, 95% CI 0.64 to 1.76; 1154 participants; low-certainty evidence). We do not know whether there is any difference in the frequency of anaemia or discontinuation of treatment between groups (very low-certainty evidence). Three trials excluded people with G6PD deficiency, and two did not provide this information. Pregnant and lactating women were either excluded or no details were provided regarding their inclusion or exclusion.

0.75 mg/kg primaquine/week for 8 weeks versus high-standard course

One RCT compared weekly primaquine with the high-standard 14-day course. G6PD-deficient patients were not randomized but were included in the weekly primaquine group. Only one G6PD-deficient participant was detected during the trial. We do not know whether weekly primaquine increases or decreases recurrences of *P vivax* compared to the 14-day regimen at 11 months' follow-up (RR 3.18, 95% CI 0.37 to 27.6; 122 participants; very low-certainty evidence). No serious adverse events and no episodes of anaemia were reported.

Three other RCTs evaluated different alternative regimens and doses of primaquine, but one of these RCTs did not have results available, and two used regimens that have not been widely used and the evidence was of very low certainty.

Authors' conclusions

Although limited data were available, the analysis did not detect a difference in recurrence between the 7-day regimen and the standard 14-day regimen of 0.5 mg/kg/day primaquine, and no serious adverse events were reported in G6PD-normal participants taking 0.5 mg/kg/day of primaquine. This shorter regimen may be useful in G6PD-normal patients if there are treatment adherence concerns. Further large high-quality RCTs are needed, such as the IMPROV trial, with more standardised comparison regimens and longer follow-up to help resolve uncertainties.

16 September 2019

Update pending

Authors currently updating

The update is due to be published in December 2019.

PLAIN LANGUAGE SUMMARY

Primaquine to cure people with *Plasmodium vivax* malaria: comparing dosing schedules

Plasmodium vivax malaria can sometimes cause potentially life-threatening illness, and the infection continues to make many people unwell. The infection includes a liver stage, and this requires primaquine to eradicate it and prevent the infection recrudescing. However, the current dosing schedule requires 14 days of daily treatment.

What are the concerns about primaquine?

Primaquine is the only drug currently recommended to treat the liver parasites in *P vivax* malaria. It can cause anaemia in people with glucose-6-phosphate dehydrogenase (G6PD) deficiency, which is a relatively common genetic blood disorder. Shorter regimens would help reduce the risk of default with the current two-week regimen.

What does the research say?

We summarized trials that compared the World Health Organization (WHO)-recommended primaquine regimen of 15 to 30 mg per day for 14 days with the same or higher doses of primaquine given over different lengths of time to determine whether alternative regimens were as successful as the recommended courses at preventing future episodes of *P vivax* malaria. We searched for trials up to 17 December 2018, and included nine randomized controlled trials (studies in which participants are assigned to one of two or more treatment groups in a random manner) in our analysis.

When using 30 mg per day compared to 15 mg per day primaquine therapy for 14 days, we do not know if there is any difference in *P vivax* recurrences at 6 months (very low-certainty evidence). No serious side effects were reported, but it is unclear whether or not there is a difference in other side effects between doses (very low-certainty evidence).

When using 30 mg primaquine per day for 7 days compared to 15 mg per day for 14 days, there may be no difference in *P vivax* recurrences at 6 to 7 months (low-certainty evidence). No serious adverse events were reported. There may be no difference in the number of side effects known to occur with primaquine between the two treatment regimens (low-certainty evidence).

We do not know whether weekly primaquine increases or decreases recurrences of *P vivax* compared to the 14-day regimen at 11 months' follow-up (very low-certainty evidence).

Further large high-quality RCTs are needed, such as the IMPROV trial, to help improve the certainty of the evidence around alternative regimens.

How up-to-date is this review?

The review authors searched for studies up to 17 December 2018.

SUMMARY OF FINDINGS

Summary of findings for the main comparison. 'Summary of findings' (main comparison)

0.5 mg/kg/day for 7 days versus standard 14-day regimen for radical cure of *P vivax* malaria

Patient or population: adults and children with confirmed clinical and parasitological *P vivax* malaria
Setting: India, Peru, Brazil
Intervention: 0.5 mg/kg/day primaquine for 7 days (adult dose 30 mg)
Comparison: standard 14-day course of primaquine (0.25 mg/kg/day, adult dose 15 mg)

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (trials)	Certainty of the evidence (GRADE)	Comments
	Risk with standard 14-day course primaquine	Risk with 0.5mg/kg/day primaquine for 7 days				
Recurrence of *P vivax* parasitaemia Follow-up: range 6 months to 7 months	89 per 1000	86 per 1000 (59 to 124)	RR 0.96 (0.66 to 1.39)	1211 (4 RCTs)	⊕⊕⊖⊖ LOW[a,b] Due to risk of bias and imprecision	There may be little or no difference between 0.5 mg/kg/day primaquine for 7 days and the standard 14-day course.
Serious adverse effects	See comment	See comment	—	1427 (5 RCTs)	—	No events reported.
Adverse events that result in the discontinuation of treatment	3 per 1000	3 per 1000 (0 to 20)	RR 1.04 (0.15 to 7.38)	1154 (4 RCTs)	⊕⊖⊖⊖ VERY LOW[c,d] due to risk of bias and imprecision	We do not know if there is any difference in adverse events that result in treatment discontinuation between 0.5 mg/kg/day primaquine for 7 days and the standard 14-day course.
Adverse effects known to occur with primaquine	44 per 1000	47 per 1000 (28 to 78)	RR 1.06 (0.64 to 1.76)	1154 (4 RCTs)	⊕⊕⊖⊖ LOW[c,e] Due to risk of bias and imprecision	There may be little or no difference in the frequency of adverse events known to occur with primaquine between 0.5 mg/kg/day primaquine for 7 days and the standard 14-day course.
Anaemia or change in haemoglobin status	0 per 1000	0 per 1000 (0 to 0)	RR 3.0 (0.51 to 174.01)	240 (1 RCT)	⊕⊖⊖⊖ VERY LOW[f,g,h]	We do not know if the occurrence of anaemia differs between the 2 treatment regimens.

					Due to risk of bias, indirectness, and imprecision	
Adverse events known to occur with chloroquine	0 per 1000	0 per 1000 (0 to 0)	RR 9.40 (0.51 to 174.01)	779 (1 RCT)	⊕⊝⊝⊝ VERY LOW[i,j,k] Due to risk of bias, indirectness, and imprecision	We do not know if there is a difference in the number of participants experiencing adverse events known to occur with chlorquine between the 2 treatment groups.

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

Abbreviations: CI: confidence interval; **RCT:** randomized controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded once for risk of bias: Rajgor 2014 IND, which contributed the most weight to the meta-analysis, was at high risk of selection bias due to no allocation concealment and high risk of attrition bias. Although Pareek 2015 IND was at risk of selection bias as well as other bias for being funded and carried out by drug company, it only contributed a small amount of weight to the meta-analysis.
[b]Downgraded once for imprecision: wide CIs - may be 34% reduction in malaria recurrences or 40% increase with 0.5 mg/kg/day primaquine for 7 days.
[c]Downgraded once for risk of bias: Rajgor 2014 IND was at high risk of selection bias due to no allocation concealment and high risk of attrition bias. Pareek 2015 IND was at risk of selection bias as well as other bias for being funded and carried out by drug company.
[d]Downgraded twice for serious imprecision: very few events (only four events occurring in one trial, Rajgor 2014 IND), very wide CIs.
[e]Downgraded once due to imprecision: wide CIs.
[f]Downgraded once due to risk of bias: Pareek 2015 IND was at risk of selection bias and other bias (funded and performed by drug company).
[g]Downgraded once for indirectness: only one study conducted in G6PD-normal adults in India (Pareek 2015 IND).
[h]Downgraded twice for serious imprecision: only one event, very wide CIs.
[i]Downgraded once for risk of bias: Rajgor 2014 IND at risk of bias selection bias due to no allocation concealment and attrition bias.
[j]Downgraded once for indirectness: only one study conducted in G6PD-normal adults in India (Rajgor 2014 IND).
[k]Downgraded twice for serious imprecision: few events, very wide CIs.

Summary of findings 2. 'Summary of findings' table 2

High-standard 14-day regimen versus standard 14-day regimen for radical cure of *P vivax* malaria

Patient or population: adults and children with confirmed clinical and parasitological *P vivax* malaria
Setting: India
Intervention: high-standard 14-day course of primaquine (0.5 mg/kg/day, adult dose 30 mg)
Comparison: standard 14-day course of primaquine (0.25 mg/kg/day, adult dose 15 mg)

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (studies)	Certainty of the evidence (GRADE)	Comments
	Risk with standard 14-day course primaquine	Risk with high-standard 14-day course primaquine				
Recurrence of *P vivax* parasitaemia follow-up: range 6 months to 7 months Blood-stage treatment: chloroquine	81 per 1000	66 per 1000 (34 to 116)	RR 0.82 (0.47 to 1.43)	639 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,b,c] due to indirectness, risk of bias, and imprecision	We do not know if there is any difference in *P vivax* recurrences between high-standard or standard 14-day courses of primaquine given with chloroquine.
Recurrence of *P vivax* parasitaemia follow-up: range 6 months Blood-stage treatment: chloroquine or an ACT	100 per 1000	111 per 1000 (17 to 709)	RR 1.11 (0.17 to 7.09)	38 (1 RCT)	⊕⊖⊖⊖ VERY LOW[d,e,f] due to indirectness, risk of bias, and imprecision	We do not know if there is any difference in *P vivax* recurrences between high-standard or standard 14-day courses of primaquine given with chloroquine or an ACT.
Serious adverse effects	0 per 1000	0 per 1000 (0 to 0)	Not estimable	816 (2 RCTs)	—	No events reported.
Adverse events that result in the discontinuation of treatment	5 per 1000	21 per 1000 (5 to 98)	RR 4.19 (0.90 to 19.60)	778 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,b,d,g] due to indirectness, risk of bias, and imprecision	We do not know if there is any difference in adverse events resulting in treatment discontinuation between high-standard or standard 14-day courses of primaquine.
Adverse effects known to occur with primaquine	13 per 1000	34 per 1000 (12 to 95)	RR 2.72 (0.98 to 7.57)	778 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,b,h] due to indirectness, risk of bias, and imprecision	We do not know if there is any difference in adverse events known to occur with primaquine between high-standard or standard 14-day courses of primaquine.
Adverse events known to occur with chloroquine	0 per 1000	0 per 1000 (0 to 0)	RR 9.43 (0.51 to 174.47)	778 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,b,h] due to indirectness, risk of bias, and imprecision	We do not know if there is any difference in adverse events associated with chloroquine between the 2 treatment groups.

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

Abbreviations: ACT: artemisinin-based combination therapy; **CI:** confidence interval; **RCT:** randomized controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded once for indirectness: only one trial conducted in India in G6PD-normal adults (Rajgor 2014 IND).
[b]Downgraded once for risk of bias: open-label - no allocation concealment, risk of selection bias; risk of attrition bias - high percentage not completing six months' follow-up with minimal explanation.
[c]Downgraded once for imprecision: wide CIs - range of 58% reduction in malaria recurrences at 6 months with high-standard 14-day course of primaquine to 43% increase in number of malaria recurrences.
[d]Downgraded once for indirectness: only one trial conducted in India in G6PD-normal adults (Saravu 2018 IND).
[e]Downgraded once for risk of bias: no blinding, high rate of loss to follow-up.
[f]Downgraded once for imprecision: wide CIs - range of 83% reduction in malaria recurrence to 609% increase in malaria recurrences with the high-standard 14-day regimen.
[g]Downgraded once for imprecision: wide CIs 0.9 to 19.6 - range of 10% reduction in adverse events with high-standard 14-day course to 186% increase in adverse events.
[h]Downgraded once for imprecision: wide CIs.

Summary of findings 3. 'Summary of findings' table 3

0.75 mg/kg primaquine/week for 8 weeks versus high-standard 14-day regimen for radical cure of *P vivax* malaria

Patient or population: adults and children with confirmed clinical and parasitological *P vivax* malaria
Setting: Pakistan
Intervention: 0.75 mg/kg primaquine/week for 8 weeks (adult dose 45 mg)
Comparison: high-standard 14-day course primaquine (0.5 mg/kg/day, adult dose 30 mg)

Outcomes	Anticipated absolute effects* (95% CI)		Relative effect (95% CI)	№ of participants (trials)	Certainty of the evidence (GRADE)	Comments
	Risk with high-standard 14-day course primaquine	Risk with once-weekly 0.75 mg/kg primaquine for 8 weeks				
Recurrence of *P vivax* malaria Follow-up: 8 months	0 per 1000	0 per 1000 (0 to 0)	RR 7.00 (0.38 to 127.32)	126 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,b,c] due to risk of bias, indirectness, and imprecision	We do not know if weekly primaquine reduces the risk of malaria recurrences when compared to the high-standard 14-day course.

Recurrence of *P vivax* malaria Follow-up: 11 months	19 per 1000	59 per 1000 (7 to 511)	RR 3.18 (0.37 to 27.60)	122 (1 RCT)	⊕⊖⊖⊖ VERY LOW[a,b,d] due to risk of bias, indirectness, and imprecision	We do not know if weekly primaquine reduces the risk of malaria recurrences when compared to the high-standard 14-day course.
Serious adverse effects	0 per 1000	0 per 1000 (0 to 0)	Not estimable	129 (1 RCT)	—	No events reported.
Anaemia (haemoglobin < 7 g/dL)	0 per 1000	0 per 1000 (0 to 0)	Not estimable	129 (1 RCT)	—	No events reported.

*__The risk in the intervention group__ (and its 95% CI) is based on the assumed risk in the comparison group and the __relative effect__ of the intervention (and its 95% CI).

Abbreviations: CI: confidence interval; **RCT:** randomized controlled trial; **RR:** risk ratio.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

[a]Downgraded by 1 for risk of bias: Leslie 2008 PAK was at high risk of bias for randomization process, allocation concealment, and incomplete outcome data.
[b]Downgraded by 1 for indirectness: only one study conducted in Pakistan, only one G6PD-deficient adult included in one trial (in weekly arm).
[c]Downgraded by 2 for serious imprecision: few events, very wide CIs.
[d]Downgraded by 2 for serious imprecision: few events, very wide CIs.

BACKGROUND

Malaria is a potentially life-threatening disease caused by the *Plasmodium* parasite, which is transmitted by the bite of an infected female *Anopheles* mosquito. Five species of *Plasmodium* malaria parasites can cause malaria disease in humans, of which *Plasmodium vivax* and *Plasmodium falciparum* are the most important (WHO 2016). In 2017, an estimated 219 million cases of malaria occurred worldwide and an estimated 435,000 people died from the disease (WHO 2018). The World Health Organization (WHO) aims to reduce malaria case load and mortality by at least 90% by 2030 (WHO 2016).

Historically, *P vivax* infection was thought to be a milder form of malaria, and researchers have focused on *P falciparum* due to the high number of deaths it causes (Bassat 2016). In recent years, it's been shown that the morbidity and mortality of *P vivax* have been underestimated, with evidence of direct fatality and contribution to mortality in patients who have other comorbidities, such as malnutrition, HIV, or coexisting infections (Baird 2013; Bhattacharjee 2013; Rizvi 2013; Singh 2013; Battle 2014; Douglas 2014; Kochar 2014; Arévalo-Herrera 2015; Baird 2015b). Repeated *P vivax* infections through childhood and adulthood also affect personal well-being, development, and education and can thus negatively impact economic development, both for the individual and the community (Mendis 2001). *P vivax* malaria in pregnancy is associated with maternal anaemia, spontaneous abortion, stillbirth, and low birthweight, with especially poor pregnancy outcomes for women with severe infection (McGready 2012; Rijken 2012; Brutus 2013).

Description of the condition

P vivax infection caused an estimated 7.5 million cases of malaria in 2017 (WHO 2018). The geographical distribution of*P vivax* malaria is more widespread than any of the other forms of human malaria, with around 35% of the world's population thought to be at risk (Howes 2016)). Co-infection with *P falciparum* is also common in many regions (Kumar 2007; Mueller 2009). As malaria control accelerates, the *P vivax* proportion in co-endemic areas tends to rise compared to that of *P falciparum*, which highlights the importance and challenge of this infection (John 2012).

P vivax is important because as many countries progress towards malaria elimination, the parasite becomes a roadblock to eradication (Cibulskis 2015; Bassat 2016). Despite a reported 45% reduction in *P vivax* malaria cases between 2010 and 2016 (WHO 2017), the parasite has several characteristics that enable it to evade control (Newby 2016). The early appearance of gametocytes in the blood, often prior to symptoms of malaria, increases the chance of onward transmission by mosquitoes (Mendis 2001). *P vivax* differs from *P falciparum* in that as well as having a blood-stage infection, hypnozoites develop in the liver that can be dormant for weeks to months before developing into an infection (White 2011). What triggers these relapses is not well-understood. There is difficulty in distinguishing between relapse (hypnozoite activation), recrudescence (subpar treatment of the initial blood-stage infection), and re-infection (new infection with *P vivax*) (Imwong 2007). A study in Papua New Guinea suggested that relapses cause four-fifths of *P vivax* infections (Robinson 2015), reinforcing the importance of relapse in sustaining transmission (White 2011). Parasites show high genetic diversity, even in countries that are at malaria elimination stage (Koepfli 2015). *P vivax* is likely underestimated worldwide, as the dormant liver stage is not detected in routine surveys (Gething 2012). Submicroscopic infections and asymptomatic infection reservoirs may also lead to underdiagnosis or misdiagnosis. A systematic review showed that across all study sites, the polymerase chain reaction (PCR) prevalence of *P vivax* was significantly higher than that identified by light microscopy (Cheng 2015). The effect this may have on *P vivax* malaria studies is unclear.

There are different strains of *P vivax* according to geographical region/endemicity areas, with relapse patterns that vary by latency (time to first relapse), likelihood of relapse, and frequency of relapses, which further complicates the assessment of efficacy of drugs on relapses (Battle 2014; White 2016). Strains commonly found in Southeast Asia and Oceania (including the 'Chesson' strain isolated from an individual infected in Papua New Guinea) have the shortest latency time to relapse, starting as early as three weeks after first infection (if untreated with a hypnozoiticidal drug) (Ehrman 1945). These areas correspond to zones 10 and 12 in Battle 2014. Indian and Pakistan strains (zone 8) exhibit heterogeneity in relapse latency, incidence, and frequency, while South American strains (zone 3) have a pattern of short latency to first relapse and less frequent relapses than in zones 10 and 12 (Battle 2014). The temperate strains (which include those from Korea in zone 11) relapse much more slowly (John 2012; Battle 2014). Strains of the type in zones 10 and 12, referred to here as 'East Asia and Oceania', are recommended to receive higher doses of primaquine (the high-standard course of 0.5 mg/kg/day rather than standard 0.25 mg/kg/day for 14 days) to prevent relapses (WHO 2015), apparently based on research done in the 1950s and 1960s (Coatney 1953; Jones 1953; Vivona 1961; Maffi 1971; Clyde 1977), although not all these studies were done with strains from the targeted geographic area.

Primaquine, an 8-aminoquinoline, has until very recently been the only drug available on the market for treating the hypnozoite stage of infection (Ashley 2014). One of the main barriers in *P vivax* treatment is the reluctance to use primaquine due to it potentially causing haemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. G6PD deficiency is the most common enzyme deficiency worldwide and affects red blood cells by leading to their premature lysis (Nkhoma 2009). G6PD deficiency is common in countries where *P vivax* malaria is endemic, with an estimated population prevalence of 8% (Howes 2012). Within G6PD deficiency, there are differing phenotypes, meaning some people may be mildly sensitive to primaquine, while others may be very sensitive and experience life-threatening haemolysis (Baird 2015a), which explains the varying responses to primaquine. In many areas where *P vivax* is predominant, testing for G6PD deficiency is not available locally (Baird 2015b). In 2018 the US Food and Drug Administration (FDA) approved a newer alternative, another 8-aminoquinoline known as tafenoquine (MMV 2018), which has shown promise in reducing relapses, but there are increased safety concerns in patients with undiagnosed G6PD deficiency compared to primaquine, due to its longer half-life (Rajapakse 2015).

Description of the intervention

People with *P vivax* malaria require treatment with an antimalarial drug to treat the blood-stage infection, and a drug to treat the hypnozoite stage (radical cure). The WHO recommends treatment with either chloroquine or an artemisinin-based combination therapy (ACT) for the blood-stage infection, with 0.25 to 0.5 mg/kg/day primaquine for 14 days for the liver stages (WHO 2015). Artemisinin-based combination therapies and chloroquine have been shown to

be effective and comparable in treating the blood-stage infection of *P vivax* malaria (Gogtay 2013).

A previous Cochrane Review showed that primaquine regimens of five days or fewer had similar recurrence rates to placebo or no primaquine. Of the comparisons included in the review, a regimen of 0.25 mg/kg/day (15 mg) a day of primaquine for 14 days had the lowest recurrence rates of *P vivax* infection (Galappaththy 2013). There were no trials at that time that compared higher doses of primaquine at 14 or 7 days.

Primaquine was first made available to North American soldiers in the 1950s (Baird 2004). Its mechanism and metabolism are not widely understood, but it has a broad spectrum of activity against the *Plasmodium* parasite. As well as preventing relapse of *P vivax* malaria by targeting the latent and developing hypnozoites in the liver, it is also used in malaria prophylaxis (Baird 2003). It is absorbed from the gastrointestinal tract, has a half-life of about four to nine hours, and crosses the placenta in pregnancy (Baird 2004). New advancements in studying *P vivax* in humanized mice may lead to a greater understanding of the mechanism of action of the drug (Mikolajczak 2015).

Adverse effects of primaquine include production of methaemoglobin, an oxidated state of haemoglobin that cannot transport oxygen to tissues. Methaemoglobinaemia (an abnormal buildup of methaemoglobin) can result in cyanosis when levels exceed 10% of the usual haemoglobin level (Vale 2009). As described above, primaquine causes haemolysis in people with G6PD deficiency, which leads to anaemia (Ashley 2014). When taken on an empty stomach it can cause abdominal pain and gastrointestinal upset (Vale 2009). Safe use of primaquine during pregnancy has not been established. The radical cure with primaquine can be delayed until after pregnancy. With regard to breastfeeding patients, a recent study showed that the levels of primaquine in breast milk may not be sufficient to cause haemolysis even in a G6PD-deficient baby (Gilder 2018), but it is not recommended at this time.

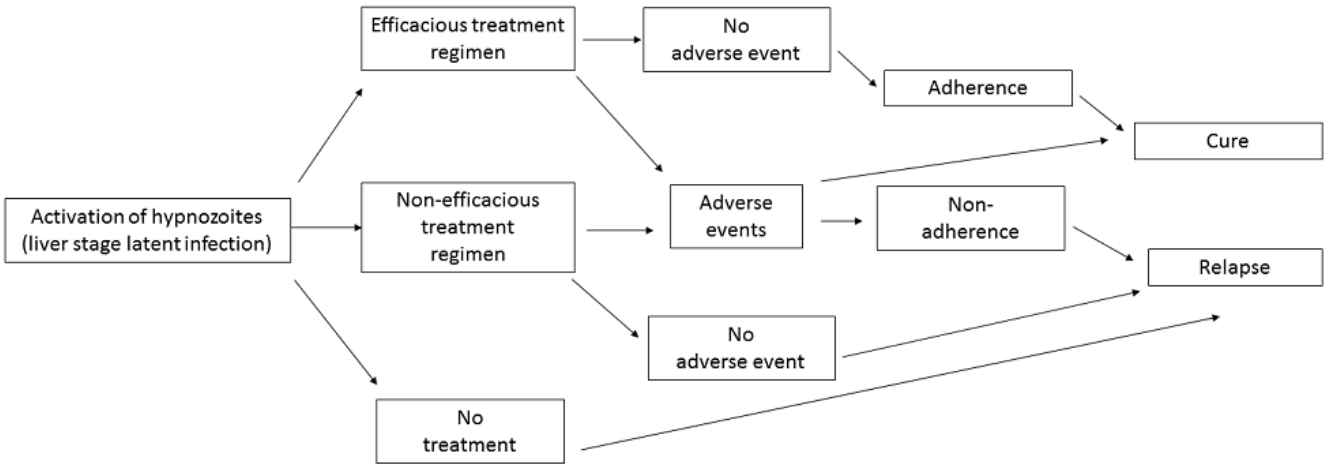
How the intervention might work

The WHO advises that 0.25 mg to 0.5 mg/kg/day of primaquine for 14 days should be used for radical cure of *P vivax* malaria in patients over six months old, excluding people with G6PD deficiency and those who are pregnant or breastfeeding (WHO 2015).

There has been suggestion of failure of the regimen of 0.25 mg/kg/day for 14 days (hereafter referred to as the 'standard 14-day regimen') of primaquine for the Chesson strain of *P vivax*, which was behind the suggestion of the increased dosing of 0.5 mg/kg/day in East Asia and Oceania (hereafter referred to as the 'high-standard 14-day regimen'). However, evidence for the choice of the high-standard regimen is not presented in the WHO treatment guidelines (WHO 2015). The previous Cochrane Review, Galappaththy 2013, found no trials that compared the high-standard 14-day regimen to the standard 14-day regimen. The WHO recommends a weekly dose of 0.75 mg/kg for eight weeks for patients with G6PD deficiency, but the evidence for this is of low quality, as there are few high-quality trials (WHO 2015).

The 14-day course of primaquine can lead to treatment adherence issues, as well as to safety concerns about haemolysis in places where G6PD testing is not available, meaning that shorter courses of primaquine are desirable. Failure to treat the hypnozoite stage of *P vivax* malaria leads to repeated relapses, morbidity, and persistent infection. The logic framework for developing efficacious and safe treatment regimens for *P vivax* is illustrated in Figure 1.

Figure 1. Logic framework: treatment outcome pathways in *Plasmodium vivax* liver hypnozoite activation.



It has long been suggested that it may be the total dose of primaquine that is important in the treatment of the hypnozoite stage rather than the length of the course (Schmidt 1977). If a higher dose of primaquine could be administered safely over a shorter period of time, it may improve adherence rates, thus reducing relapse rates and morbidity and mortality resulting from *P vivax* infection. There are small trials from the 1970s that suggest that shorter, higher-dose regimens were as efficacious as the 14-day courses (Clyde 1977; Saint-Yves 1977). At the time of the previous Cochrane Review (Galappaththy 2013), there were no recent large high-quality trials that had investigated the use of higher doses given over seven days. We planned to include any such trials in this Cochrane Review.

Why it is important to do this review

The use of primaquine for radical cure of *P vivax* malaria continues to pose a therapeutic dilemma for healthcare providers in areas without adequate screening for G6PD status. Clinicians must either choose to give primaquine and risk haemolysis if the patient is G6PD-deficient, or withhold treatment and accept the complications of ongoing parasite infection and relapses. This is why when clinicians choose to treat with primaquine they prefer a lower dose over a more prolonged period, which then risks difficulties with adherence and thus reduced effectiveness.

We know from the previous Cochrane Review on primaquine with chloroquine for radical cure that the standard 14-day regimen of 0.25 mg/kg/day (15 mg per day or 210 mg total dose) is better than shorter regimens of similar daily doses and placebo (Galappaththy 2013). In fact, the regimen of 0.25 mg/kg/day for 5 days of primaquine did not reduce recurrences compared to treating with chloroquine alone.

A major problem with the radical cure of *P vivax* is difficulty with the adherence of the 14-day course of primaquine, which has led to many countries shortening the regimen. Peru was one such example, although a study revealed that patients often still discontinued the therapy after around three days, when they started to feel better (Grietens 2010). A study that compared directly observed therapy (DOT) for 14 days of primaquine versus non-DOT primaquine found that the *P vivax* recurrence rate was significantly lower in the DOT group (Takeuchi 2010). These problems have led to a more urgent call for shorter treatment regimens. Various trials are investigating regimens that revise dosing and duration of treatment in order to improve adherence and reduce the potential for incomplete treatment and development of resistance. As mentioned previously, the significance of the total cumulative primaquine dose given, rather than the length of the course, is one avenue of investigation. In areas where G6PD screening is present, using higher dosing regimens over shorter time periods, if at least similarly efficacious, could improve adherence and reduce morbidity associated with *P vivax* parasitaemia.

World Health Organization guidelines suggest a higher dosing regimen of primaquine for the tropical, frequent-relapsing strain of *P vivax* in East Asia and Oceania (WHO 2015), although the previous Cochrane Review, Galappaththy 2013, did not find any trials assessing this. Investigating the evidence base for this is therefore important. The 2015 WHO guidelines also suggest an alternate dosing regimen of weekly primaquine, which may be safer in patients with G6PD deficiency. As the previous Cochrane Review included data from only one trial assessing this, it is useful to investigate whether there is further evidence to substantiate this guidance.

In this Cochrane Review, we have excluded comparisons between blood-stage drug (chloroquine/ACT) with and without primaquine, as the rationale for primaquine use has been sufficiently demonstrated in a previous Cochrane Review (Galappaththy 2013). Similarly, we have not included comparisons between different blood-stage drugs in which the same dose of primaquine was used; an update to an existing Cochrane Review, Gogtay 2013, is in progress and will address this. However, we planned to stratify our results according to partner drug, as there is increasing evidence that primaquine is metabolized via the cytochrome P450 2D6 (CYP2D6) pathway (Bennett 2013), and efficacy may thus be affected if the blood-stage antimalarial drug is a CYP2D6 inhibitor (Baird 2018).

This review excluded comparisons of regimens that do not use the control of the standard or high-standard regimen of 14 days of primaquine. Also, it did not include comparisons of primaquine regimens of 0.25 mg/kg/day for less than 14 days, as Galappaththy 2013 has already assessed these shorter regimens of the same daily dose.

There is currently a lack of consensus among studies as to what the minimum time frame for follow-up of relapse in *P vivax* malaria should be. The WHO guidance on clinical trials in malaria sets out standard follow-up for blood (or schizontal) stage infection as 28 days after treatment commencement, but has no clear definition on the follow-up period for radical cure in primaquine studies. It states that "follow up varies from three months to a year in the literature, and should be adapted to regional parasite characteristics" (WHO 2009). In a recent review, John 2012 described relapse of the tropical frequently relapsing strain of *P vivax* as typically three weeks, but this varies according to blood-stage treatment: "three weeks following quinine therapy" and "six to eight weeks following chloroquine" (White 2011). With exposure to primaquine - even if radical cure is not achieved - relapses may occur at longer intervals (Sutanto 2013). In the Cochrane Review (Galappaththy 2013), the follow-up period started 30 days after completing primaquine treatment. Relapse is frequently defined as the presence of *P vivax* parasites more than 28 to 30 days after the full course of primaquine in people living in a non-endemic area (Looareesuwan 1997). Due to the varying lengths of treatment and relapse time in *P vivax* malaria, 28 days from treatment completion may not allow true assessment of radical cure. It also makes assessment of the weekly primaquine regimen difficult, as the follow-up time should start before the eight-week treatment course has finished. In this Cochrane Review we planned to assess parasitaemia at 3, 6, and 12 months' follow-up, in keeping with WHO guidance. We intended to describe the length of follow-up across studies, and then group them into meaningful lengths of follow-up, depending on the regimen.

We intended to answer the following questions by comparing the new regimens to the standard 14-day regimen of primaquine (0.25 mg/kg/day; 15 mg adult dose) or the high-standard 14-day regimen (0.5 mg/kg/day; 30 mg adult dose) recommended for East Asia/Oceania.

- Is the high-standard 14-day regimen more efficacious and safer compared to the standard 14-day course in all areas, or only in areas where it is recommended (East Asia and Oceania)?
- Are shorter, higher-dose regimens (0.5 mg/kg/day for 7 days) as efficacious and safe as the standard 14-day regimen?
- Are weekly dosing regimens (0.75 mg/kg or 45 mg adult dose/week for 8 weeks) as efficacious and safe as the standard or high-standard 14-day regimen?

OBJECTIVES

To assess the efficacy and safety of alternative primaquine regimens for radical cure of *P vivax* malaria compared to the standard or high-standard 14 days of primaquine (0.25 or 0.5 mg/kg/day), as well as comparison of these two WHO-recommended regimens.

METHODS

Criteria for considering studies for this review

Types of studies

Randomized controlled trials (RCTs). We excluded quasi-RCTs.

Types of participants

Adults and children with confirmed clinical and parasitological (light microscopy or PCR, or both) diagnosis of *P vivax* malaria. We included trials that excluded people with G6PD deficiency and trials that included populations that had or had not been screened for G6PD deficiency. People with mixed malaria infections were excluded.

Types of interventions

Intervention

Any regimen of either chloroquine or an artemisinin-based combination therapy (ACT) plus primaquine with any of the following.

- Daily doses higher than 0.25 mg/kg/day for 14 days.
- Shorter regimens with the same total dose.
- Weekly dosing regimens.

Control

WHO-defined standard regimen of 14 days of primaquine at 0.25 mg/kg/day (15 mg adult dose) in most areas, or high-standard regimen of 0.5 mg/kg/day (30 mg adult dose) in East Asia and Oceania, plus either chloroquine or an ACT.

We included comparisons between the two WHO recommended 14 day regimes (0.25mg/kg/day and 0.5mg/kg/day). We included trials that used chloroquine or ACT as the treatment for blood-borne infection, and we planned to stratify by the blood schizonticidal agent.

Types of outcome measures

Primary outcomes

- *P vivax* parasitaemia (detected by light microscopy or PCR, or both) at 3, 6, and 12 months' follow-up. We planned to describe this as recurrences of *P vivax* malaria due to the previously mentioned difficulties in distinguishing between relapse and re-infection.

Secondary outcomes

- *P vivax* parasitaemia (detected by light microscopy or PCR, or both) at one to three months' follow-up.

Adverse events

- Serious adverse events (fatal, life-threatening, or requiring hospitalization).
- Adverse events that result in discontinuation of treatment.
- Events known to occur with primaquine (cyanosis, leucopenia, methaemoglobinaemia, hypertension, cardiac arrhythmia, abdominal pain, nausea, vomiting, or haemolysis) or those due to a comparator drug used along with primaquine.
- Anaemia or change in haemoglobin status.
- Other adverse events.

Search methods for identification of studies

We attempted to identify all relevant studies regardless of language or publication status (published, unpublished, in press, or in progress).

Electronic searches

We searched the following databases using the search terms and strategy described in Appendix 1: the Cochrane Infectious Diseases Group Specialized Register (17 December 2018); the Cochrane Central Register of Controlled Trials (CENTRAL, 2018, Issue 12, published in the Cochrane Library); MEDLINE (PubMed, 1946 to 17 December 2018); Embase (Ovid, 1947 to 17 December 2018); and LILACS (Bireme, 1982 to 17 December 2018). We also searched the World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/), and ClinicalTrials.gov (clinicaltrials.gov/ct2/home), for trials in progress, on 17 December 2018, using "primaquine" and "vivax" as search terms.

Searching other resources

We checked the reference lists of all studies identified by the above methods for additional potentially relevant studies. We contacted researchers working in the field and the WHO for unpublished and ongoing trials. We also searched the reference lists and included studies of the Cochrane Review by Galappaththy 2013.

Data collection and analysis

Selection of studies

Two review authors independently screened the titles and abstracts of the search results to identify potentially eligible trials, coding the articles as either 'retrieve' or 'do not retrieve'. We obtained the full-text reports of potentially eligible trials and assessed them for inclusion in the review using a predesigned eligibility form based on the inclusion criteria. Any discrepancies were resolved through discussion or by consulting a third review author if necessary. Where necessary, we contacted the trial authors for clarification of trial methods. We listed the excluded trials and the reasons for their exclusion in a 'Characteristics of excluded studies' table. Where there were multiple reports relating to the same trial, we planned to include all reports and collate data. We detailed the trial selection process in a PRISMA diagram.

Data extraction and management

Two review authors independently extracted data from the included trials using a data extraction form designed specifically for this review, in keeping with Cochrane guidance (Higgins 2011).

For each included trial we extracted a minimum of the following data where available.

- Study design.
- Endemicity/population demographics.
- G6PD status of participants (known/unknown).
- CYP2D6 status (if available).
- Blood-stage antimalarial drug choice.
- Dose/duration/timing of treatment arms.
- Supervised or non-supervised therapy.
- Duration of follow-up.
- Adverse events.

- Reported outcomes.

Any differences in data extraction were resolved through discussion or by consulting a third review author if necessary. We entered the extracted data into Review Manager 5 (RevMan 2014). Where necessary, we contacted the authors of primary trials regarding missing data or methodological details of the trial. We noted any limitations in the included studies.

We grouped comparisons as illustrated in Table 1.

Assessment of risk of bias in included studies

Two review authors independently assessed the risk of bias of each included trial using the Cochrane 'Risk of bias' assessment tool, discussing any differences of opinion. In the case of missing or unclear information, we contacted the trial authors for clarification. We summarized the results in the 'Risk of bias' tables in the 'Characteristics of included studies' tables (Higgins 2011).

Measures of treatment effect

For dichotomous data, we compared interventions using risk ratios (RRs) to measure treatment effect. Where trial authors presented data as odds ratios, we recalculated the effect. We defined statistical significance as $P < 0.05$ and calculated 95% confidence intervals (CIs) for all results. For comparable trials, we performed meta-analyses if there were sufficient data.

Unit of analysis issues

We split trials that included more than two comparison groups and analysed them as individual pair-wise comparisons. If there was a shared control group, we split the control group so that participants were only counted once in the overall meta-analysis.

Dealing with missing data

We analysed missing data using available-case analysis if we judged the trial to be at low risk of bias for incomplete outcome data. We attempted to contact trial authors to obtain missing or unclear data. If the missing data rendered the result uninterpretable, we excluded the data from meta-analyses and clearly stated the reason for exclusion. If the missing data meant that results were interpretable but likely to be at high risk of bias, we used imputation methods to investigate the impact of the missing data. We analysed extracted data on an intention-to-treat basis where there were no missing data.

Assessment of heterogeneity

We inspected forest plots for overlapping CIs. We also applied the Chi^2 test as a statistical test for the presence of heterogeneity, with a P value of 0.10 used to indicate statistical significance, and we computed the I^2 statistic to quantify the percentage of the variability in effect estimates that was due to heterogeneity rather than sampling error (chance). We investigated possible causes of heterogeneity by subgroup analysis. If substantive heterogeneity persisted, defined as an I^2 statistic value of greater than 50%, we used a random-effects meta-analysis.

Assessment of reporting biases

We planned to examine the likelihood of reporting bias using funnel plots, however the number of included trials was insufficient to permit this.

Data synthesis

We analysed the data using Review Manager 5 (RevMan 2014). We assessed the certainty of the evidence for each outcome measure using the GRADE approach, and we constructed 'Summary of findings' tables using GRADEpro GDT (GRADEpro GDT 2015). We stratified results according to blood-stage partner drug (if different blood-stage antimalarials were used, which only occurred for one comparison). Length of follow-up varied with regimens and between studies. We described regimens and follow-up periods and defined sensible groupings for follow-up. We also performed subgroup analyses according to geographical region/endemicity and directly observed therapy (DOT) or non-DOT. We stratified results by length of follow-up. We had planned to perform a subgroup analysis according to CYP2D6 status, however data were insufficient to permit this.

Subgroup analysis and investigation of heterogeneity

We grouped the analysis by drug regimen. We described the interventions and outcomes in all included trials. We conducted an inventory of length of follow-up against each drug regimen and then grouped *P vivax* parasitaemia recurrence by appropriate groupings for length of follow-up.

Sensitivity analysis

We planned to assess the risk of bias of studies that contributed data to the meta-analyses for the prespecified outcomes with sensitivity analyses against concealment of allocation.

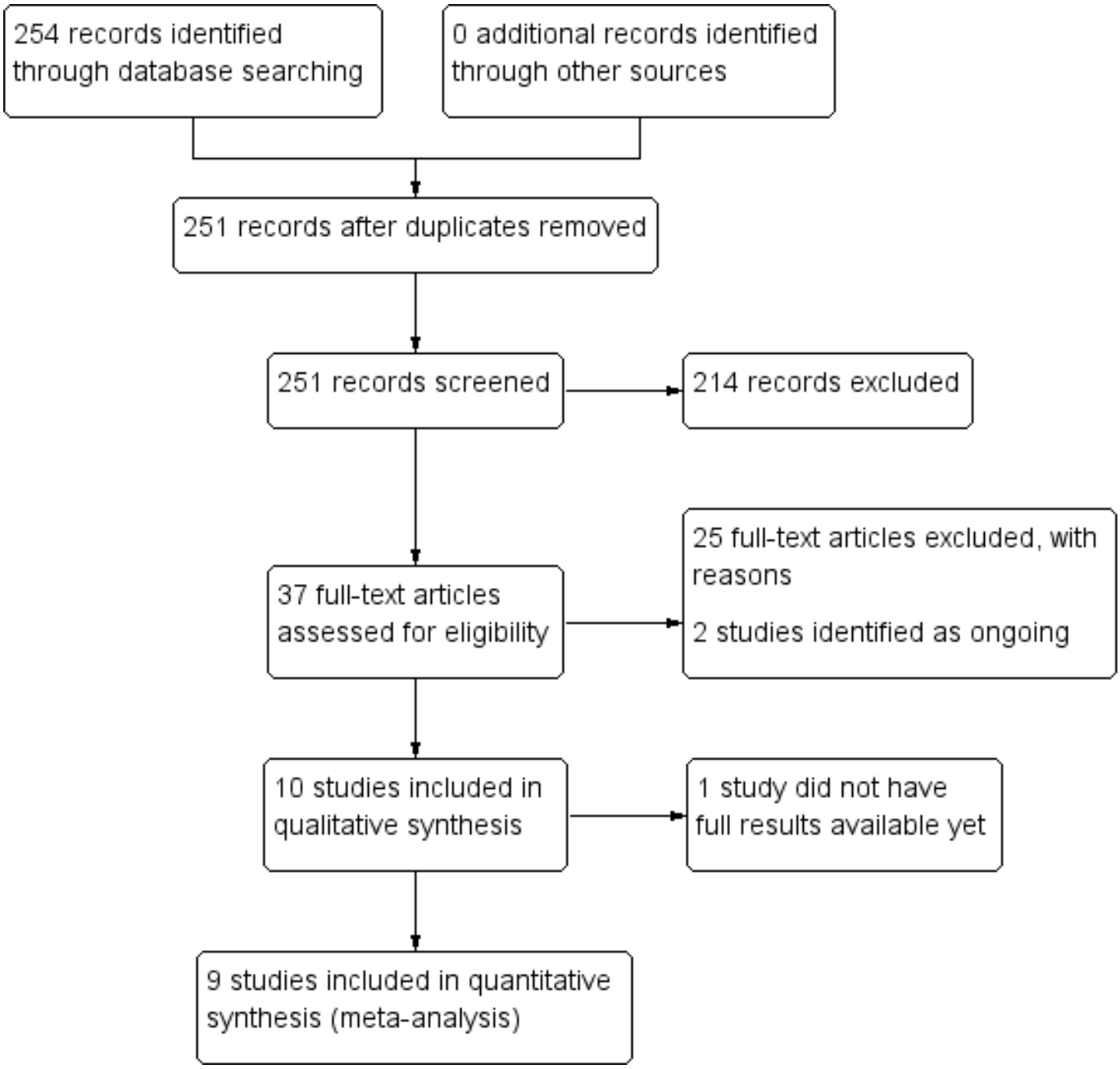
RESULTS

Description of studies

Results of the search

Our database search, conducted up to 17 December 2018, identified 251 studies (after removal of 3 duplicates). We excluded 214 articles during abstract screening, and selected 37 studies for full-text review. We excluded 25 studies with reasons provided; identified two trials as ongoing; and included 10 studies in the review. The search results are presented in a PRISMA diagram in Figure 2.

Figure 2. Study flow diagram.



Included studies

Although 10 studies (of 10 trials) met our inclusion criteria, one study was deemed ineligible for data extraction and analysis. There were only partially available results available in a conference abstract for Chu 2016 THA, meaning that we could not assess risk of bias or analyse results. We contacted the author for the full results but this was declined pending future publication. We included nine studies (of nine trials) in our quantitative analysis.

Four trials were conducted in South America: one in Colombia (Carmona-Fonseca 2009 COL), one in Brazil (Abdon 2001 BRA), and two in Peru (Solari-Soto 2002 PER; Durand 2014 PER). Five trials were conducted in Asia: one in Pakistan (Leslie 2008 PAK), one in Thailand (Bunnag 1994 THA), and three in India (Rajgor 2014 IND; Pareek 2015 IND; Saravu 2018 IND). All nine trials included data for adults, and four trials included children under the age of 10 years (Solari-Soto 2002 PER; Leslie 2008 PAK; Carmona-Fonseca 2009 COL; Durand 2014 PER). No trials had information on children under one year old.

Seven trials excluded pregnant women, and two trials did not specify whether or not pregnant women were included (Bunnag 1994 THA; Solari-Soto 2002 PER). Six trials specified that lactating women were excluded, while the remaining three trials did not provide details regarding this (Bunnag 1994 THA; Solari-Soto 2002 PER; Carmona-Fonseca 2009 COL). Only one trial included people with G6PD deficiency (Leslie 2008 PAK). Six trials excluded people with

G6PD deficiency (Bunnag 1994 THA; Carmona-Fonseca 2009 COL; Durand 2014 PER; Rajgor 2014 IND; Pareek 2015 IND; Saravu 2018 IND), and two trials did not specify whether or not people with G6PD deficiency were included (Abdon 2001 BRA; Solari-Soto 2002 PER). All of the trials used microscopy for diagnosis of parasitaemia. Four trials carried out PCR genotyping of *vivax* parasitaemia as well (Durand 2014 PER; Rajgor 2014 IND; Pareek 2015 IND; Saravu 2018 IND).

Two trials used different doses or regimens of chloroquine within trial arms, but as both confirmed that parasitaemia had resolved following treatment, we still included them in the review (see Characteristics of included studies) (Bunnag 1994 THA; Abdon 2001 BRA). None of the included trials described the CYP2D6 status of participants.

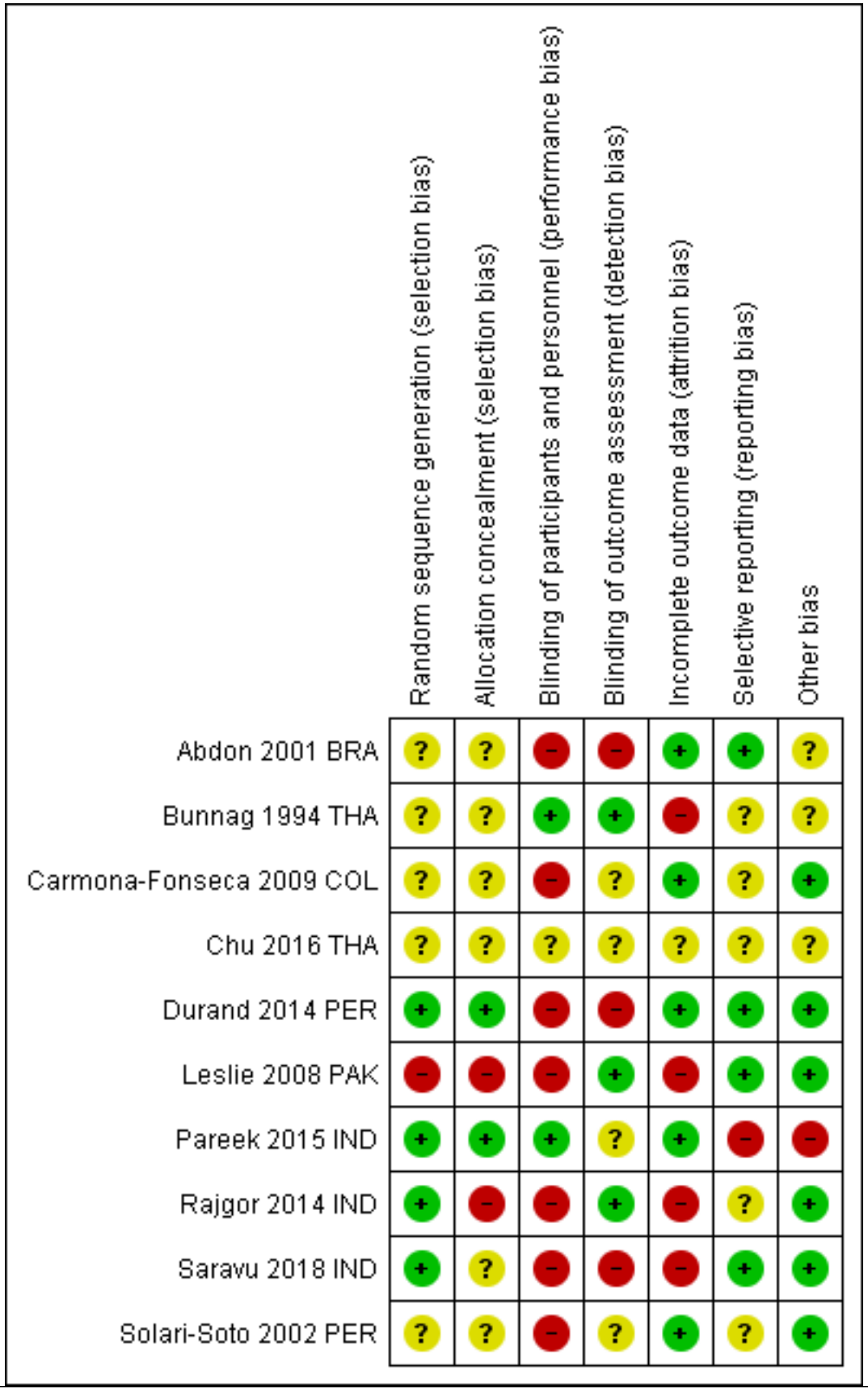
Excluded studies

We excluded 25 studies during full-text screening; see details in Characteristics of excluded studies.

Risk of bias in included studies

A summary of the 'Risk of bias' assessments is presented in Figure 3. Full details are shown in the Characteristics of included studies tables.

Figure 3. 'Risk of bias' summary: review authors' judgements about each 'Risk of bias' item for each included study.



Allocation

Four trials described adequate methods of treatment randomization and were judged to be at low risk of selection bias (Durand 2014 PER; Rajgor 2014 IND; Pareek 2015 IND; Saravu 2018 IND). We assessed one trial as being at high risk of bias as it used two different methods of randomization depending on location, using house numbers or sequential patient numbers (Leslie 2008 PAK). Four trials did not detail the randomization process (Bunnag 1994 THA; Abdon 2001 BRA; Solari-Soto 2002 PER; Carmona-Fonseca 2009 COL).

Two trials used sealed envelopes to conceal allocation and so were assessed as being at low risk of bias (Durand 2014 PER; Pareek 2015 IND). We assessed two trials with no concealment of treatment allocation as at high risk of bias (Leslie 2008 PAK; Rajgor 2014 IND), while five trials provided no information on whether or not allocation concealment was used(Bunnag 1994 THA; Abdon 2001 BRA; Solari-Soto 2002 PER; Carmona-Fonseca 2009 COL; Saravu 2018 IND).

Blinding

Seven trials were open-label and were assessed as at high risk of performance bias (Abdon 2001 BRA; Solari-Soto 2002 PER; Leslie 2008 PAK; Carmona-Fonseca 2009 COL; Durand 2014 PER; Rajgor 2014 IND; Saravu 2018 IND); two of these trials reported blinding of the microscopists who analysed the blood work (Leslie 2008 PAK; Rajgor 2014 IND). Two trials reported blinding of participants and personnel and were classified as being at low risk of bias (Bunnag 1994 THA; Pareek 2015 IND).

Incomplete outcome data

Five trials had low rates of attrition with losses accounted for and so were judged as at low risk of attrition bias (Abdon 2001 BRA; Solari-Soto 2002 PER; Carmona-Fonseca 2009 COL; Durand 2014 PER; Pareek 2015 IND). We assessed four trials as at high risk of attrition bias. Bunnag 1994 THA had unexplained, significant loss to follow-up (more than three-quarters of participants by the end of the trial), making the results uninterpretable. Leslie 2008 PAK had a higher loss to follow-up in the intervention group compared to the control group (6% loss versus 1% loss). Rajgor 2014 IND had a high percentage of missing results at six months. Saravu 2018 IND had a high percentage of loss to follow-up in both arms by six months.

Selective reporting

We judged four trials to have adequately reported on either prespecified or expected outcomes (Abdon 2001 BRA; Leslie 2008 PAK; Durand 2014 PER; Saravu 2018 IND). Risk of reporting bias was unclear for five trials as no protocols were available (Bunnag 1994 THA; Solari-Soto 2002 PER; Carmona-Fonseca 2009 COL; Rajgor 2014 IND; Chu 2016 THA). We assessed Pareek 2015 IND as being at high risk of reporting bias because compliance was added as an outcome, primaquine levels were not reported as planned, and PCR results were not well-detailed.

Other potential sources of bias

We judged Pareek 2015 IND to be at high risk of other bias as it was funded by the drug company that manufactured the primaquine preparations, and the authors were employees of the company. We assessed six trials as at low risk of other bias (Solari-Soto 2002 PER; Leslie 2008 PAK; Carmona-Fonseca 2009 COL; Durand 2014 PER; Rajgor 2014 IND; Saravu 2018 IND). We assessed two trials for which funding was not detailed as at unclear risk of other bias (Bunnag 1994 THA; Abdon 2001 BRA).

Effects of interventions

See: **Summary of findings for the main comparison** 'Summary of findings' (main comparison); **Summary of findings 2** 'Summary of findings' table 2; **Summary of findings 3** 'Summary of findings' table 3

High-standard 14-day regimen versus standard 14-day regimen

The WHO recommends higher doses of primaquine (0.5 mg/kg/day) for 14 days in East Asia and Oceania. We intended to examine whether this high-standard regimen was more efficacious in areas where it is currently recommended (because of assumed resistance - East Asia, Oceania) as well as in all other areas where resistance is not thought to occur.

Two trials compared the high-standard 14-day course with the standard (0.25 mg/kg/day) 14-day course, both carried out in adults in India (Rajgor 2014 IND; Saravu 2018 IND). Both trials excluded pregnant/lactating and G6PD-deficient patients. In Rajgor 2014 IND, participants were treated with chloroquine, with the primaquine regimen (which was supervised) given after completion of the chloroquine course. In Saravu 2018 IND, participants were treated with either chloroquine or an ACT (artesunate with doxycycline or artemether-lumefantrine), and (unsupervised) primaquine was given after completion of the blood-stage treatment. We planned to stratify results according to blood-stage treatment; however, Saravu 2018 IND combined the results for both blood-stage treatments, so we were unable to separate results according to partner drug. Only the blood-stage drugs given to participants who had recurrences were described. For this reason, results from the two studies are not combined and are presented separately.

Efficacy

In Rajgor 2014 IND, 21 participants out of 317 in the high-standard 14-day group had a recurrence of *vivax* malaria compared with 26 out of 322 in the standard 14-day group at 6 months' follow-up, giving an 18% reduction in recurrence of parasitaemia in the high-standard group (risk ratio (RR) 0.82, 95% confidence intervals (CI) 0.47 to 1.43; 639 participants; very low-certainty evidence; Analysis 1.1). *Vivax* malaria recurrences were also investigated by PCR to determine whether they were true relapses or new infections. After this adjustment, results showed an 83% increase in *vivax* malaria cases in the high-standard group (RR 1.83, 95% CI 0.62 to 5.40; Analysis 1.2).

Rajgor 2014 IND was at high risk of bias for allocation concealment. However, as we chose not to combine the data with Saravu 2018 IND for this analysis, a sensitivity analysis could not be done.

In Saravu 2018 IND, 2 out of 18 participants in the high-standard 14-day group had a recurrence of *P vivax* malaria compared to 2 out of 20 in the standard 14-day group at 6 months' follow-up (RR 1.11, 95% CI 0.17 to 7.09; Analysis 1.1). Both of the recurrences in the high-standard 14-day group were given chloroquine. Of the recurrences in the standard 14-day group, one participant received chloroquine and one participant received artesunate and doxycy-

cline. Polymerase chain reaction genotyping suggested that all four participants had true relapses of infection.

It should be noted that because Saravu 2018 IND was a small pilot trial, if we had not stratified according to blood-stage treatment, results would have been largely the same as for Rajgor 2014 IND alone.

Adverse effects

In Rajgor 2014 IND there were no serious adverse events were reported in either study arm (778 participants). In the high-standard 14-day group, 8 out of 380 participants discontinued treatment due to adverse events, compared to 2 out of 398 in the standard 14-day group (RR 4.19, 95% CI 0.90 to 19.60; 778 participants; very low-certainty evidence; Analysis 1.4). In the high-standard arm, 13 out of 380 participants experienced adverse events known to occur with primaquine, compared to 5 out of 398 in the standard arm (RR 2.72, 95% CI 0.98 to 7.57; 778 participants; very low-certainty evidence; Analysis 1.5). In the high-standard arm, 4 out of 380 participants experienced adverse events known to occur with the blood-stage antimalarial chloroquine, compared to 0 out of 398 in the standard group (RR 9.43, 95% CI 0.51 to 174.47; 778 participants; very low-certainty evidence; Analysis 1.6). This could suggest a trend towards a higher occurrence of adverse events in the high-standard 14-day regimen.

No significant adverse events were noted in either group in Saravu 2018 IND.

0.5 mg/kg/day for 7 days versus standard 14-day regimen

This comparison aimed to investigate whether shorter, higher-dose regimens of primaquine over 7 days are as efficacious as standard treatment over 14 days to determine whether the total dose rather than the length of treatment is an important factor (total dose 210 mg).

Five trials in India and South America compared 0.5 mg/kg/day of primaquine for 7 days versus the standard (0.25 mg/kg/day) 14-day regimen (same total dose 210 mg) (Abdon 2001 BRA; Solari-Soto 2002 PER; Durand 2014 PER; Rajgor 2014 IND; Pareek 2015 IND). Pareek 2015 IND used a sustained-release preparation of primaquine in two of the study arms (0.5 mg/kg/day sustained release and 0.25 mg/kg/day sustained release) and standard primaquine at 0.25mg/kg/day in a third arm. We included the 0.5 mg/kg/day sustained release in the analysis and combined the results with the standard preparation at the same dose used for the other trials, but used only the standard-preparation group of 0.25 mg/kg/day in the study as the control group and did not include the arm of 0.25mg/kg/day sustained release preparation.

Three trials excluded people with G6PD deficiency, while two trials did not provide this information (Bunnag 1994 THA; Solari-Soto 2002 PER). All but one trial excluded women who were pregnant or lactating (Solari-Soto 2002 PER did not provide details). Participants were a mixture of adults and children over one year old. All trials used microscopy for diagnosis, and only Pareek 2015 IND did not use supervised treatment. Two trials gave chloroquine and primaquine courses simultaneously (Abdon 2001 BRA; Durand 2014 PER), while the other three trials administered primaquine following the chloroquine course. No trials stratified by age, so results were combined.

Efficacy

There was minimal difference in the number of malaria recurrences between groups at 6 to 7 months' follow-up (RR 0.96, 95% CI 0.66 to 1.39; 1211 participants; low-certainty evidence; Analysis 2.1). One trial only followed participants for two months (Solari-Soto 2002 PER), and so was not part of the main analysis.

We had planned to perform a sensitivity analysis based on risk of bias for allocation concealment (which would have involved removing Rajgor 2014 IND from the meta-analysis), but we decided that as the remaining trials were all at high risk of bias for blinding and thus quality was generally low, we would not conduct a sensitivity analysis but address these issues in our GRADE assessment.

Two trials PCR-adjusted their results to differentiate between relapses and new infections at 6 to 7 months' follow-up. In Durand 2014 PER, PCR-adjusted results showed a 31% reduction in recurrence (24% reduction with light microscopy) with the regimen of 0.5 mg/kg/day for 7 days compared with the standard 14-day course, while in Rajgor 2014 IND, PCR-adjusted results showed a 159% increase in recurrence (25% increase in recurrence with light microscopy) with the regimen of 0.5 mg/kg/day for 7 days compared to the standard 14-day regimen (Analysis 2.2). We decided that these results could not be combined in a meta-analysis, as PCR techniques can differ, and there were high levels of heterogeneity.

We performed a subgroup analysis according to geographic region (Analysis 2.3). For trials in South America, the regimen of 0.5 mg/kg/day for 7 days led to a 30% reduction in *P vivax* recurrences compared to a 19% increase in recurrences for trials in Asia, although confidence intervals were wide and included no effect for both subgroups (South America: RR 0.70, 95% CI 0.39 to 1.26; Asia: RR 1.19, 95% CI 0.73 to 1.94). Only one trial did not use directly observed therapy (DOT) (Pareek 2015 IND). Subgroup analysis (Analysis 2.4) showed that with DOT there was minimal difference in recurrences at 6 to 7 months between treatment regimens (RR 0.98, 95% CI 0.67 to 1.43) compared to a reduction of about half of recurrences with the regimen of 0.5 mg/kg/day for 7 days when treatment was not supervised (RR 0.48, 95% CI 0.04 to 5.20).

Adverse effects

No serious adverse events were reported in either group (1427 participants). The number of participants experiencing adverse events leading to discontinuation of treatment was similar in both groups (RR 1.04, 95% CI 0.15 to 7.38; 1154 participants; Analysis 2.6), as were adverse events known to occur with primaquine (RR 1.06, 95% CI 0.64 to 1.76; 1154 participants; Analysis 2.7). One trial reported on change in haemoglobin status (Pareek 2015 IND), with 1 participant out of 120 in the group receiving 0.5 mg/kg/day for 7 days becoming anaemic, versus no participants out of 120 in the standard 14-day regimen group (RR 3.0, 95% CI 0.51 to 174.01; 240 participants; very low-certainty evidence; Analysis 2.8). Only one study reported on adverse events known to occur with chloroquine (Rajgor 2014 IND), with more occurring in the group receiving 0.5 mg/kg/day for 7 days than the standard 14-day group (RR 9.40, 95% CI 0.51 to 174.01; 779 participants; very low-certainty evidence; Analysis 2.9).

0.75 mg/kg primaquine/week for 8 weeks versus high-standard 14-day regimen

This comparison aimed to investigate whether a higher once-weekly dosing regimen, which may be more beneficial for people with

G6PD deficiency, was as efficacious as the high-standard 14-day regimen.

One trial compared weekly 0.75 mg/kg primaquine (45 mg adult dose) for 8 weeks with the high-standard 14-day regimen (0.5 mg/kg/day) (Leslie 2008 PAK). G6PD-deficient participants were not randomized but were included in the weekly group, although there only was one G6PD-deficient person included. Pregnant and lactating women were excluded. Treatment was supervised. It was not specified whether chloroquine and primaquine were given concurrently.

Efficacy

Recurrences were more common in the weekly group at 8 months' follow-up (RR 7.0, 95% CI 0.38 to 127.32; 126 participants; Analysis 3.1). Recurrences remained more common in the weekly group at 11 months' follow-up (RR 3.18, 95% CI 0.37 to 27.6; 122 participants; Analysis 3.1). Leslie 2008 PAK was at high risk of bias for allocation concealment, but a sensitivity analysis could not be done as it was the only trial found for this comparison.

Adverse effects

No serious adverse events were reported in either study arm (Analysis 3.2). No participants had anaemia defined as haemoglobin less than 7 g/dL (Analysis 3.3).

Other regimens

0.375 mg/kg/day for 14 days versus standard 14-day regimen

Bunnag 1994 THA compared 0.375 mg/kg/day (adult dose 22.5 mg) primaquine daily for 14 days with the standard regimen of 0.25 mg/kg/day for 14 days. There was a high loss to follow-up, with 167 participants enrolled and only 38 completing 18 months' follow-up, although the loss was equal in both groups at the end of follow-up. At 6 months' follow-up there were no episodes of *P vivax* in the experimental group (0/40) and two recurrences in the standard-regimen group (2/33) (RR 0.17, 95% CI 0.01 to 3.34; 73 participants; Analysis 4.1), although only about half of enrolled participants were followed up at this time point. No further recurrences were described in either group up to the end of follow-up at 18 months, but as described, the high level of unexplained dropout makes interpretation difficult.

No formal assessment of adverse events was reported, but it is mentioned in the study narrative that there was no drop in haematocrit or haemoglobinuria in either group.

1.17 mg/kg/day for 3 days versus standard 14-day regimen

One trial delivered the total dose of primaquine (1.17 mg/kg/day or 70 mg adult dose, total dose 210 mg) over 3 days versus the standard (0.25 mg/kg/day) 14-day regimen (Carmona-Fonseca 2009 COL). Recurrences of *P vivax* malaria were more common in the group receiving 1.17 mg/kg/day for 3 days than in the standard 14-day group at 4 months' follow-up (RR 3.88, 95% CI 2.11 to 7.11; 129 participants; Analysis 5.1).

Adverse events were not reported, although it was noted that there were no serious adverse events from co-administering primaquine and chloroquine.

1 mg/kg/day for 7 days versus high-standard 14-day regimen

This comparison aimed to investigate whether shorter, higher doses of primaquine over 7 days are as effective as the high-standard 14-day regimen to determine whether the total dose rather than the length of treatment is the important factor for East Asia and Oceania regimen (total dose 420 mg primaquine). Only one included trial compared 1 mg/kg/day (adult dose 60 mg) of primaquine for 7 days with the high-standard 14-day course (0.5 mg/kg/day) (Chu 2016 THA), administering the regimen with either chloroquine or an ACT (4 arms). Results are still awaited, but a conference report of the trial reports that out of 680 participants there was no difference between the two regimens. No further details are currently available.

DISCUSSION

Summary of main results

High-standard 14-day regimen versus standard 14-day regimen

See Summary of findings 2

We included 2 RCTs that compared 0.5 mg/kg/day primaquine (daily adult dose 30 mg) for 14 days with 0.25 mg/kg/day (daily adult dose 15 mg) for 14 days, both conducted in India. People with G6PD deficiency and pregnant or lactating women were excluded. One trial did not account for whether participants were given chloroquine or an ACT for blood-stage treatment. We do not know if there is any difference in *P vivax* recurrences at 6 months with the high-standard 14-day course compared to the standard 14-day course when given with chloroquine (very low-certainty evidence). We do not know if there is any difference in *P vivax* relapses at 6 months with the high-standard 14-day course compared to the standard 14-day course when given with chloroquine or an ACT (very low-certainty evidence).

No serious events were reported in either trial. We do not know whether there is a difference in adverse events between the high-standard 14-day course and the standard 14-day course (very low-certainty evidence).

0.5 mg/kg/day for 7 days versus standard 14-day regimen

See Summary of findings for the main comparison

We included 5 RCTs that compared 0.5 mg/kg/day (adult dose 30 mg) primaquine for 7 days with the standard 14-day regimen (0.25 mg/kg/day). There may be little or no difference in *P vivax* recurrences at 6 to 7 months when using the same total dose (210 mg) over 7 days as compared to 14 days (low-certainty evidence). No serious adverse events were reported. There may be little or no difference in the number of adverse events known to occur with primaquine when using the shorter regimen as compared to the longer regimen (low-certainty evidence).

We do not know whether there is any difference in the frequency of anaemia or discontinuation of treatment between groups (very low-certainty evidence). Three trials excluded people with G6PD deficiency, and two did not provide this information, so we do not know the effect of the higher daily dose regimen in this group. Pregnant and lactating women were either excluded or this information was not provided.

0.75 mg/kg primaquine/week for 8 weeks versus high-standard 14-day regimen

See Summary of findings 3

We included 1 RCT that compared 0.75 mg/kg (daily adult dose 45 mg) weekly primaquine for 8 weeks with the high-standard 14-day regimen (0.5 mg/kg/day, daily adult dose 30 mg). G6PD-deficient participants were not randomized but were included in the weekly primaquine group. Only one G6PD-deficient participant was detected during the trial and was included in the weekly group. We do not know whether weekly primaquine reduces recurrence of *P vivax* compared to the high-standard 14-day regimen at 8 to 11 months' follow-up (very low-certainty evidence).

No serious adverse events and no episodes of anaemia were reported.

Some other included trials evaluated alternative regimens and doses of primaquine, but these regimens have not been widely used, and the evidence available from stand-alone trials was of very low certainty.

Overall completeness and applicability of evidence

We initially thought we could evaluate whether the high-standard 14-day regimen (0.5 mg/kg/day) was more effective in all areas rather than just areas where recommended by the WHO due to reported resistance or strain differences (East Asia and Oceania) (WHO 2015). However, we only found two trials that compared the high standard 14-day regimen to the standard 14-day regimen, both of which were conducted in India. A recent retrospective case review in French Guiana (also an area where the high-standard regimen is not currently recommended) found that recurrences were similar in both standard and high-standard 14-day regimens (Valdes 2018). We did not find any RCTs that evaluated whether the high-standard 14-day regimen was more effective compared to the standard 14-day regimen for the tropical, frequently relapsing strain of *P vivax* in East Asia and Oceania, so we are unable to comment on its efficacy.

A difficulty encountered in including and comparing studies was the variation in dosing and length of follow-up in studies.

In general, there were few well-conducted RCTs that used an evidence-based standard primaquine regimen (15 mg/kg/day for 14 days) as a comparator. Some trials used the high-standard 0.5 mg/kg/day for 14 days regimen as a comparator, which is recommended by the WHO in East Asia and Oceania, which is why we included these trials. However, there is limited clear evidence in this review for the increased high-standard 14-day regimen. We found two randomized clinical trials that compared its efficacy to the standard regimen, both of which were conducted in India (Rajgor 2014 IND; Saravu 2018 IND), where the regimen is not recommended.

We also found that trials continue to be conducted where placebo is used instead of an alternative primaquine regimen, which is contrary to the evidence available demonstrating its superiority for reducing recurrences (Galappaththy 2013). This may be because there is continued reluctance to use primaquine in some national programmes.

We excluded studies where individuals had mixed malaria infections so as to assess the efficacy of treatment on *P vivax* malaria alone. Areas endemic for *P vivax* malaria may also be co-endemic for *P falciparum* or *Plasmodium ovale* infection, or both. However, it should be noted that as part of our screening process we did not identify any studies where participants with mixed malaria were included, so we do not think that narrowing our search criteria impacted the directness of our results.

Although the evidence is currently of low certainty, it does appear that using 0.5 mg/kg/day with the same total dose (210 mg) over 7 days may be non-inferior to the regimen of 0.25 mg/kg/day for 14 days. It may be that these shorter regimens promote course completion. Although no serious adverse events were reported due to few reported events for any other adverse effects, it is difficult to draw conclusions as to whether there may be increased adverse events for this higher dosing until more data are available. This higher-dose regimen was not tested in G6PD-deficient patients in any of the RCTs meeting our inclusion criteria. This remains a concern in settings where testing is not available.

There was a general lack of detailed safety data for trials, which is interesting given that safety is a particular concern with primaquine use. Only one included RCT investigated the weekly primaquine regimen that is currently recommended by WHO for G6PD-deficient individuals, and only one G6PD-deficient participant was actually included in the treatment group.

Certainty of the evidence

The overall certainty of evidence for all of the outcomes was either low or very low. All results were downgraded for imprecision due to wide CIs for all of the meta-analyses performed.

The efficacy comparison for the high-standard 14-day regimen versus the standard 14-day regimen was also downgraded for indirectness. Results were based on two trials in adults in India (Rajgor 2014 IND; Saravu 2018 IND). Rajgor 2014 IND was at risk of bias as there was no allocation concealment and unexplained loss to follow-up; this study also contributed most to the meta-analysis for the comparison of 0.5 mg/kg/day for 7 days versus standard 14-day regimen, so this study was also downgraded. Saravu 2018 IND was a small pilot study where participants were given either chloroquine or an ACT for the blood stage, and which blood-stage treatment they were given was not stated. Saravu 2018 IND was downgraded for imprecision, indirectness, and risk of bias (not blinded and high rate of loss to follow-up).

We downgraded the comparison of 0.75 mg/kg weekly primaquine versus high-standard 14-day regimen for indirectness as it was based on just one study conducted in Pakistan (Leslie 2008 PAK), with only one G6PD-deficient patient participating. Leslie 2008 PAK was at risk of bias due to the randomization process used, lack of allocation concealment, and incomplete outcome data. We downgraded efficacy outcomes for this comparison for serious imprecision due to few events and very wide CIs.

Potential biases in the review process

The strictness of our inclusion criteria to not include trials where the total dose was less than the total dose of the standard regimen and the necessity of having the comparison arm be one of the WHO-recommended regimens may have meant that some relevant comparisons were excluded.

We changed the protocol to include the high-standard 14-day regimen that WHO recommends in East Asia and Oceania as a control regimen, as we realized that some trials had used this as the comparator, and we felt that these comparisons were useful. However, this may have introduced bias, as per our results the evidence base for RCTs showing the efficacy of this regimen is limited.

The difficulty in determining between relapse and re-infection with *P vivax* remains a recognized challenge for assessing the efficacy of drugs for radical cure.

Agreements and disagreements with other studies or reviews

Our findings that 210 mg over 7 days may be as efficacious as 210 mg over 14 days are similar to the findings of other systematic reviews that examined both randomized and non-randomized studies (Carmona-Fonseca 2015; Zuluaga-Idarraga 2015). Other reviews also commented on the difficulty of comparing results due to the varying treatment regimens and length of follow-up used in clinical trials (John 2012; Carmona-Fonseca 2015; Zuluaga-Idarraga 2015).

AUTHORS' CONCLUSIONS

Implications for practice

Although limited data were available, no difference was detected for efficacy between the regimen of 0.5 mg/kg/day for 7 days and the standard (0.25 mg/kg/day) 14-day regimen in G6PD-normal patients.

No serious adverse events were reported in G6PD-normal patients taking 0.5 mg/kg/day of primaquine.

Implications for research

Further high-quality randomized controlled trials are needed with more standardized comparison regimens and length of follow-up, in particular investigating the use of the high-standard 14-day regimen, same total dose over 7 days, and weekly regimens in G6PD-deficient patients. Trials such as IMPROV will help resolve some of the uncertainties.

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CHARACTERISTICS OF STUDIES

Characteristics of included studies *[ordered by study ID]*

Abdon 2001 BRA

Methods	RCT July 1994 to June 1995
Participants	120 participants enrolled. Inclusion criteria: • Confirmed parasitological diagnosis for *P vivax* malaria. • Age older than 12 years. • Staying in Belém (study area) until the end of the follow-up period (180 days). Exclusion criteria • Pregnant and nursing mothers were excluded. • Patients who used antimalarials at least 2 weeks prior to the start of current treatment. • Carriers of mixed malaria. Diagnosis: microscopy G6PD status not stated No details CYP2D6 status.
Interventions	• Chloroquine 10 mg/kg single dose + primaquine 0.5 mg/kg/day for 7 days. • Chloroquine 150 mg (25 mg/kg total dose) over 3 days, 10 mg/kg day 1, 7.5 mg/kg days 2 and 3 + primaquine 15 mg/day 14 days. (Additional arm chloroquine 10 mg/kg + primaquine 0.5 mg/kg for 5 days not included as total dose (150 mg) less than standard treatment (210 mg)) Although different doses of chloroquine in the 2 arms, all participants had negative parasitaemia within 72 hours. Primaquine and chloroquine given concurrently. Supervised treatment.
Outcomes	• Relapse. • Safety.

Abdon 2001 BRA *(Continued)*

	Follow-up 180 days
Notes	Location: Belém, state of Pará, Brazil Setting: not stated Source of funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details supplied on randomization process.
Allocation concealment (selection bias)	Unclear risk	No details supplied on allocation.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label.
Blinding of outcome assessment (detection bias) All outcomes	High risk	Open-label.
Incomplete outcome data (attrition bias) All outcomes	Low risk	One loss to follow-up as moved out of area.
Selective reporting (reporting bias)	Low risk	Unable to find protocol but relapse and standard errors (SEs) reported as would be expected.
Other bias	Unclear risk	Funding not stated.

Bunnag 1994 THA

Methods	RCT Dates not provided.
Participants	167 participants enrolled. Inclusion criteria: • 15 to 60 years. Exclusion criteria: • History of previous treatment. • G6PD deficiency. • Mixed infections. Diagnosis: microscopy No details on pregnant/breastfeeding women.

Bunnag 1994 THA *(Continued)*

	No details CYP2D6 status.
Interventions	• Chloroquine + 22.5 mg/day primaquine for 14 days. • Chloroquine + 15 mg/day primaquine for 14 days. Open randomization to chloroquine treatment – either 300 mg or 450 mg on day 1 of admission. Re-allocated after recovery of acute symptoms (double-blind RCT). Chloroquine course completed and parasitological clearance confirmed prior to randomization to primaquine group (exact time between treatment courses not specified). Supervised treatment in hospital.
Outcomes	• Relapse. • Safety. Follow-up 6 months
Notes	Location: Thailand Setting: not stated Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	1st step chloroquine is open randomization, then PQ stage randomized. No details on randomization process.
Allocation concealment (selection bias)	Unclear risk	No details.
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Reported as double-blind.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Reported as double-blind.
Incomplete outcome data (attrition bias) All outcomes	High risk	Unexplained high loss to follow-up.
Selective reporting (reporting bias)	Unclear risk	No protocol.
Other bias	Unclear risk	Funding not disclosed.

Carmona-Fonseca 2009 COL

Methods	RCT September 2003 to September 2006

Carmona-Fonseca 2009 COL *(Continued)*

Participants	133 patients enrolled across 2 arms (total 188 counting arms not included in review) Inclusion criteria: • Age > 2 years. • *P vivax* parasitaemia of > 1000 asexual forms/L. • Willingness to participate. • A normal quantitative G6PD screening test was required for those administered > 0.25 mg/kg/day primaquine base, and only individuals with normal G6PD levels were included in the study. Exclusion criteria: • Pregnant women. • Those with associated acute infectious diseases. • A history of antimalarials intake during the previous 2 weeks. • Presence of diarrhoea or vomiting (> 5 episodes in 24 hours). • Symptoms or signs of severe malaria (according to WHO 2006). • Hypersensitivity to antimalarials or severe undernutrition. • Exclusion from the study also followed intake of any antimalarial different from those provided by the researchers. • Failure to attend follow-up appointments. • Treatment failure during the primary episode (first 28 days of follow-up). • Consent withdrawal. Diagnosis: microscopy No details CYP2D6 status or breastfeeding mothers.
Interventions	• Chloroquine (10 mg/kg day 1, 7.5 mg/kg days 2 and 3) + primaquine 1.17 mg/kg/day for 3 days (total 210 mg). • Chloroquine (10 mg/kg day 1, 7.5 mg/kg days 2 and 3) + primaquine 0.25 mg/kg/day for 14 days. (Additional arms: 0.83 mg/kg day for 3 days (total dose 149.4 mg) and 0.58 mg/kg day for 3 days (total dose 104.4 mg) not included as total dose less than standard treatment) Primaquine given simultaneously with chloroquine. Supervised treatment.
Outcomes	• Recurrence of *P vivax* malaria (parasitaemia after day 28). Follow-up 120 days
Notes	Location: Colombia Setting: patients that attended the local health clinics in Turbo and El Bagre Funding: not stated

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Details of randomization not given.
Allocation concealment (selection bias)	Unclear risk	No details supplied.

Carmona-Fonseca 2009 COL *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	High risk	Not blinded.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No mention on blinding in blood smear assessment.
Incomplete outcome data (attrition bias) All outcomes	Low risk	2 lost per group, no explanations given, but less than 5% of total across groups.
Selective reporting (reporting bias)	Unclear risk	Protocol not found. No safety data were provided (which might have been expected to have been provided).
Other bias	Low risk	Looks like government funding.

Chu 2016 THA

Methods	RCT
Participants	680 enrolled. G6PD normal. No further details
Interventions	• Chloroquine + primaquine 7 days (1 mg/kg/day). • Chloroquine + primaquine 14 days (0.5 mg/kg/day). • Dihydroartemisinin-piperaquine + primaquine 7 days (1 mg/kg/day). • Dihydroartemisinin-piperaquine + primaquine 14 days (0.5 mg/kg/day). No further details
Outcomes	• Relapse. Follow-up: 1 year
Notes	Location: Thailand Setting: no details Funding: no details

Risk of bias

Bias	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Unclear risk	No details.
Allocation concealment (selection bias)	Unclear risk	No details.

Chu 2016 THA *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	Unclear risk	No details.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details.
Incomplete outcome data (attrition bias) All outcomes	Unclear risk	No details.
Selective reporting (reporting bias)	Unclear risk	No details.
Other bias	Unclear risk	No details.

Durand 2014 PER

Methods	RCT March 2006 to August 2008
Participants	360 participants Inclusion criteria: • Microscopy-confirmed diagnosis of monoinfection with *P vivax* between 250 and 100,000 asexual parasites/mL (determined by microscopic examination of thick and thin peripheral blood smears). • Fever defined as axillary temperature 37.5 °C or history of fever, or both. • > 1 year old. Exclusion criteria: • Pregnant and lactating women. • Patients with chronic illnesses. • Patients with symptoms of severe malaria. • Patients with G6PD deficiency. Diagnosis: light microscopy Parasite genotyping with PCR also performed - 5 microsatellite loci used to determine whether homologous relapse.
Interventions	• Chloroquine (10 mg/kg day 1 and 2, 5 mg/kg day 3) + primaquine 0.5 mg/kg/day 7 days. • Chloroquine (10 mg/kg day 1 and 2, 5 mg/kg day 3) + primaquine 0.25 mg/kg/day for 14 days. (Additional arm of chloroquine + primaquine 0.5 mg/kg/day for 5 days excluded as total dose 150 mg, which was less than standard treatment.) Supervised. Primaquine administered concurrently with chloroquine.
Outcomes	• Relapse between days 35 and 210. • Relapses (homologous only).

Durand 2014 PER *(Continued)*

	Follow-up: 210 days
Notes	Location: Peru Setting: Padre Cocha and the San Juan Health Centers and Santa Clara Health Center The periphery of the city of Iquitos, which is located on the river bank of the Amazon River and is the largest city in the Peruvian rainforest Funding: the US Department of Defense Global Emerging Infections Surveillance and Response System (DoD-GEIS), the National Institute of Health of Peru, and the Pan-American Health Organization/US Agency for International Development (PAHO-USAID) Americas Malaria Initiative/Amazonic Network of Antimalarial Drug Resistance, AMI/RAVREDA project

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Computer-generated block randomization table.
Allocation concealment (selection bias)	Low risk	The treatment allocation for each participant was placed in a sealed envelope, kept in an orderly manner, and opened only at the time of enrolment of a new participant to prevent selection bias by study physicians.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label.
Blinding of outcome assessment (detection bias) All outcomes	High risk	Open-label – no mention of blood smear blinding.
Incomplete outcome data (attrition bias) All outcomes	Low risk	8% to 10% loss following randomization, but all accounted for.
Selective reporting (reporting bias)	Low risk	Study protocol registered. Unable to find outcomes in protocol, but expected outcomes were reported on.
Other bias	Low risk	We did not detect any other sources of bias.

Leslie 2008 PAK

Methods	RCT September 2004 to July 2006
Participants	129 Afgan refugees Inclusion criteria: • Patients diagnosed with *P vivax* parasitaemia at study basic health units (BHUs). • Patients over 3 years of age. • Patient permanently resident in the village. Exclusion criteria:

Leslie 2008 PAK *(Continued)*

	• Pregnancy or lactation. • Severe clinical anaemia (7 g/dL). • *P falciparum* or *P vivax* (mixed infections), or both. • Intake of any antimalarial drug in the 2 weeks prior to consultation. • Patients unavailable for the duration of follow-up (11 months). • Patients with concomitant infections or disease likely to mask treatment response. Diagnosis: microscopy
Interventions	• Chloroquine (25 mg/kg in divided doses over 3 days) + primaquine 0.75 mg/kg once weekly for 8 weeks. • Chloroquine (25 mg/kg in divided doses over 3 days) + primaquine 0.5 mg/kg/day for 14 days. (Additional arm chloroquine + weekly placebo not included) Supervised. Not specified whether primaquine given concurrently with chloroquine.
Outcomes	• *P vivax* malaria relapse. • The number of subsequent episodes and anaemia rates during and up to 2 weeks post-treatment as well as any notable adverse events. Follow-up: 9 months (11 months participation: 8 weeks treatment + 9 months follow-up)
Notes	Location: Pakistan Setting: Adizai, Baghicha, and Khagan villages, close to Peshawar, Northwest Frontier Province, Pakistan where Afghan refugees have been resident for more than 20 years Funding: UNDP/World Bank/WHO Special Program for Research in Tropical Diseases; Gates Malaria Partnership)

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Two randomization methods were used. In Baghicha and Khagan villages, participants were randomized by household, whereas in Adizai, randomization was at the individual level. Randomization lists for each village were generated using a random number list (MS Excel, Microsoft Corp, Seattle, USA) by staff not involved in patient recruitment. Participants were randomized on enrolment by study staff in the BHUs based on house number or sequential patient numbers, depending on the study site.
Allocation concealment (selection bias)	High risk	Participants were randomized on enrolment by study staff in the BHUs based on house number or sequential patient numbers, depending on the study site.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Blood slides were double-read by 2 microscopists working independently, who were blinded to the other's result.
Incomplete outcome data (attrition bias)	High risk	Higher loss to follow-up in intervention group (6% to 8% versus 1% to 1.8%).

Leslie 2008 PAK *(Continued)*

All outcomes		
Selective reporting (reporting bias)	Low risk	Trial protocol available, all planned outcomes reported on.
Other bias	Low risk	We did not detect any other sources of bias.

Pareek 2015 IND

Methods	RCT
Participants	358 participants Inclusion criteria: • Patients of either sex. • Aged between 18 and 65 years. • Body weight > 40 kg. • Microscopically confirmed *P vivax* malaria with ≥ 1000 asexual parasites/µL of blood. • Axillary temperature ≥ 37.5°C (≥ 99.5°F). • Presence of at least 5 of the following signs and symptoms of uncomplicated malaria: chills, nausea, vomiting, headache, malaise, diarrhoea, anorexia, abdominal cramps, myalgia, and arthralgia. Exclusion criteria: • Mixed malarial infections. • Severe or complicated malaria (as defined by the WHO). • G6PD deficiency. • Any other significant concomitant illness. • Patients with history of dark urine or significant haemoglobinuria related to previous primaquine treatment or those with history of methaemoglobinaemia. • Patients with protracted vomiting and oliguria. • Those with underlying condition compromising bone marrow function or having a tendency to granulocytopenia. • Patients taking cardioactive drug or potentially haemolytic drugs or drugs that could interact with study drugs. • Patients having history of hypersensitivity to any of the study-related drugs. • Those on another investigational drug. • History/presence of substance abuse. • Pregnant or lactating women or women of childbearing potential not using medically accepted means of birth control. Diagnosis: microscopy
Interventions	• Chloroquine (3-day course, dose not specified) + primaquine 30 mg sustained release 7 days. • Chloroquine (3-day course, dose not specified) + primaquine 15 mg 14 days. (Additional arm of chloroquine + primaquine 15 mg sustained release for 14 days not included in review) Primaquine given following completion of chloroquine course. Not supervised.
Outcomes	• Relapse. • Compliance.

Pareek 2015 IND *(Continued)*

	• Safety. PCR genotyping done to see if true relapse (no details on genotyping method). Follow-up: 5 months (6 months participation)
Notes	Location: India Setting: multicentre, no details as to centres involved Funding: funded by drug manufacturer Ipca Laboratories Ltd. Anil Pareek and Nitin Chandurkar are the employees of Ipca Laboratories Ltd who sponsored this trial.

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Randomization codes were generated using computer-generated block randomization method.
Allocation concealment (selection bias)	Low risk	Patient-specific sealed boxes of medicine were provided to each study site. (Sequentially numbered, sealed, opaque envelopes (from protocol)).
Blinding of participants and personnel (performance bias) All outcomes	Low risk	Double-blind, double-dummy.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	No details as to whether microscopy was blinded or whether there was double reading of smears.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Loss to follow-up equal between groups. Relapses counted as discontinued patients, but numbers provided so can be assessed.
Selective reporting (reporting bias)	High risk	Compliance added as an outcome, but original outcomes also reported on. Not clear why they have concluded that compliance increased with SR, as participants had to take 3 sets of pills as did those who took dummy versions, so all participants took 3 sets of drugs. No measurement of levels of PQ (pharmacokinetics), although states that PQ SR should have therapeutic concentration over 24 hours as part of the concept. PCR results are not well-detailed.
Other bias	High risk	The study was sponsored by Ipca Laboratories Ltd, who manufactures the drugs, and the principal investigators are employees of the company.

Rajgor 2014 IND

Methods	RCT August 2001 to February 2004
Participants	1159 participants enrolled.

Rajgor 2014 IND *(Continued)*

	Inclusion criteria: • Adult patients, male and female (18 years of age or older). • Peripheral blood smear diagnosis of *P vivax.* • Willing to undergo hospitalization for the entire duration of primaquine treatment. • Willing to provide informed consent. • Willing to undergo investigations and come for regular follow-up. • Normal G6PD. • Haemoglobin ≥ 10 g/dL. Exclusion criteria: • Mixed infection with *P falciparum.* • Pregnancy and lactation. • Evidence of significant hepatic, renal, or cardiac disease as diagnosed by history, clinical examination, and laboratory tests whenever necessary. • Any other condition that would interfere with patient's participation in the study or compliance with the treatment. Diagnosis: microscopy
Interventions	• Chloroquine (10 mg/kg day 1 and 2, 5 mg/kg day 3) + primaquine 30 mg/day 7 days. • Chloroquine (10 mg/kg day 1 and 2, 5 mg/kg day 3) + primaquine 30 mg/day 14 days. • Chloroquine (10 mg/kg day 1 and 2, 5 mg/kg day 3) + primaquine 15 mg/day 14 days. (Additional no-primaquine arm not included in analysis) Supervised treatment. Primquine treatment commenced after chloroquine treatment (day 4).
Outcomes	• Recurrence of *vivax* malaria. • Safety. Follow-up: 6 months The secondary outcome also included comparison of number of participants classified as relapse and re-infection by the 3 methods to determine the concordance between the methods used and the genetic diversity observed based on PCR sequencing method. The cases of recurrence were classified as relapse or re-infection based on the 3 methods, the month of recurrence, and the 2 genotyping methods: PCR-RFLP and PCR sequencing.
Notes	Location: Mumbai, India Setting: inpatient assessment in Mumbai Funding: Indian Council of Medical Research

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	A simple, computer-generated randomization scheme was used for the randomization of participants into the 3 PQ regimen groups.
Allocation concealment (selection bias)	High risk	This was an open-label study, and no concealment of treatment allocation was followed.

Rajgor 2014 IND *(Continued)*

Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label.
Blinding of outcome assessment (detection bias) All outcomes	Low risk	Although the study was not blinded in terms of treatment administration, the person seeing the slides and carrying out other outcome assessments was blinded to the treatment group by coding of the samples.
Incomplete outcome data (attrition bias) All outcomes	High risk	High percentage of participants not completing 6 months' follow-up across all groups. Minimal explanation for discontinuation of participants.
Selective reporting (reporting bias)	Unclear risk	No registered protocol found - reported on expected outcomes of efficacy and adverse effects. Trial carried out 2001 to 2004 but not published until 2014.
Other bias	Low risk	We did not detect any other sources of bias.

Saravu 2018 IND

Methods	RCT, open-label, pilot study March 2017 to August 2017
Participants	50 participants enrolled. Patients presenting to Kasturba Hospital, Manipal and Dr TMA Pai Hospital, Udupi, India Inclusion criteria: • *P vivax* malaria monoinfection. • Age 18 years and over. • Fever > 37.5°C tympanic or oral, or a history of fever within previous 3 days. • Willing to give informed consent. Exclusion criteria: • Pregnant or lactating, or both. • Patients with G6PD deficiency. • Mixed infection with *P vivax* and *P falciparum.* Primaquine given after blood-stage treatment. Diagnosis: microscopy, but PCR also performed to genotype recurrences No details CYP2D6
Interventions	Blood-stage treatment: either CQ or ACT (artesunate with doxycycline or artemether-lumefantrine as per the treating clinician's judgement of severity) • Primaquine 0.5 mg/kg/day for 14 days • Primaquine 0.25 mg/kg/day for 14 days Drug therapy not supervised.
Outcomes	1. Recurrence (2. Primaquine levels in the blood at 7 days)

Saravu 2018 IND *(Continued)*

	Follow-up 6 months
Notes	Location: Udupi district of Karnataka State, India Setting: typical tropical climatic conditions. Malaria incidence throughout the year with peaks around June to July. Urban and rural settings in catchment area. Source of funding: seed Grant Award from Manipal McGill Center for Infectious Diseases

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Block randomization – 5 blocks of 10, randomization within each block done by a lottery method.
Allocation concealment (selection bias)	Unclear risk	No details.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label.
Blinding of outcome assessment (detection bias) All outcomes	High risk	Open-label.
Incomplete outcome data (attrition bias) All outcomes	High risk	High percentage of loss to follow-up by 6 months in both arms – results difficult to interpret.
Selective reporting (reporting bias)	Low risk	Outcomes reported as per protocol.
Other bias	Low risk	Supported by a seed Grant Award from Manipal McGill Center for Infectious Diseases, MAHE, Manipal.

Solari-Soto 2002 PER

Methods	RCT October 1998 to January 1999
Participants	60 participants enrolled. Inclusion criteria: • Confirmed diagnosis of *P vivax* malaria (febrile and positive *P vivax* blood smear). Exclusion criteria: • Patients who had received antimalarial medication in the 4 weeks prior to diagnosis. • Children under 5 years. • Patients with severe concomitant diseases. No details about inclusion/exclusion of G6PD-deficient/pregnant/breastfeeding patients.

Solari-Soto 2002 PER *(Continued)*

	Diagnosis: microscopy
Interventions	• Chloroquine (10 mg/kg day 1 and 2, 5 mg/kg day 3) + primaquine 0.25 mg/kg/day for 14 days. • Chloroquine (10 mg/kg day 1 and 2, 5 mg/kg day 3) + primaquine 0.5 mg/kg/day for 7 days. Directly observed therapy. Primaquine given after chloroquine course.
Outcomes	• Relapse. • Adverse events. Follow-up: 60 days (total enrolment 60 days)
Notes	Location: Peru Setting: patients treated at San Martín de Pangoa Hospital, Junín Funding: US Naval Medical Research Institute Detachment

Risk of bias

Bias	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	No details on randomization process.
Allocation concealment (selection bias)	Unclear risk	No details on allocation process.
Blinding of participants and personnel (performance bias) All outcomes	High risk	Open-label.
Blinding of outcome assessment (detection bias) All outcomes	Unclear risk	Samples double-checked, but no details as to whether blinded.
Incomplete outcome data (attrition bias) All outcomes	Low risk	Missing data accounted for, similar in each group.
Selective reporting (reporting bias)	Unclear risk	No details.
Other bias	Low risk	We did not detect any other sources of bias.

Abbreviations: ACT: artemisinin-based combination therapy; CQ: chloroquine; CYP2D6: cytochrome P450 2D6; G6PD: glucose-6-phosphate dehydrogenase; PCR: polymerase chain reaction; PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; PQ: primaquine; RCT: randomized controlled trial; SE: standard error; SR: sustained release; WHO: World Health Organization.

Characteristics of excluded studies *[ordered by study ID]*

Study	Reason for exclusion
Adak 2001	No PQ comparison group.

Study	Reason for exclusion
Alvarez 2006	Comparison regimens are of a lower total dose than the control (15 mg/day for 3 days or 7 days) – shown to be inferior in Galappaththy 2013.
Alvarez Sanchez 2007	Low-dose, shorter regimens of PQ.
Betuela 2012	Only one treatment group received primaquine.
Chu 2017	Wrong outcomes: primary outcome of this analysis was the fractional haematocrit reduction up to day 14 after enrolment.
Chu 2018	No primaquine comparison arm.
Clyde 1977	Not an RCT, observational single-arm trial.
Contacos 1974	Not an RCT.
da Silva 1984	Not properly randomized (randomized according to whether the end of the notes code is odd or even), low-dose comparison PQ group.
Gogtay 1999	Low-dose 15 mg for shorter time period (5 days) – shown to be ineffective in Galappaththy 2013.
Goller 2007	Not an RCT – logistic regression using already-published RCTs and observational studies (not primary trial).
Kim 2012	Wrong comparator: low-dose for 5 days - shown to be ineffective in Galappaththy 2013.
Kimura 1996	Not an RCT.
Krudsood 2008	Artesunate only as blood-stage treatment (does not meet inclusion criteria) and follow-up only 28 days.
Leslie 2004	No PQ comparison group: supervised versus unsupervised therapy.
Leslie 2008b	Duplicate of Leslie 2008 PAK; conference abstract title only for session at ASTMH 57th Annual Meeting.
Maneeboonyang 2011	Not randomized, participants were sequentially allocated into either the directly observed therapy (DOT) group or the self-administered therapy (SAT) group. No PQ comparison group, supervised versus non-supervised therapy.
Miller 1974	Not an RCT.
Pasaribu 2013	No PQ comparison group.
Pukrittayakamee 2000	No PQ comparison group.
Sabchareon 1981	No blood-stage antimalarial treatment used in primaquine comparison group according to inclusion criteria.
Saint-Yves IF 1977	Presumptive treatment of 45 mg PQ given to all participants before randomization.
Takeuchi 2010	No PQ comparison group: supervised versus non-supervised therapy.
Villalobos-Salcedo 2000	Wrong comparator: lower dose of PQ in comparison group (total dose 150 mg) - shown to be ineffective in Galappaththy 2013.

Study	Reason for exclusion
Warrasak 2018	No primaquine comparison arm, ophthalmological outcomes.

Abbreviations: PQ: primaquine; RCT: randomized controlled trial.

Characteristics of ongoing studies *[ordered by study ID]*

NCT01814683

Trial name or title	Improving the radical cure of *vivax* malaria: a multicentre randomised comparison of short and long course primaquine regimens
Methods	RCT, multicentre Participant, care provider, and investigator blinding
Participants	Aged 6 months and older. Inclusion criteria: • Participant (or parent/guardian of children below age of consent) is willing and able to give written informed consent to participate in the trial; verbal consent in the presence of a literate witness is required for illiterate patients. In addition, written assent (or verbal assent in the presence of a literate witness for illiterates) from children 12 to 17 years as per local practice. • Monoinfection with *P vivax* of any parasitaemia in countries that use chloroquine as blood schizonticidal therapy. Mixed infections with *P vivax* and *P falciparum* can be enrolled in countries that use an artemisinin combination therapy. • Diagnosis based on rapid diagnostic tests. • Over 6 months of age. • Weight 5 kg or greater. • Fever (axillary temperature 37.5°C) or history of fever in the last 48 hours. • Able (in the investigator's opinion) and willing to comply with the study requirements and follow-up. Exclusion criteria: • Female participant who is pregnant, lactating, or planning pregnancy during the course of the study. • Inability to tolerate oral treatment. • Previous episode of haemolysis or severe haemoglobinuria following primaquine. • Signs/symptoms indicative of severe/complicated malaria or warning signs requiring parenteral treatment - haemoglobin concentration less than 9 g/dL. • Known hypersensitivity or allergy to the study drugs. • Blood transfusion in last 90 days, since this can mask G6PD-deficient status. • A febrile condition due to diseases other than malaria (for example, measles, acute lower respiratory tract infection, severe diarrhoea with dehydration). • Presence of any condition which in the judgement of the investigator would place the participant at undue risk or interfere with the results of the study (for example, serious underlying cardiac, renal, or hepatic disease; severe malnutrition; HIV/AIDS; or severe febrile condition other than malaria); co-administration of other medication known to cause haemolysis or that could interfere with the assessment of antimalarial regimens. • Currently taking medication known to interfere significantly with the pharmacokinetics of primaquine and the schizonticidal study drugs. • Prior antimalarial medications in the previous 7 days. Locations: Afghanistan, Ethiopia, Indonesia, Vietnam

NCT01814683 *(Continued)*

	Estimated enrolment: 3150 participants
Interventions	1. Standard blood schizonticidal therapy plus 7 days of supervised primaquine (7 mg/kg total dose) administered once per day (1.0 mg/kg once daily) followed by 7 days of placebo 2. Standard blood schizonticidal therapy plus 14 days of supervised primaquine (7 mg/kg total dose) administered once per day (0.5 mg/kg) (3. Standard blood schizonticidal therapy plus 14 days placebo)
Outcomes	• Incidence rate (per person-year) and risk of symptomatic recurrent *P vivax* [Time Frame: 12 months]. The incidence rate (that is, per person-year) of symptomatic recurrent *P vivax* parasitaemia (detected by microscopy) over 12 months of follow-up in the 7- versus 14-day primaquine groups for all sites combined and stratified by site • Incidence rate (per person-year) of recurrent *P vivax* parasitaemia; haematological recovery; serious adverse drug reaction, primaquine tolerability, risk of severe anaemia in G6PD-deficient arm, cost-effective analysis with respect to the use of G6PD tests
Starting date	July 2014
Contact information	Ric Price, University of Oxford; ric.price@ndm.ox.ac.uk
Notes	Esimated completion date: December 2019 Protocol published (see IMPROV Study Group 2015; listed under NCT01814683) clinicaltrials.gov/ct2/show/NCT01814683

NCT01837992

Trial name or title	Evaluation of safety and efficacy of two primaquine dosing regimens for the radical treatment of *Plasmodium vivax* malaria in Vanuatu and Solomon Islands
Methods	RCT, open-label
Participants	Children and adults aged 12 months to 60 years. Solomon Islands and Vanuatu. Inclusion criteria: • Age 12 months to 60 years. • Melanesian background and living in local area. • Microscopically (based on field microscopy) or RDT-confirmed *P vivax* regardless of parasite density. Mixed infections (*P falciparum-P vivax* and *P malariae-P vivax*) can be included. Exclusion criteria: • Any signs of severe malaria (see WHO definitions) including: impaired consciousness, respiratory distress, severe anaemia (haemoglobin < 5), multiple seizures, frequent vomiting/inability to swallow tablets, prostration, jaundice, hypotension, abnormal bleeding, or hypoglycaemia. • Clinical evidence of non-malarial illness (such as pneumonia or otitis media). • Severe malnutrition (weight-for-age nutritional Z score < 60th percentile). • Permanent disability that prevents or impedes study participation. • Treatment with primaquine in the previous 14 days. • Residence or planned travel outside the study area during the follow-up period (precluding supervised treatment and follow-up procedures). • Known or suspected pregnancy. • Currently breastfeeding.

NCT01837992 *(Continued)*	• A positive rapid test for G6PD deficiency (Binax or Carestart RDT).
Interventions	1. Primaquine dose of 0.5 mg/kg/day for 14 consecutive days and standard age-based dosage 3-day course of artemether-lumefantrine 2. Primaquine dose of 0.25 mg/kg for 14 consecutive days and standard age-based dosage 3-day course of artemether-lumefantrine (3. Participants will receive a standard 3-day treatment course of artemether-lumefantrine at the standard age-based dosage, but will not receive primaquine until the time of confirmed recurrent parasitaemia or completion of 3 months follow-up)
Outcomes	• Efficacy: numbers of *P vivax* relapses per person-years of follow-up [Time Frame: 12 months]. Total number of microscopically diagnosed (including both symptomatic and asymptomatic infections), PCR-confirmed relapses with *P vivax* in participants in each treatment arm over the 3-month follow-up period, expressed as number of relapses per person-years of follow-up. • Safety and toxicity: mild, moderate, and severe adverse events, haemolysis, methaemoglobinaemia.
Starting date	May 2013
Contact information	Dr Ivo Mueller; mueller@wehi.edu.au
Notes	Estimated completion date May 2015. Contacted for results - no response. Protocol available at clinicaltrials.gov/ct2/show/NCT01837992

Abbreviations: G6PD: glucose-6-phosphate dehydrogenase; RCT: randomized controlled trial; RDT: rapid diagnostic test; WHO: World Health Organization.

DATA AND ANALYSES

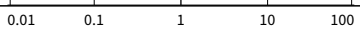
Comparison 1. High-standard 14-day regimen versus standard 14-day regimen

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Recurrence at 6 months' follow-up	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 6 months (chloroquine blood-stage treatment)	1	639	Risk Ratio (M-H, Fixed, 95% CI)	0.82 [0.47, 1.43]
1.2 6 months (chloroquine or ACT blood-stage treatment)	1	38	Risk Ratio (M-H, Fixed, 95% CI)	1.11 [0.17, 7.09]
2 Recurrence (PCR-adjusted)	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
2.1 6 to 7 months	1	639	Risk Ratio (M-H, Fixed, 95% CI)	1.83 [0.62, 5.40]
3 Serious adverse effects	1	778	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
4 Adverse events that result in discontinuation of treatment	1	778	Risk Ratio (M-H, Fixed, 95% CI)	4.19 [0.90, 19.60]

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
5 Adverse effects known to occur with primaquine	1	778	Risk Ratio (M-H, Fixed, 95% CI)	2.72 [0.98, 7.57]
6 Adverse events known to occur with chloroquine	1	778	Risk Ratio (M-H, Fixed, 95% CI)	9.43 [0.51, 174.47]

Analysis 1.1. Comparison 1 High-standard 14-day regimen versus standard 14-day regimen, Outcome 1 Recurrence at 6 months' follow-up.

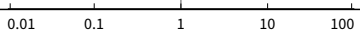
Study or subgroup	0.5mg/kg/ day PQ 14 days n/N	0.25mg/kg/ day PQ 14 days n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.1.1 6 months (chloroquine blood-stage treatment)					
Rajgor 2014 IND	21/317	26/322		100%	0.82[0.47,1.43]
Subtotal (95% CI)	**317**	**322**		**100%**	**0.82[0.47,1.43]**
Total events: 21 (0.5mg/kg/day PQ 14 days), 26 (0.25mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.7(P=0.48)					
1.1.2 6 months (chloroquine or ACT blood-stage treatment)					
Saravu 2018 IND	2/18	2/20		100%	1.11[0.17,7.09]
Subtotal (95% CI)	**18**	**20**		**100%**	**1.11[0.17,7.09]**
Total events: 2 (0.5mg/kg/day PQ 14 days), 2 (0.25mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.11(P=0.91)					
Test for subgroup differences: Chi^2=0.09, df=1 (P=0.76), I^2=0%					



Favours 0.5mg/kg/day PQ 14 days | Favours 0.25mg/kg/day PQ 14 days

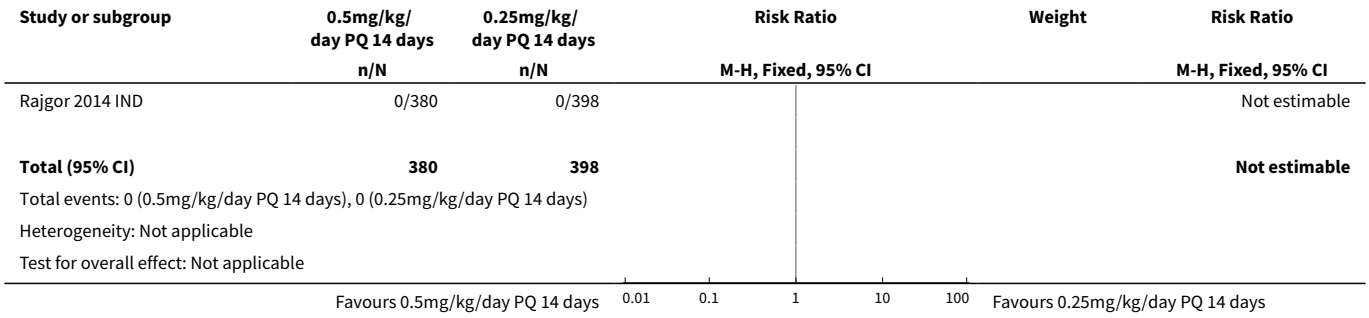
Analysis 1.2. Comparison 1 High-standard 14-day regimen versus standard 14-day regimen, Outcome 2 Recurrence (PCR-adjusted).

Study or subgroup	0.5mg/kg/ day PQ 14 days n/N	0.25mg/kg/ day PQ 14 days n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
1.2.1 6 to 7 months					
Rajgor 2014 IND	9/317	5/322		100%	1.83[0.62,5.4]
Subtotal (95% CI)	**317**	**322**		**100%**	**1.83[0.62,5.4]**
Total events: 9 (0.5mg/kg/day PQ 14 days), 5 (0.25mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.09(P=0.27)					

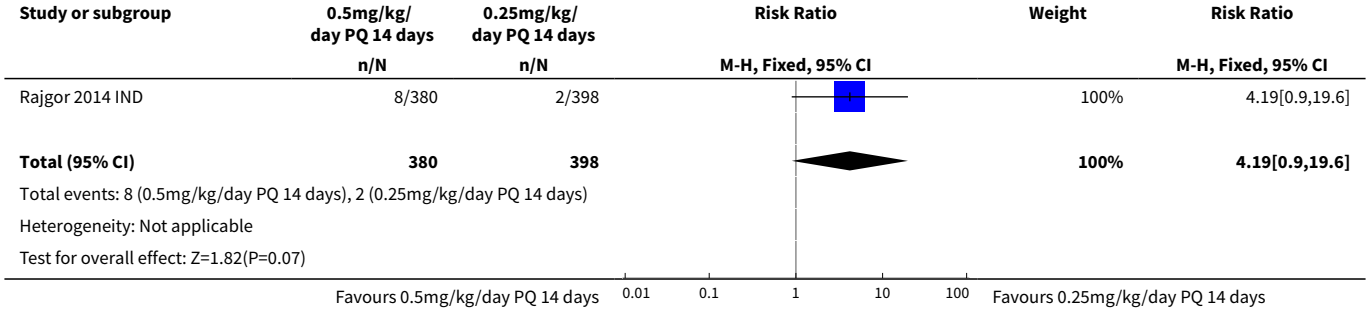


Favours 0.5mg/kg/day PQ 14 days | Favours 0.25mg/kg/day PQ 14 days

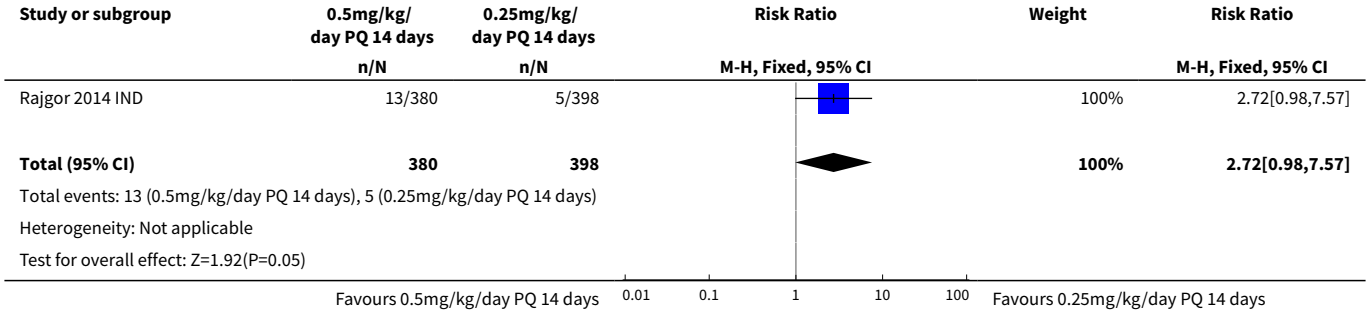
Analysis 1.3. Comparison 1 High-standard 14-day regimen versus standard 14-day regimen, Outcome 3 Serious adverse effects.



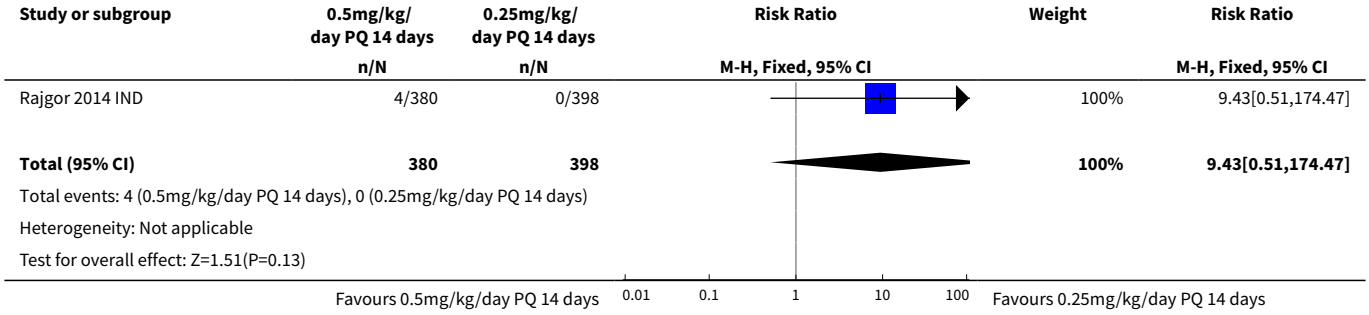
Analysis 1.4. Comparison 1 High-standard 14-day regimen versus standard 14-day regimen, Outcome 4 Adverse events that result in discontinuation of treatment.



Analysis 1.5. Comparison 1 High-standard 14-day regimen versus standard 14-day regimen, Outcome 5 Adverse effects known to occur with primaquine.



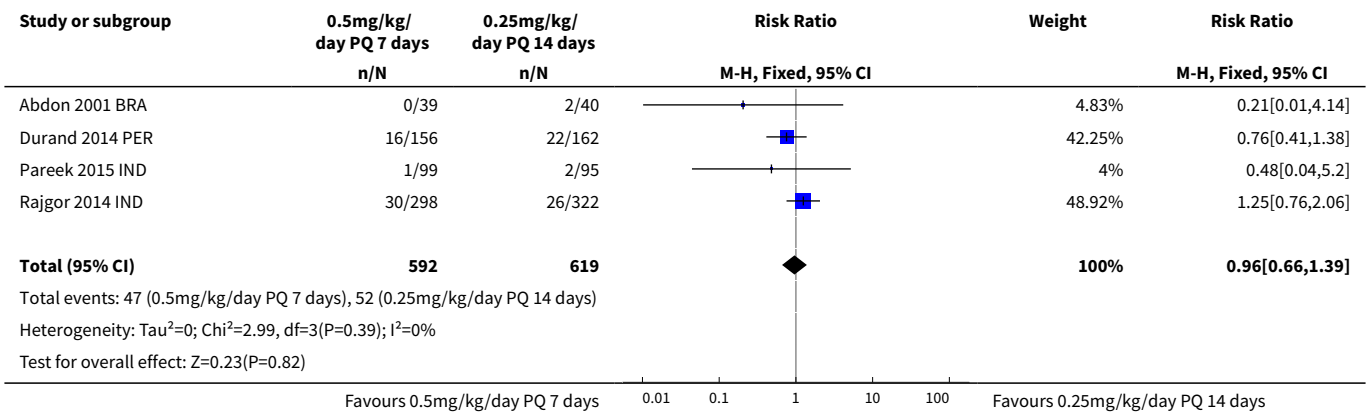
Analysis 1.6. Comparison 1 High-standard 14-day regimen versus standard 14-day regimen, Outcome 6 Adverse events known to occur with chloroquine.



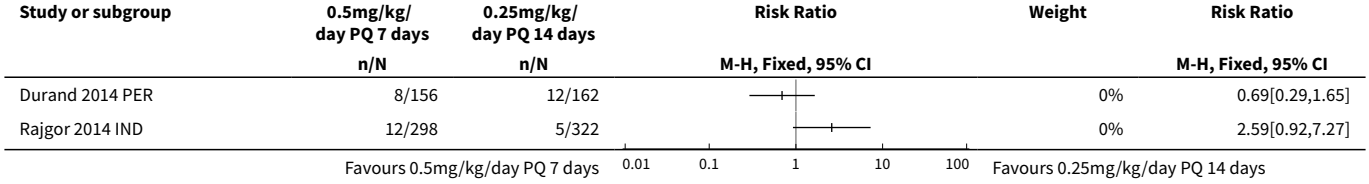
Comparison 2. 0.5 mg/kg/day for 7 days versus standard 14-day regimen

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Recurrence by 6 to 7 months' follow-up	4	1211	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.66, 1.39]
2 Recurrence by 6 to 7 months' follow-up (PCR-adjusted)	2		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
3 Recurrence by 6 to 7 months subgrouped by geographical region	4	1211	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.66, 1.39]
3.1 South America	2	397	Risk Ratio (M-H, Fixed, 95% CI)	0.70 [0.39, 1.26]
3.2 Asia	2	814	Risk Ratio (M-H, Fixed, 95% CI)	1.19 [0.73, 1.94]
4 Recurrence by 6 to 7 months subgrouped by directly observed therapy (DOT) versus non-DOT	4	1211	Risk Ratio (M-H, Fixed, 95% CI)	0.96 [0.66, 1.39]
4.1 DOT	3	1017	Risk Ratio (M-H, Fixed, 95% CI)	0.98 [0.67, 1.43]
4.2 Non-DOT	1	194	Risk Ratio (M-H, Fixed, 95% CI)	0.48 [0.04, 5.20]
5 Serious adverse effects	5	1427	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
6 Adverse events that result in discontinuation of treatment	5	1427	Risk Ratio (M-H, Fixed, 95% CI)	1.04 [0.15, 7.38]
7 Adverse effects known to occur with primaquine	4	1154	Risk Ratio (M-H, Fixed, 95% CI)	1.06 [0.64, 1.76]
8 Anaemia or change in haemoglobin status	1	240	Risk Ratio (M-H, Fixed, 95% CI)	3.0 [0.12, 72.91]
9 Adverse events known to occur with chloroquine	1	779	Risk Ratio (M-H, Fixed, 95% CI)	9.40 [0.51, 174.01]

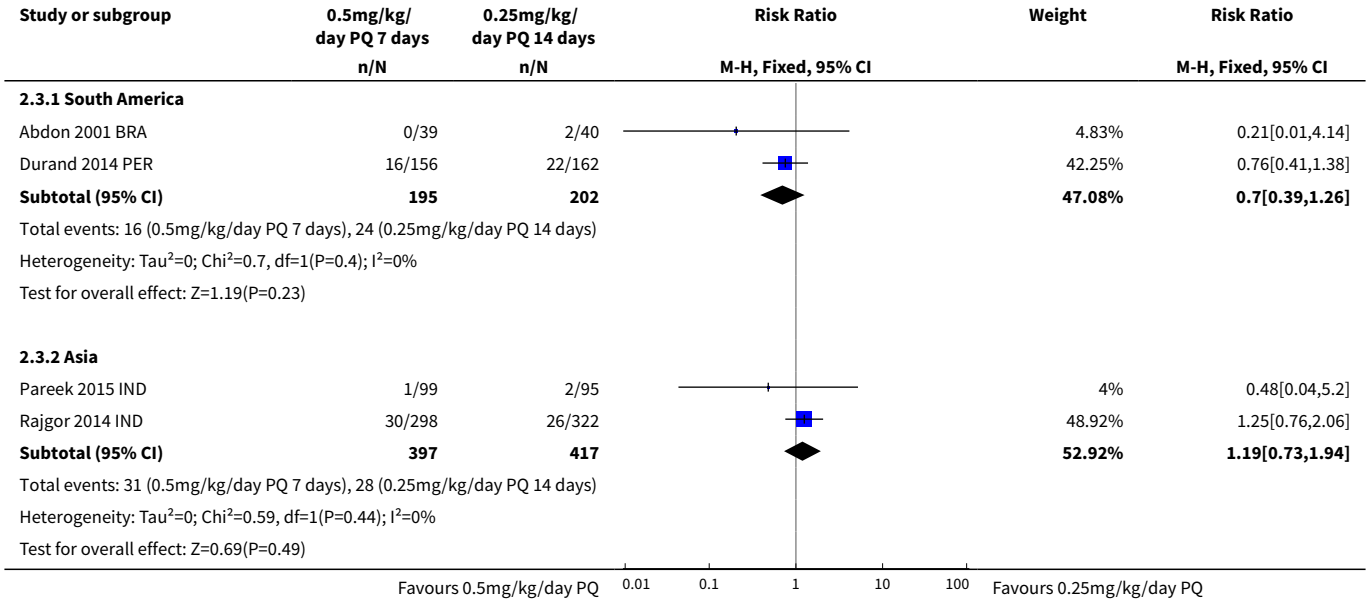
Analysis 2.1. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 1 Recurrence by 6 to 7 months' follow-up.

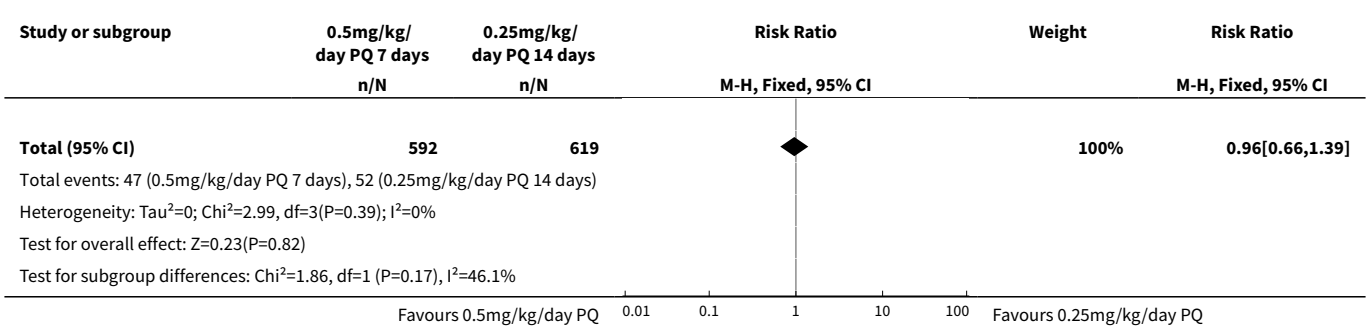


Analysis 2.2. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 2 Recurrence by 6 to 7 months' follow-up (PCR-adjusted).

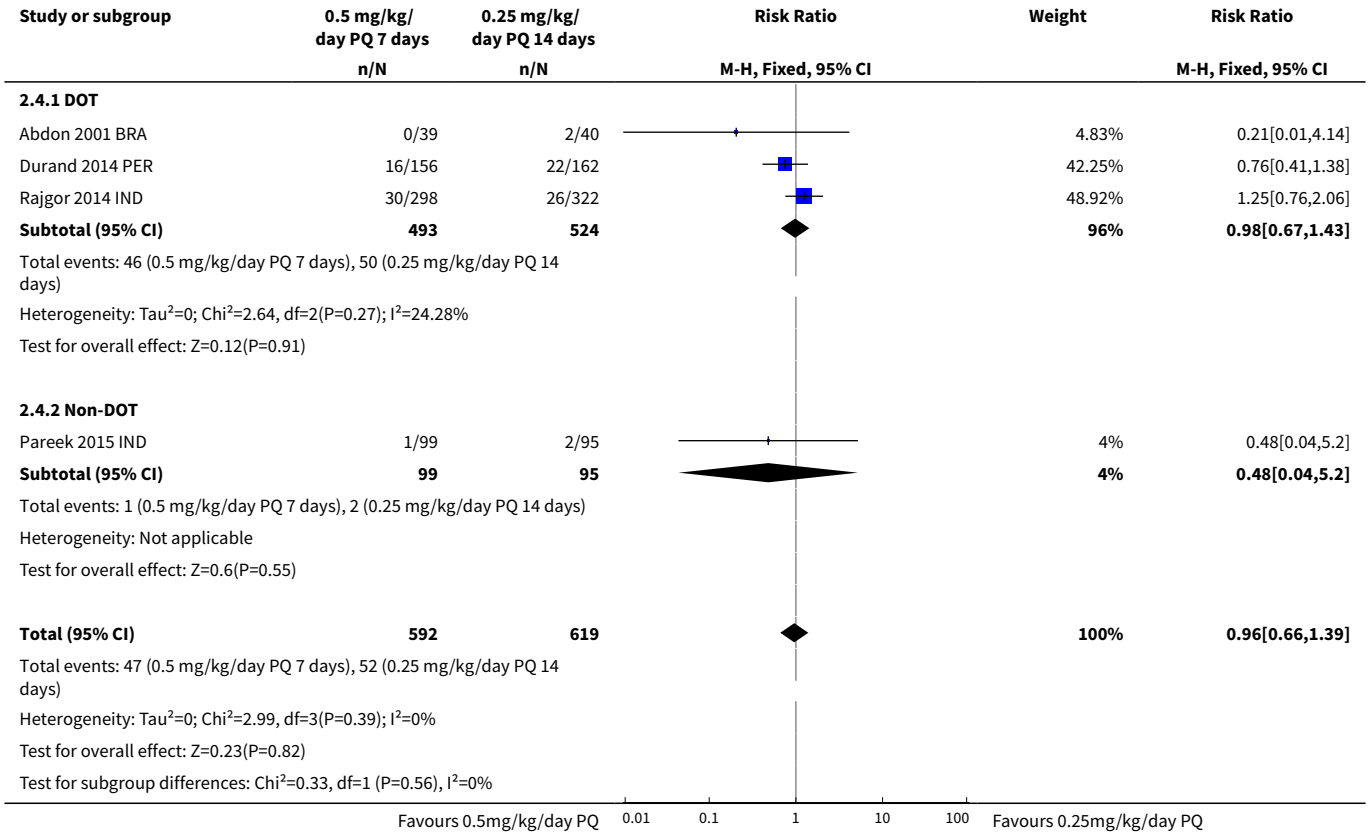


Analysis 2.3. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 3 Recurrence by 6 to 7 months subgrouped by geographical region.

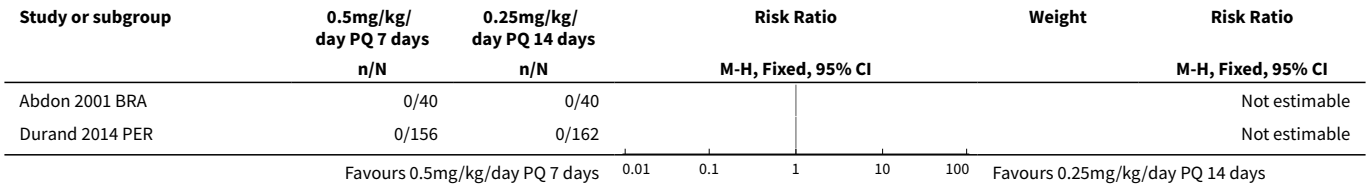




Analysis 2.4. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 4 Recurrence by 6 to 7 months subgrouped by directly observed therapy (DOT) versus non-DOT.



Analysis 2.5. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 5 Serious adverse effects.



Study or subgroup	0.5mg/kg/ day PQ 7 days n/N	0.25mg/kg/ day PQ 14 days n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Pareek 2015 IND	0/99	0/95			Not estimable
Rajgor 2014 IND	0/381	0/398			Not estimable
Solari-Soto 2002 PER	0/28	0/28			Not estimable
Total (95% CI)	**704**	**723**			**Not estimable**
Total events: 0 (0.5mg/kg/day PQ 7 days), 0 (0.25mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					

Favours 0.5mg/kg/day PQ 7 days 0.01 0.1 1 10 100 Favours 0.25mg/kg/day PQ 14 days

Analysis 2.6. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 6 Adverse events that result in discontinuation of treatment.

Study or subgroup	0.5mg/kg/ day PQ 7 days n/N	0.25mg/kg/ day PQ 14 days n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Abdon 2001 BRA	0/40	0/40			Not estimable
Durand 2014 PER	0/156	0/162			Not estimable
Pareek 2015 IND	0/99	0/95			Not estimable
Rajgor 2014 IND	2/381	2/398		100%	1.04[0.15,7.38]
Solari-Soto 2002 PER	0/28	0/28			Not estimable
Total (95% CI)	**704**	**723**		**100%**	**1.04[0.15,7.38]**
Total events: 2 (0.5mg/kg/day PQ 7 days), 2 (0.25mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Z=0.04(P=0.97)					

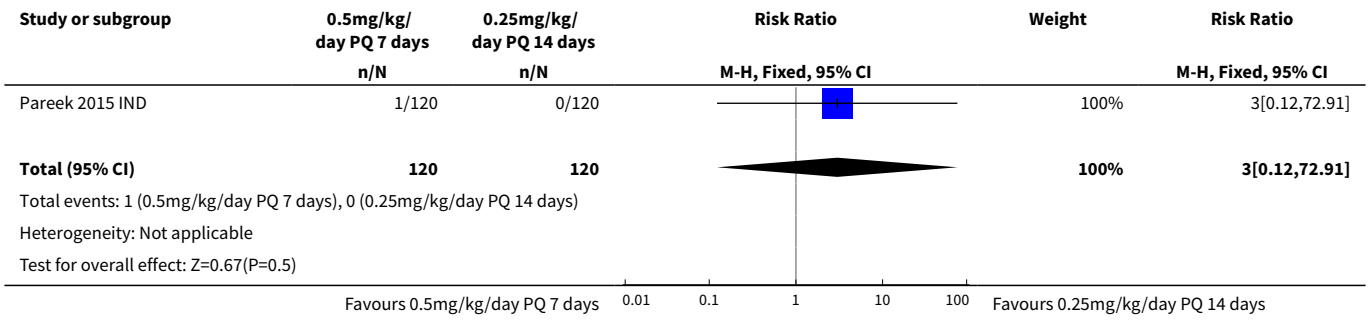
Favours 0.5mg/kg/day PQ 7 days 0.01 0.1 1 10 100 Favours 0.25mg/kg/day PQ 14 days

Analysis 2.7. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 7 Adverse effects known to occur with primaquine.

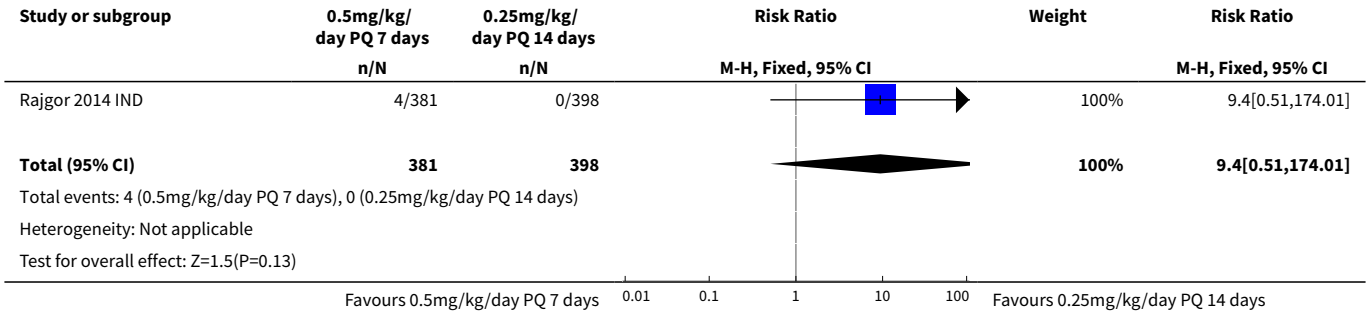
Study or subgroup	0.5mg/kg/ day PQ 7 days n/N	0.25mg/kg/ day PQ 14 days n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Abdon 2001 BRA	6/39	11/40		42.18%	0.56[0.23,1.36]
Pareek 2015 IND	11/120	10/120		38.83%	1.1[0.49,2.49]
Rajgor 2014 IND	10/381	5/398		18.99%	2.09[0.72,6.06]
Solari-Soto 2002 PER	0/28	0/28			Not estimable
Total (95% CI)	**568**	**586**		**100%**	**1.06[0.64,1.76]**
Total events: 27 (0.5mg/kg/day PQ 7 days), 26 (0.25mg/kg/day PQ 14 days)					
Heterogeneity: $Tau^2=0$; $Chi^2=3.54$, df=2(P=0.17); $I^2=43.55\%$					
Test for overall effect: Z=0.22(P=0.82)					

Favours 0.5mg/kg/day PQ 7 days 0.01 0.1 1 10 100 Favours 0.25mg/kg/day PQ 14 days

Analysis 2.8. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 8 Anaemia or change in haemoglobin status.



Analysis 2.9. Comparison 2 0.5 mg/kg/day for 7 days versus standard 14-day regimen, Outcome 9 Adverse events known to occur with chloroquine.

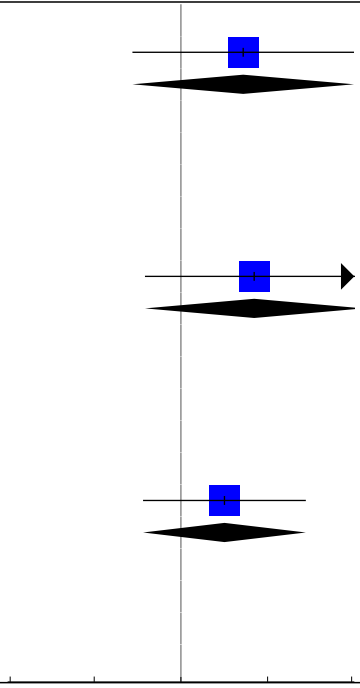


Comparison 3. 0.75 mg/kg primaquine/week for 8 weeks versus high-standard 14-day regimen

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Recurrence	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 5 months	1	129	Risk Ratio (M-H, Fixed, 95% CI)	5.23 [0.28, 99.15]
1.2 8 months	1	126	Risk Ratio (M-H, Fixed, 95% CI)	7.0 [0.38, 127.32]
1.3 11 months	1	122	Risk Ratio (M-H, Fixed, 95% CI)	3.18 [0.37, 27.60]
2 Serious adverse effects	1	129	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
3 Anaemia (haemoglobin < 7 g/dL)	1	129	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]

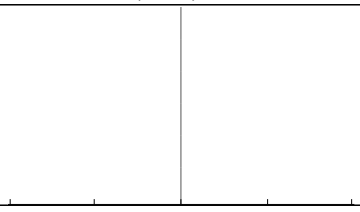
Analysis 3.1. Comparison 3 0.75 mg/kg primaquine/week for 8 weeks versus high-standard 14-day regimen, Outcome 1 Recurrence.

Study or subgroup	0.75mg/kg PQ weekly 8wks n/N	0.5mg/kg/ day PQ 14 days n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
3.1.1 5 months					
Leslie 2008 PAK	3/74	0/55		100%	5.23[0.28,99.15]
Subtotal (95% CI)	**74**	**55**		**100%**	**5.23[0.28,99.15]**
Total events: 3 (0.75mg/kg PQ weekly 8wks), 0 (0.5mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.1(P=0.27)					
3.1.2 8 months					
Leslie 2008 PAK	4/71	0/55		100%	7[0.38,127.32]
Subtotal (95% CI)	**71**	**55**		**100%**	**7[0.38,127.32]**
Total events: 4 (0.75mg/kg PQ weekly 8wks), 0 (0.5mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.31(P=0.19)					
3.1.3 11 months					
Leslie 2008 PAK	4/68	1/54		100%	3.18[0.37,27.6]
Subtotal (95% CI)	**68**	**54**		**100%**	**3.18[0.37,27.6]**
Total events: 4 (0.75mg/kg PQ weekly 8wks), 1 (0.5mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Z=1.05(P=0.29)					
Test for subgroup differences: Chi^2=0.2, df=1 (P=0.91), I^2=0%					



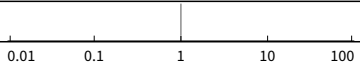
Analysis 3.2. Comparison 3 0.75 mg/kg primaquine/week for 8 weeks versus high-standard 14-day regimen, Outcome 2 Serious adverse effects.

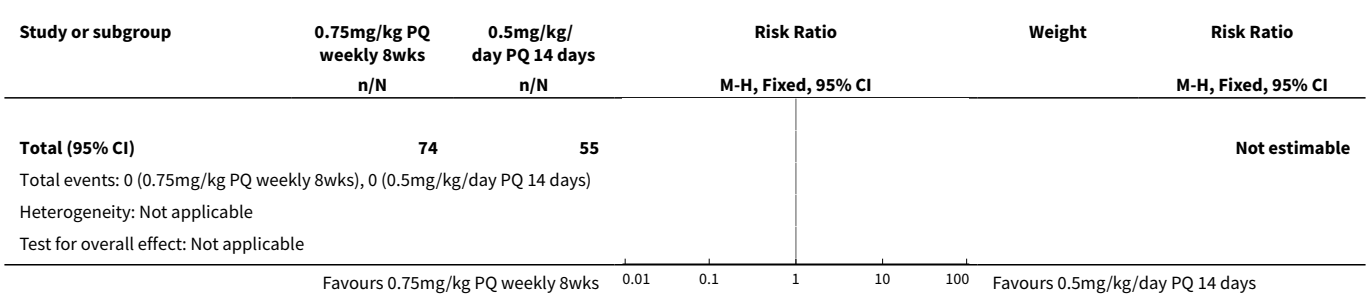
Study or subgroup	0.75mg/kg PQ weekly 8wks n/N	0.5mg/kg/ day PQ 14 days n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Leslie 2008 PAK	0/74	0/55			Not estimable
Total (95% CI)	**74**	**55**			**Not estimable**
Total events: 0 (0.75mg/kg PQ weekly 8wks), 0 (0.5mg/kg/day PQ 14 days)					
Heterogeneity: Not applicable					
Test for overall effect: Not applicable					



Analysis 3.3. Comparison 3 0.75 mg/kg primaquine/week for 8 weeks versus high-standard 14-day regimen, Outcome 3 Anaemia (haemoglobin < 7 g/dL).

Study or subgroup	0.75mg/kg PQ weekly 8wks n/N	0.5mg/kg/ day PQ 14 days n/N	Risk Ratio M-H, Fixed, 95% CI	Weight	Risk Ratio M-H, Fixed, 95% CI
Leslie 2008 PAK	0/74	0/55			Not estimable

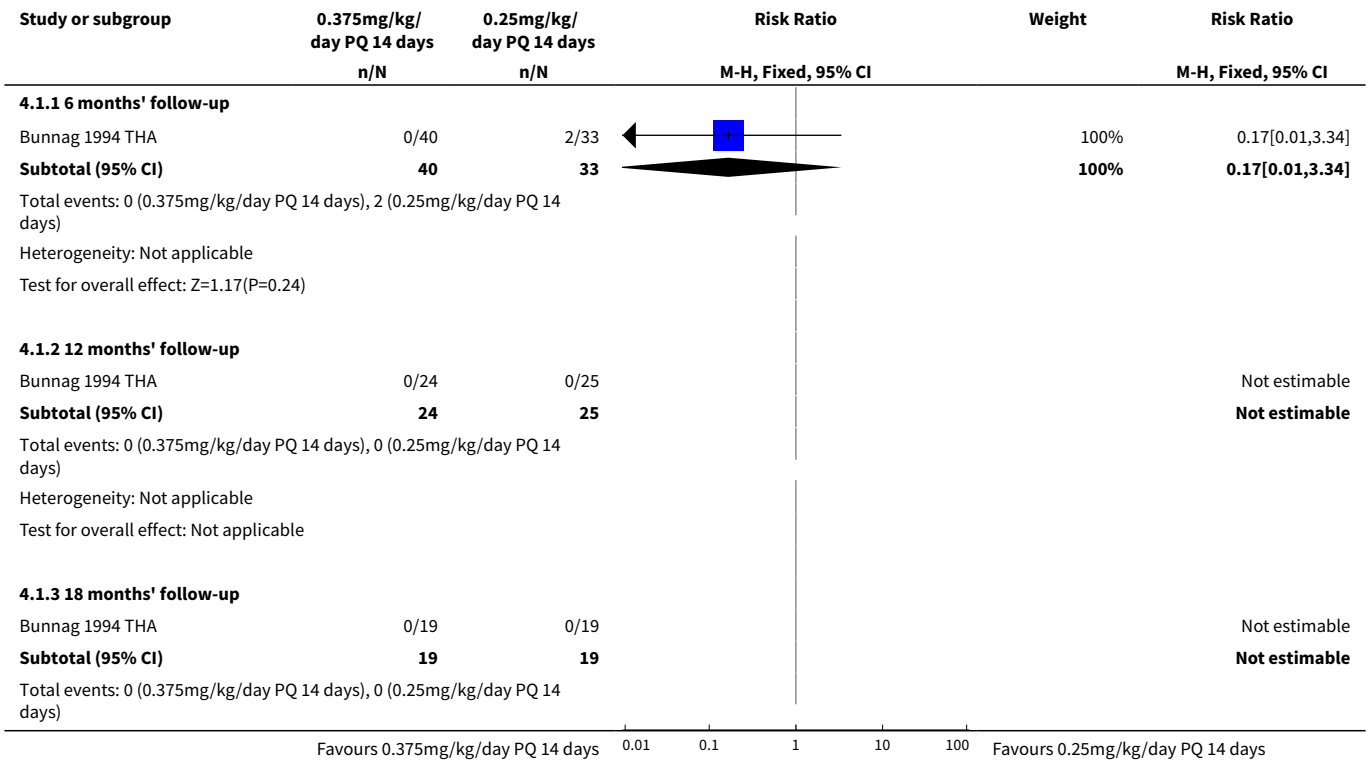


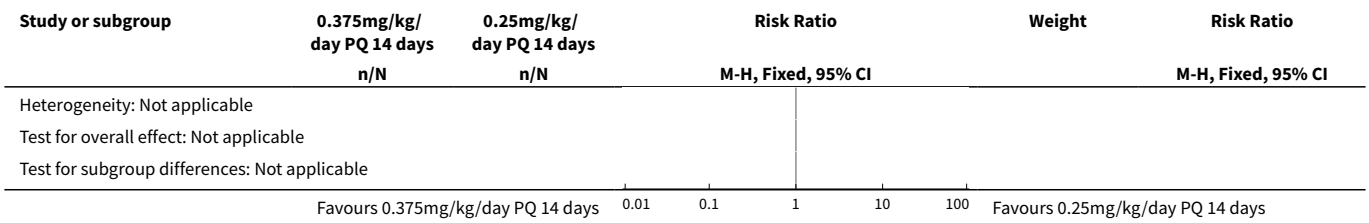


Comparison 4. 0.375 mg/kg/day primaquine for 14 days versus standard 14-day regimen

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Recurrence	1		Risk Ratio (M-H, Fixed, 95% CI)	Subtotals only
1.1 6 months' follow-up	1	73	Risk Ratio (M-H, Fixed, 95% CI)	0.17 [0.01, 3.34]
1.2 12 months' follow-up	1	49	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]
1.3 18 months' follow-up	1	38	Risk Ratio (M-H, Fixed, 95% CI)	0.0 [0.0, 0.0]

Analysis 4.1. Comparison 4 0.375 mg/kg/day primaquine for 14 days versus standard 14-day regimen, Outcome 1 Recurrence.

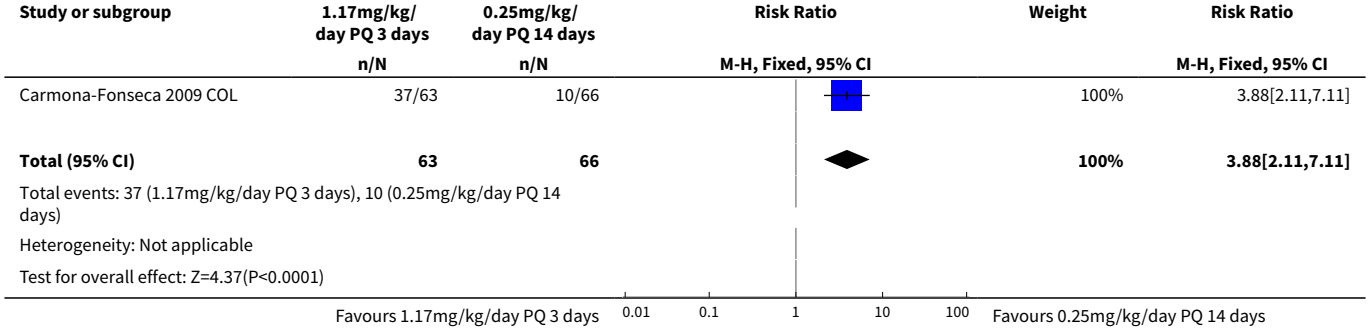




Comparison 5. 1.17 mg/kg/day primaquine for 3 days versus standard 14-day regimen; follow-up 4 months

Outcome or subgroup title	No. of studies	No. of participants	Statistical method	Effect size
1 Recurrence	1	129	Risk Ratio (M-H, Fixed, 95% CI)	3.88 [2.11, 7.11]

Analysis 5.1. Comparison 5 1.17 mg/kg/day primaquine for 3 days versus standard 14-day regimen; follow-up 4 months, Outcome 1 Recurrence.



ADDITIONAL TABLES

Table 1. Data extraction: grouping of comparisons to address the review's objectives

Objective	Intervention	Control
Are higher doses (0.5 mg/kg/day or 30 mg/day primaquine for 14 days) more effective in all areas, or only in areas where they are standard treatment (East Asia and Oceania)?	Blood-stage antimalarial drug with primaquine 0.5 mg/kg/day (adult dose 30 mg) for 14 days (total dose 420 mg). Both intervention and control groups must have received the same treatment for the blood-borne stage of infection, that is, either CQ or ACT.	Blood-stage antimalarial drug with standard 14-day course primaquine (0.25 mg/kg/day, adult dose 15 mg, total dose 210 mg). Both intervention and control groups must have received the same treatment for the blood-borne stage of infection, that is, either CQ or ACT.
Are shorter, higher-dose regimens of primaquine over 7 days as effective as treatment over 14 days (is the total dose	Blood-stage antimalarial drug with primaquine 0.5 mg/kg/day (adult dose 30 mg) for 7 days (total dose 210 mg) or 1 mg/kg/day (adult dose 60 mg) for 7 days (total dose 420 mg).	Blood-stage antimalarial drug with standard 14-day course primaquine (0.25 mg/kg/day, adult dose 15 mg, total dose 210 mg) or high-standard 14-day course primaquine (0.5 mg/kg/day, adult dose 30 mg, total dose 420 mg).

Table 1. Data extraction: grouping of comparisons to address the review's objectives *(Continued)*

rather than the length of treatment the important factor)?	Both intervention and control groups must have received the same treatment for the blood-borne stage of infection, that is, either CQ or ACT.	Both intervention and control groups must have received the same treatment for the blood-borne stage of infection, that is, either CQ or ACT.
Are weekly dosing regimens (0.75 mg/kg/week or 45 mg/week for 8 weeks) as effective?	Blood-stage antimalarial drug with primaquine 0.75/kg (45 mg) per week for 8 weeks (total dose 360 mg)	Blood-stage antimalarial drug with standard 14-day course primaquine (0.25 mg/kg/day, adult dose 15 mg, total dose 210 mg) or high-standard 14-day course primaquine (0.5 mg/kg/day, adult dose 30 mg, total dose 420 mg). Both intervention and control groups must have received the same treatment for the blood-borne stage of infection, that is, either CQ or ACT.

Abbreviations: ACT = artemisinin-based combination therapy; CQ = chloroquine.

APPENDICES

Appendix 1. Detailed search strategies

PubMed	MEDLINE
1	primaquine [Title/Abstract]
2	"Primaquine"[Mesh]
3	1 or 2
4	"plasmodium vivax" [Title/Abstract]
5	"Plasmodium vivax"[Mesh]
6	"vivax malaria " [Title/Abstract]
7	"Malaria, Vivax"[Mesh]
8	4 or 5 or 6
9	3 and 8
10	"Randomized Controlled Trial" [Publication Type] OR "Controlled Clinical Trial" [Publication Type]
11	randomized or placebo [Title/Abstract]
12	randomly or trial or groups [Title/Abstract]
13	"drug therapy" [Subheading]
14	10 or 11 or 12 or 13
15	9 and 14

Cochrane Library

Issue 12 2018

ID Search

#1 primaquine: ti,ab,kw: (Word variations have been searched)

#2 MeSH descriptor: [Primaquine] explode all trees

#3 #1 or #2

#4 "plasmodium vivax": ti, ab,kw (Word variations have been searched)

#5 MeSH descriptor : [Malaria, Vivax] explode all trees

#6 MeSH descriptor: [Plasmodium vivax] explode all trees

#7 #4 or #5 or #6

#8 #3 and #7

Embase

1947-Present, updated daily

---

1 "primaquine".mp.

2 primaquine/

3 1 or 2

4 plasmodium vivax.mp. or Plasmodium vivax/

5 malaria vivax.mp. or Plasmodium vivax malaria/

6 4 or 5 or 6

7 controlled clinical trial.mp. or Controlled Clinical Trial/

8 randomized controlled trial.mp. or Randomized Controlled Trial/

9 (randomized or placebo or double-blind* or single-blind*).mp.

10 randomization/

11 crossover procedure/

12 7 or 8 or 9 or 10 or 11

13 3 and 6 and 12

LILACS

Search on : primaquine [Words] and malaria vivax or plasmodium vivax [Words]

ClinicalTrials.gov and WHO ICTRP

primaquine and vivax

CONTRIBUTIONS OF AUTHORS

Rachael Milligan (RM): data collection and management, analysis and interpretation of results, review writing.

Andre Daher (AD): data collection and management, analysis and interpretation of results, review writing.

Patricia Graves (PMG): interpretation of results, review writing.

All review authors read and approved the final manuscript.

DECLARATIONS OF INTEREST

RM has no known conflicts of interest.
AD has no known conflicts of interest.
PMG has no known conflicts of interest.

SOURCES OF SUPPORT

Internal sources

- Liverpool School of Tropical Medicine, UK.

External sources

- Department for International Development, UK.

  Project number 300342-104

DIFFERENCES BETWEEN PROTOCOL AND REVIEW

We changed the inclusion criteria for trials to add 30 mg (0.5 mg/kg/day) for 14 days, as this is a World Health Organization-recommended regimen, and some trials use it as the control group for this reason.

Chapter III

Introduction Chapter III

The use of routine health information systems

The gold standard to evaluate the efficacy of medical interventions is the randomized clinical trial (RCT); these usually assess an efficacy outcome in a controlled scenario. RCT designs minimize bias and confounding factors, and provide robust causal inferences comparing two interventions (Friedman et al., 2010). Although there is a clear need to produce this sort of evidence for regulatory purposes before any large-scale use of a medical intervention, these results may lack external validity, meaning that the same results may not be reproduced in other conditions than the ideal scenario.

As renewed interest of evaluating the impact of large scale interventions in public health develops, methodologies for broader assessment are needed. Public health policies need to consider outcomes in the real-world setting, i.e. the effectiveness of the intervention (Thriemer et al., 2018). Effectiveness evaluations have tended to use intermittent surveys, but there are compelling arguments to expand the use of routine health information systems to enable assessment of the outcomes of programmatic interventions or policy changes (Wagenaar et al., 2015).

Routine health information systems tend to generate numerous observations over an extended period, and a wide range of health facilities, in real time. On the other hand, intermittent surveys are expensive, can only be undertaken intermittently and mostly rely

on external research teams, preventing local capacity building and sustainability of surveillance. Certain features of routine health information systems may allow research designs that demonstrate causal inferences of a programme intervention or policy change that surveys might have less power to demonstrate, especially in low endemicity settings (Ashton et al., 2017).

Concerns about the use of routine health information systems focus on potential measurement error; incomplete or inaccurate collected data may bias estimations of the intervention's impact (Wagenaar et al., 2015, Ashton et al., 2017). This was particularly illustrated in malaria studies in Africa, where case definitions were based on clinical examination without parasitological confirmation and treatment provided by the private sector was not captured in the routine health system. Widespread use of the malaria rapid diagnosis test and the implementation of electronic capturing data systems have changed this (AbouZahr and Boerma, 2005). The Global Technical Strategy for Malaria 2016-2030 emphasizes the need urges to transform malaria surveillance into a core intervention (World Health Organization., 2015b).

The Brazilian Ministry of Health has recorded all notified cases of malaria since 2003 in an online surveillance system (Sistema de Vigilância Epidemiológica em malária-SIVEP-mal) (Braz et al., 2016). This surveillance system was designed to track outbreaks and assess the effectiveness of local malaria control activity, and eventually, prospective interventions (Santelli et al., 2012). It is estimated that 99.6% of malaria cases notified in Brazil were captured by the system in 2014 (Secretaria de Vigilância em Saúde, 2015). High rates of completeness are driven by the compulsory notification of malaria in Brazil and because notification is necessary to access free of charge malaria treatment, which is

provided exclusively by the National Health System (Sistema Único de Saúde-SUS) (Braz et al., 2016) for each microscopy or rapid diagnostic test confirmed case of malaria. Although, SIVEP provide a unique source of rich data, there is a fundamental limitation to its use to evaluate interventions. Successive observations of the same patient over a period of time are not linked by a personal unique identifier, making the evaluation of individual outcomes challenging. We have explored how computational tools can address this issue.

Record linkage

Assembling information from difference sources is routine in almost all research settings. Drawing conclusions based on information recorded in two distinct logbooks is easy and feasible, but only if there is a link between the two records, using a unique identifier. Linking pairs of records based on agreement using a specific identifier is called deterministic record linkage (Machado, 2004).

Deterministic record linkage relies on unique identifiers (ID), limiting its use when they are absent. It may also miss the link if there is an ID error, meaning that this approach does not necessarily adequately reflect the uncertainty that exist due to inherent quality assurance problems.

In contrast, probabilistic record linkage is based on the similarity of the records, and does not require a unique identifier. The underlying assumption is that if there is enough information on a record as name, date of birth, and address, these items of information may be as suitable to link records as a unique ID. This approach in public health dates backs to the 40's (Dunn, 1946). Its use as a way of recording follow up of the patients

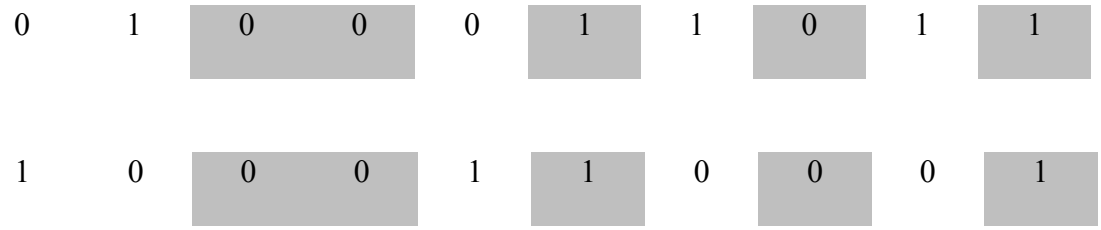
based on hospital records appeared for the first time in the medical literature in the end of the 50's (Newcombe et al., 1959).

Since then, many approaches have been developed: calculating an edit distance; name phonetic algorithms; the q-gram approach, which divides strings into chunks of size q; other distance metrics such as the Jaro or Jaro–Winkler (Sayers et al., 2016), and finally the bloom filters. All of these methods have certain principles in common, the need to use a score and a definition on what is an acceptable threshold of similarity, in other words, criteria to classify the records either as unmatched or matches (Fellegi and Sunter, 1969).

The Dice score was described in 1945 as a measure of ecological association between species (Dice, 1945). It is consistently used in computational science to define the acceptance threshold in probabilistic record linkage, or a measure of association between records. The Dice score is the simple formula $2h/A+B$, where h is the number of coincidences, A = number of "0s" and B = number of "1s".

In this work, we used Dice scores and Bloom filters. The latter were initially designed in the 70's as a spell correcting tool, but currently are used to screen vast amounts of data with applications in many fields of computational science (Broder A., 2005). Bloom filter uses the array of bit underlying the data, i.e. a sequence of 0 and 1, and compares with the array of bit in another group. The ***Figure 7*** illustrate a hypothetical pair of arrays of 10 bits, each one corresponding to one record, and its Dice score.

Figure 7. Illustration of two hypothetical arrays of 10 bits each.



The coincident bits are grey. Dice score = 2*5/11+9 = 0.5

The study evaluation of malaria recurrence rate in Brazil

The data matching in the study *evaluation of malaria recurrence rate in Brazil, an observational retrospective cohort* was done using bloom filters to exclude duplicate records of the same patient in the National System of Malaria Surveillance (SIVEP malaria) data set from 1st July 2014 till 31st May 2015. As we were looking for recurrences, we compared this data set with itself, and therefore, each record was duplicate. This process is called deduplication.

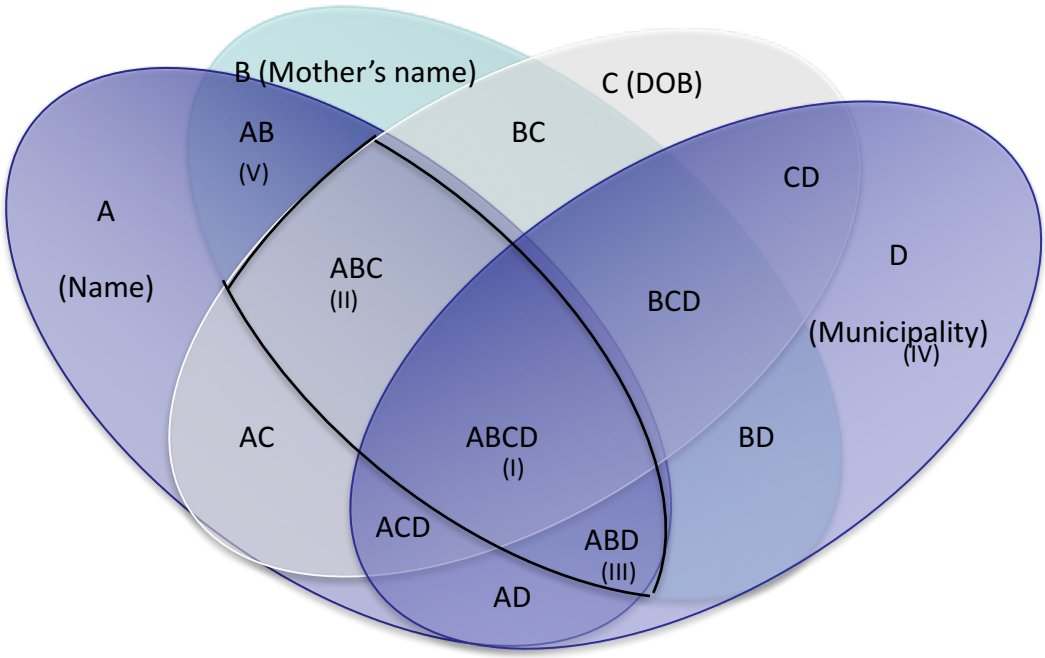
The procedure used in this study included the filter of 240 bites (***Figure 8***). The results are converted into a threshold score using the Dice index. The designing of the data matching strategy was a critical methodological step to define the relevant probabilistic record linkage threshold within this dataset.

Figure 8. Division of the 240 bites array.

```
#
# I - ABCD
#
field_1 = [s1_X,50]
field_2 = [s2_X,50]
field_3 = [day,30]
field_4 = [month,30]
field_5 = [year,30]
field_6 = [ufr,25]
field_7 = [munr,25]
p = Filtro_de_Bloom_parametrizado(7,
                                  field_1,
                                  field_2,
                                  field_3,
                                  field_4,
                                  field_5,
                                  field_6,
                                  field_7)
f_I.write( p +  ',' + str(idn) + ',m' + "\n")
'''
NOME       -- A
NM_MAE     -- B
DT_NASC    -- C
MUN_RESI   -- D
'''
```

The data used in this study record linkage is schematically presented in Figure 9. The final match was based the area surrounded by the black line.

Figure 9. Schematically presented record linkage



Evaluating the data matching strategy

After a record linkage procedure, each record is classified either as a match or an unmatched record. The match result can be either a false match or a true match, excessive false matches mean lack of specificity. On the other hand, unmatched records that are "missed true matches" denote lack of sensitivity.

Using current computational processors, a deterministic record linkage (based on unique ID) can only miss a true match or provide a false match due to an error in the unique ID. Dealing with routine health information systems and probabilistic record linkage is more challenging. The underlying assumption is that other information rather than a unique ID can be compared to produce a match, and the highest score (100% similarity) is a true match. However, if we rely only on the highest score, many true matches will be missed (low sensitivity) due to the inherent quality of the routine health information systems, that includes misspelling and other errors. In this scenario, setting the threshold score is a critical point.

A dataset that has a subset of records with a unique ID is the ideal situation to test and set the probabilistic record linkage threshold score. A deterministic record linkage based on the unique ID is the gold standard that allows defining the accuracy of the procedure (Silveira and Artmann, 2009). This ideal setting is very unlikely to happen, and other strategies have been put in place, none of them are universally useful or accepted, although many produce satisfactory results (Blakely and Salmond, 2002, Oliveira et al., 2016, Fonseca et al., 2010).

The National System of Malaria Surveillance (SIVEP malaria) data set does not has a subset of records with a unique ID and a data matching strategy was defined prior to the procedure.

The national guidelines require the health agent to record all vivax positive slide with in a 60 days' period as a follow up slides. This data does not allow the matching of the records, but it provides an estimate of the recurrences in Brazil. The objective of the data matching strategy was to be more sensitive than the health agent reports in the field (number of follow up slides- LVC- SIVEP). Match counts lower than reported cases in the field were considered to be inadequate sensitivity, or regarded as not able to identify all true matches. The specificity of the record matching was confirmed by visual inspection, in order to quantify the false matches.

Similarly to a drug dose range study, threshold scores were chosen from a wide range that included a low specificity (low scores and vast number of matches) to a low sensitivity (higher scores and smaller number of matches than the health agent reports in the field). Each intersection of figure 2 (ABCD; ABD; ABC) was tested as shown in Table 1.

Table 1. Threshold scores tested to define the data matching strategy.

		Probabilistic record linkage fields		
	Strategy	**ABCD**	**ABD**	**ABC**
Dice score (x 10000)	I	8000	8500	8500
	II	8000	9500	9500
	III	9500	9500	9500
	IV	9500	10000	10000

A= Name; B= mother's name; C= Date of Birth; D = Municipality of residence

Results of data matching

The main limitation to Strategy IV (higher scores in the ABC) was the very low completeness of the field date of birth in the state of Acre. This was one of states with the highest malaria incidence in Brazil during the year studied, however this strategy resulted in a very low number of matches. The strategy III, II and I were selected as these were more sensitive than the health agent reports in the field.

These were then individually inspected to see whether the pairs were true matches. Both I and II had too many false matches. Complete visual inspection was performed in the dataset that was processed according to the strategy III, namely Matches ABCD (threshold score 9.500) and ABD and ABC (threshold score 9.500).

In this dataset, 241 matches (out of 17983) were identified as false matches (1.3%), denoting good specificity. Some of these false matches had more than one patient grouped as the same match. These matches were reorganized and they generated 493 new matched records. The replacement and manual matching procedures are recorded in the investigator file. After the replacement of the false matches the dataset was locked and analysis performed. Herein, the results are presented and discussed (Daher et al., 2019).

In conclusion, during the current era, computational data analysis capabilities are growing exponentially. It makes the use of the routine health information methodologically easier and more valuable, as it increases the external validity of the results and the feasibility of the studies in low endemic areas, as Brazil. Besides the methodological and financial advantages of the use of routine health information, its widespread use in operational

research promotes improvement in data collection quality and strengthens the local health care organization.

Many methods are available to link the records of these health information systems, including bloom filters that process the data in its minimal unit, an array of bits. Despite the use of technology proving the sensitivity and specificity of the method is challenging. The linkage strategy is a crucial methodological point to appraise these features of the evaluation. In this study, the selected strategy had an acceptable sensitivity according to the threshold defined. There were not excessive false matches denoting good specificity. However, as each data set presents dissimilarities in the completeness and accuracy of the data, there is not a universally useful methodology, and the most suitable one must be appraised on a case-by-case basis.

Evaluation of Plasmodium vivax malaria recurrence in Brazil.

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Evaluation of *Plasmodium vivax* malaria recurrence in Brazil

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Abstract

Background: Control of vivax malaria in endemic areas requires management of recurrence. The Brazilian National Malaria Surveillance System (SIVEP-Malária) records every case of malaria in Brazil, but is not designed to differentiate between primary and recurrent infections. The aim of this study was to explore whether the information provided by SIVEP-Malária could be used to identify *Plasmodium vivax* recurrences, its risk factors and evaluate the effectiveness of short course primaquine (7–9 days: total dose 3–4.2 mg/kg) in preventing relapses.

Methods: In this observational retrospective cohort study, data matching of SIVEP-Malária records was undertaken using bloom filters to identify potential recurrences defined as microscopically-confirmed *P. vivax* episodes from the same individual occurring within a year. Generalized Estimation Equation (GEE) models were used to determine predictors of recurrence. Extended Cox-based conditional Prentice–Williams–Peterson models (PWP) models were used to evaluate time to recurrence.

Results: Between June 1, 2014 and May 31, 2015, 26,295 episodes fulfilled the criteria of potential recurrence among 154,970 reported malaria episodes. Age ≤ 3 years, being male, literate, not-indigenous and having domestic working activities were identified as risk factors for recurrence. There was no difference in time to recurrence or recurrence frequency between patients treated with 14-day or 7–9 day primaquine regimens (HR = 1.02, 0.96–1.09) and RR = 0.97 (0.90–1.04), respectively. The use of chloroquine alone was associated with a 1.43 (1.29–1.58, $p < 0.0001$) increased risk of *P. vivax* recurrence compared to patients who used chloroquine combined with short-course primaquine, the Brazilian standard of care. This was RR = 2.06 (1.48–2.86, $p < 0.0001$), RR = 1.90 (1.60–2.25, $p = 0.0001$) and RR = 1.14 (1.00–1.29, $p = 0.05$) for recurrences occurring between 3–28, 29–60 and > 60 days, respectively. PWP models showed that the time to recurrence was longer in recipients of both primaquine and artemisinin-based combination therapy (ACT) compared to patients treated with chloroquine alone or with concomitant primaquine, HR = 2.2 (1.62–2.99, $p < 0.0001$), HR = 1.27 (0.97–1.66, $p = 0.08$), respectively.

Conclusion: Short course primaquine was as effective as 14-day regimens and associated with a halving of the risk and delay in time to recurrence of *P. vivax* infections in comparison to chloroquine alone. The study demonstrates the feasibility of using record linkage on routine surveillance data to identify potential *P. vivax* recurrences, associated risk factors and impact of treatment.

Keywords: Malaria, Recurrences, Plasmodium, Vivax, Falciparum, Primaquine, Chloroquine, Artemisinin-based combination therapy, ACT, Record link

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Background

Malaria control was first implemented in Brazil in 1905. Initial success led to the eradication campaign in 1965 [1], which lasted until the late 60s. The lowest number of annual cases, 36,900, was recorded in 1961 [2]. During the 90s, partly because of a growing population in the Amazon Region, over half a million cases per year were recorded, peaking in 1999 with 637,470 cases [3]. Since then, following the introduction of renewed malaria control efforts, including vector control and early diagnostics and treatment with artemisinin-based combination therapy (ACT) [4, 5], a drop in malaria cases has been observed with a nadir of 143,552 malaria cases in 2014. *Plasmodium vivax* infections accounted for 84% of all cases, highlighting the growing importance of this specie.

Brazil is currently focusing on the elimination of both *Plasmodium falciparum* and *P. vivax* [6, 7]. Understanding *P. vivax* recurrences is critical for malaria control in endemic areas. In Brazil, cases of malaria are recorded in the National System of Malaria Surveillance (SIVEP Malária). Although it is believed that over 99% of cases are recorded in this system, it is not designed to differentiate between primary and recurrent malaria episodes. This study aimed to explore if and how routinely collected data from the Health Surveillance System can be used to describe epidemiological patterns such as the event rate, the time interval between repeated episodes of vivax malaria, and risk factors of *P. vivax* recurrence, such as age or *P. falciparum* triggering a *P. vivax* episode.

The study also investigated the effectiveness of a 7–9 days primaquine and its comparison with a 14 days primaquine regimen, the synergic effects of concomitant use of primaquine with chloroquine and the influence of the use of ACT on the time to *P. vivax* recurrence.

Methods

National malaria treatment guidelines Brazil

The current first-line treatment for vivax infection is 3 days of chloroquine (600 mg on day 1, and 450 mg on days 2 and 3) with concomitant use of a short course of primaquine (7–9 days: total dose 3–4.2 mg/kg). The prescription of 14-day regimens of primaquine is indicated only if the health care provider can monitor the adherence. Pregnant women standardly receive the 3-day treatment course of chloroquine but do not receive primaquine. Only if they experience more than one episode of clinical malaria during a single pregnancy, they receive weekly chloroquine chemoprophylaxis for 12 weeks or until delivery. The current first-line treatment for uncomplicated falciparum malaria is artemether-lumefantrine [8] combined with a single dose of primaquine (maximum total dose 45 mg).

Population and study design

This observational retrospective cohort study used records of the National System of Malaria Surveillance (SIVEP Malária) from nine States in the Brazilian Legal Amazon Region between 1st June 2014 and 31st May 2015 inclusive. The SIVEP Malaria is an online surveillance system from the Ministry of Health that records all notified cases of malaria in Brazil since 2003 [9]. This surveillance system was designed to identify outbreaks and track the effectiveness of malaria control.

It is estimated that 99.6% of malaria cases notified in Brazil were captured by the system in 2014 [5]. High rates of notification are driven by the compulsory notification of malaria in Brazil and because notification is necessary to access free malaria treatment, which is provided exclusively by the National Health System (Sistema Único de Saúde-SUS) [9] for each microscopy or rapid diagnostic tests (RDT) confirmed case of malaria. Only malaria cases in specific populations, such as illegal gold miners working in border areas, may be under represented in the National System of Malaria Surveillance.

Recurrences and data matching

National guidelines state that any malaria positive smears conducted within 60 days of previous *P. vivax* infections and within 40 days for *P. falciparum* should be recorded as 'follow up' smears [8]. This information relies upon the reports provided by the patient to the health worker because the SIVEP Malaria system does not include a unique individual patient identifier, making it difficult to easily identify recurrent events.

Bloom filters matching strategies [10] were used to link records of different clinic visits made by the same patient to determine the interval in days between visits. A filter of 240 bites was defined, using a combination of patient's name (A), patient's mother's name (B), date of birth (C), and municipality of residency (D) (Venn diagram presented in Additional file 1). Absent identifiers were replaced with random values to avoid unwanted similarity. The degree of similarity between two filters was assessed using the Dice Coefficient calculated on the two binary vectors [11]. The score was scaled to a maximum of 10,000. Different combinations of identifiers were used in separate reduplication runs: (a) ABCD, (b) ABD and (c) ABC. These were then individually inspected to see whether the pairs were coherent. This procedure allowed the exclusion of false matches.

Evaluation of the population and recurrences

Baseline characteristics available from the SIVEP form (see Additional file 2) were evaluated. Age categories were matched to the primaquine dose bands of the

Brazilian treatment guidelines. A map of the incidence rate of vivax recurrences (cases per 1000) at the local governmental level of the Federative Republic of Brazil (municipalities) was obtained using Tableau Desktop (version 10.3).

The effect of baseline variables on recurrent events was expressed as relative risks (RR) obtained from log-binomial regression models with random effect for patients using Generalized Estimation Equation (GEE). Time between recurrent events was stratified as 3–28, 29–60 and >60 days based on the chloroquine half-life and per national definitions of recurrence. The same model was used to estimate the synergic effect of the concomitant use of primaquine and chloroquine on *P. vivax* recurrence.

Extended Cox-based conditional Prentice–Williams–Peterson (PWP) survival models for repeated ordered events were used for the analysis of time to vivax recurrence [12]. The time interval between sequential malaria episodes were graphically displayed using Kaplan–Meier curves and results presented as median (95% CI) time to recurrence. Two-sided p-values were used and statistical significance defined as <0.05. Analysis was conducted using R (version 3.2.5) and IBM SPSS statistics (version 24.0).

Ethics statement

The study protocol was reviewed and approved by the Ethics Committee at National Institute of Infectious Disease, Oswaldo Cruz Foundation (No. 1.591.434 CAAE 56245716.1.0000.5262) and complied with procedures of the Health Surveillance Secretary (SVS)—Ministry of Health of Brazil to access datasets containing personal information. The investigators ensured confidentiality of all records. The study is registered at the Brazilian Register of Clinical Trials (RBR-3n947j), a primary repository of WHO.

Results

Data matching results

Between June 1, 2014 and May 31, 2015 inclusive, 26,295 recurrences involving 18,185 patients were identified among 154,970 reported malaria episodes involving 128,675 patients using the Bloom Filter matching strategy. The strategy selected had ABCD, ABD and ABC Dice threshold scores of 9500. Individual record inspection identified 241 out of 17,983 matches as false matches. Some of these involved multiple potential matches between episodes from multiple patients (i.e. one visit matching to more than one patient). These 241 potential false matches were manually inspected and corrected, which resulted in 493 additional matches.

The original SIVEP Malaria dataset had 28,062 recurrent malaria episodes, however 1767 episodes were recurrences occurring within 2 days from the initial report and were excluded from the analysis giving a total of 26,295 recurrences that were used in the model (Additional file 3). The baseline characteristics are presented in Table 1.

Plasmodium vivax malaria recurrence

The final combination of parameters of the strategy selected resulted in more matches than the number of recurrences reported by the health agents. The incidence rates of *P. vivax* recurrence varied considerably at the municipality level, reflecting the widespread area over which malaria transmission occurs in Brazil [13] (Fig. 1).

Risk factors for *Plasmodium vivax* recurrences

Among those who took primaquine, the risk of recurrence decreased with increasing age. Compared to children aged ≤ 3 years, children aged 4–8 years and older adults had a 20% and 38% lower risk (4–8 years: RR = 0.80, 95% CI 0.75–0.86, $p<0.0001$; adults RR = 0.62, 0.57–0.67, $p<0.0001$, respectively). Being male increased the risk of *P. vivax* recurrence (RR = 1.11, 1.07–1.14, $p<0.0001$). The native indigenous population were at lower risk compared to other groups. The risk of vivax recurrence was higher in all education levels compared to those who were illiterate. Individual with domestic working activities were at the highest risk of recurrence compared to other occupations.

Similar risk factors were identified for time to recurrence. This reduced with each successive episode of *P. vivax* malaria (Table 2 and Fig. 2). Other factors associated with shorter time to recurrence included age ≤ 3 years, male gender, no hypnozoite treatment, and domestic occupation (Table 3). Previous *P. falciparum* infection was not associated with a shorter time to recurrence HR = 1.04, 0.93; 1.16, p = 0.50.

Effectiveness of 7 days versus 14 days primaquine on *P. vivax* recurrence incidence

Overall, 6226 (4.9%) patients were prescribed a 14-day course of primaquine and 120,608 (95.1%) a 7-day course (total dose 3–4.2 mg/kg). All received 3-days of chloroquine for *P. vivax* malaria. The risk of *P. vivax* recurrence was similar between the two regimens: 14-day: 887/6226 (14.3%) vs 7–9 day: 17,610/12,0608 (14.6%), RR = 0.97 (0.90–1.04), p = 0.96. Similar conclusions could be drawn from the PWP survival model which also showed no difference in the time to recurrence (HR = 1.02, 0.96–1.09, p = 0.52).

Table 1 Population baseline characteristics of the study population

Variables	Categories	Patients n = 128,675 (%)	Malaria cases n = 153,203 (%)								
			Results per specie					Parasitaemia			
			Vivax	Falciparum	Mixed F + V	Malariae	Non falciparum	Less than half cross	Half to one cross	Two crosses	Three–four crosses
Age	≤ 3 years	7231 (5.62)	8584 (5.6)	690 (0.45)	34 (0.02)	0 (0)	77 (0.05)	1900 (1.24)	2791 (1.82)	4082 (2.67)	507 (0.33)
	4–8 years	9884 (7.68)	10,837 (7.07)	1060 (0.69)	54 (0.04)	1 (0)	69 (0.05)	3039 (1.98)	3799 (2.48)	4686 (3.06)	406 (0.27)
	9–11 years	6344 (4.93)	6930 (4.52)	676 (0.44)	26 (0.02)	1 (0)	41 (0.03)	2192 (1.43)	2477 (1.62)	2785 (1.82)	166 (0.11)
	12–14 years	7036 (5.47)	7637 (4.98)	680 (0.44)	42 (0.03)	0 (0)	32 (0.02)	2260 (1.48)	2866 (1.87)	3065 (2)	156 (0.1)
	15–35 years	39,704 (30.86)	41,928 (27.37)	4467 (2.92)	333 (0.22)	16 (0.01)	152 (0.1)	12,328 (8.05)	14,754 (9.64)	18,412 (12.02)	1167 (0.76)
	Older than 36 years	58,476 (45.44)	57,099 (37.27)	11,284 (7.37)	370 (0.24)	18 (0.01)	65 (0.04)	24,565 (16.04)	24,878 (16.25)	18,198 (11.88)	1069 (0.7)
Gender	Female	51,327 (39.89)	52,861 (34.5)	7658 (5)	313 (0.2)	5 (0)	197 (0.13)	19,469 (12.71)	20,956 (13.69)	19,037 (12.43)	1282 (0.84)
	Male	77,346 (60.11)	80,153 (52.32)	11,198 (7.31)	546 (0.36)	31 (0.02)	239 (0.16)	26,814 (17.51)	30,608 (19.99)	32,191 (21.02)	2189 (1.43)
Race	White	8825 (6.97)	9198 (6)	1182 (0.77)	59 (0.04)	0 (0)	21 (0.01)	2893 (1.89)	3203 (2.09)	4022 (2.63)	311 (0.2)
	African background	5024 (3.97)	4983 (3.25)	872 (0.57)	46 (0.03)	3 (0)	11 (0.01)	1548 (1.01)	1768 (1.15)	2400 (1.57)	184 (0.12)
	Asian background	1310 (1.03)	1356 (0.89)	161 (0.11)	14 (0.01)	0 (0)	2 (0)	369 (0.24)	441 (0.29)	656 (0.43)	64 (0.04)
	Mixed background	88,927 (70.25)	91,756 (59.89)	14,344 (9.36)	608 (0.4)	23 (0.02)	162 (0.11)	34,902 (22.79)	34,355 (22.44)	35,081 (22.91)	2230 (1.46)
	Native indigenous	22,492 (17.77)	23,139 (15.1)	2250 (1.47)	129 (0.08)	8 (0.01)	240 (0.16)	6119 (4)	11,017 (7.19)	7766 (5.07)	583 (0.38)
	Not informed	14,594 (11.34)	2583 (1.69)	48 (0.03)	3 (0)	2 (0)	0 (0)	453 (0.3)	781 (0.51)	1303 (0.85)	99 (0.06)
Years in School	Illiterate	77,141 (59.95)	14,523 (9.48)	2199 (1.44)	94 (0.06)	3 (0)	80 (0.05)	5721 (3.74)	5694 (3.72)	5033 (3.29)	347 (0.23)
	Incomplete/complete fundamental studies	20,725 (16.11)	78,780 (51.42)	11,958 (7.81)	534 (0.35)	28 (0.02)	236 (0.15)	27,919 (18.23)	31,415 (20.52)	30,006 (19.6)	1826 (1.19)
	Incomplete/complete high school	2227 (1.73)	22,637 (14.78)	2961 (1.93)	152 (0.1)	5 (0)	23 (0.02)	7892 (5.15)	8428 (5.5)	8889 (5.81)	531 (0.35)
	Graduation	13,988 (10.87)	1180 (0.77)	157 (0.1)	3 (0)	0 (0)	2 (0)	454 (0.3)	444 (0.29)	413 (0.27)	29 (0.02)
	Not informed	7231 (5.62)	15,895 (10.38)	1582 (1.03)	76 (0.05)	0 (0)	95 (0.06)	4298 (2.81)	5584 (3.65)	6887 (4.5)	738 (0.48)

Table 1 (continued)

Variables	Categories	Patients n = 128,675 (%)	Malaria cases n = 153,203 (%)								
			Results per specie					Parasitaemia			
			Vivax	Falciparum	Mixed F + V	Malariae	Non falciparum	Less than half cross	Half to one cross	Two crosses	Three–four crosses
Economic activity	Domestic	12,062 (9.37)	13,056 (8.52)	1595 (1.04)	77 (0.05)	0 (0)	27 (0.02)	4353 (2.84)	4991 (3.26)	5093 (3.33)	275 (0.18)
	Others	75,468 (58.65)	78,955 (51.54)	10,939 (7.14)	374 (0.24)	13 (0.01)	164 (0.11)	28,306 (18.49)	30,073 (19.64)	29,699 (19.4)	2092 (1.37)
	Agriculture livestock	28,192 (21.91)	28,253 (18.44)	4393 (2.87)	186 (0.12)	4 (0)	153 (0.1)	10,213 (6.67)	12,469 (8.14)	9597 (6.27)	512 (0.33)
	Tourism	2759 (2.14)	2883 (1.88)	287 (0.19)	18 (0.01)	2 (0)	8 (0.01)	649 (0.42)	1103 (0.72)	1326 (0.87)	110 (0.07)
	Mining	5999 (4.66)	5722 (3.73)	1125 (0.73)	172 (0.11)	10 (0.01)	23 (0.02)	1486 (0.97)	1334 (0.87)	3812 (2.49)	368 (0.24)
	Vegetal extractivism	1048 (0.81)	955 (0.62)	208 (0.14)	18 (0.01)	7 (0)	3 (0)	293 (0.19)	354 (0.23)	508 (0.33)	31 (0.02)
	Fishing hunting	2758 (2.14)	2758 (1.8)	282 (0.18)	10 (0.01)	0 (0)	56 (0.04)	890 (0.58)	1101 (0.72)	970 (0.63)	75 (0.05)
	Road dam construction	389 (0.3)	433 (0.28)	28 (0.02)	4 (0)	0 (0)	2 (0)	94 (0.06)	140 (0.09)	223 (0.15)	8 (0.01)

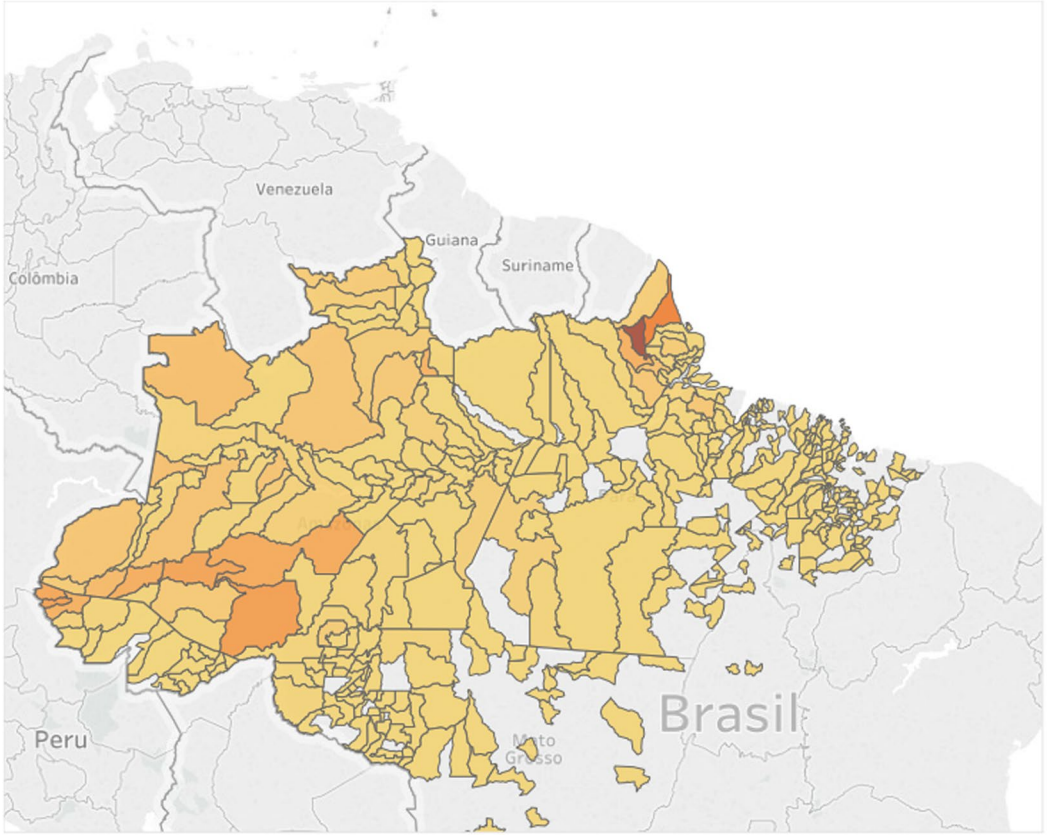

Fig. 1 Incidence of *Plasmodium vivax* recurrence (cases per 1000)

Effectiveness of the concomitant use of primaquine on *P. vivax* recurrence incidence

As stated previously, primaquine treatment is not recommended in Brazil for pregnant women and chloroquine chemoprophylaxis for pregnant women is only given after the second malaria episode. This enabled the use of the pregnant population with a single vivax recurrence as a comparator to assess the effect of hypnozoite treatment.

Pregnant patients who did not take short-course primaquine had a 1.43 (95% CI [1.29–1.58]) risk of *P. vivax* recurrence compared to non-pregnant patients who took primaquine. This was RR = 2.06 (1.48–2.86, p < 0.0001), RR = 1.90 (1.60–2.25, p = 0.0001) and RR = 1.14 (1.00–1.29, p = 0.05) for recurrences occurring between 3–28, 28–60 and > 60 days. The time to a *P. vivax* recurrence was also shorter in pregnant women (HR = 1.53, 1.39–1.69, p < 0.0001).

Overall, 399 patients received ACT with a primaquine total dose of 3–4.2 mg/kg starting after the third day of ACT to treat mixed *P. vivax* and *P. falciparum* infections. There was a reduced risk of *P. vivax* recurrence in those taking ACT and sequential primaquine compared to pregnant patients treated with chloroquine only (RR = 0.70, 0.52–0.93, p = 0.015). *Plasmodium vivax* recurrence also took longer in patients taking ACT and sequential primaquine than chloroquine alone treated patients HR = 2.2 (1.62–2.99, p < 0.0001), and those treated with chloroquine and with concomitant primaquine, HR = 1.27 (0.97–1.66, p = 0.08), but this difference was of borderline statistical significance.

Discussion

This study demonstrated the potential for the use of routinely collected public health surveillance records to evaluate the epidemiology and risk factors of recurrent *P. vivax* malaria in Brazil. The computational tools available allowed the processing and successful automated matching of over 150,000 case records to identify potential recurrent infections. This provided access to unique data that allowed us to identify associated risk factors and impact of treatment using routine surveillance data. The analysis showed that short 7–9 day courses of primaquine with concomitant use of chloroquine was more effective than chloroquine alone, and equally as effective as the 14-day regimen in preventing *P. vivax* recurrences. ACT-primaquine combinations were also more effective

Table 2 Median time to sequential episodes of vivax malaria

Recurrences	Number of vivax recurrences	Median time (95% CI) to recurrence (days)
1st	17,460	71 (70.19–71.81)
2nd	4967	65 (63.83–66.17)
3rd	1429	60 (58.33–61.67)
4th	452	56 (52.08–59.92)
5th	150	54 (46.41–61.59)
6th	49	41 (31.47–50.53)
7th	15	38 (29.51–46.49)
8th	6	46 (34.24–57.76)
Overall		69 (68.37–69.63)

than chloroquine alone or chloroquine–primaquine combinations in delaying recurrence, although the latter was not statistically significant.

An important part of this analysis included matching of case records in the absence of a unique patient identifier to link different episodes that occurred in the same individual during a 1-year period. Although there are limitations in the quality of the routine data in many surveillance systems in resource poor settings, using computational approaches to explore the large datasets may help generate evidence to support public health policies [14]. Bloom filters are designed to screen vast amounts of data with applications in many fields of computational science [15] and are aimed at detecting duplicate entries. The quality of data entry is a major factor in defining the best matching strategy and, therefore, there is no standard strategy suitable for all datasets. This meant that, depending on the completeness of the dataset using high Dice's coefficients in the matching strategy led to a low number of matches (low sensitivity). However, the visual inspection of the lower thresholds had too many false matches (low specificity). The current strategy identified a greater frequency of recurrences than the one reported by the health agents based on patient's recall.

Brazil recommends a short 7–9 days courses (total dose 3–4.2 mg/kg). Fourteen days of primaquine is only recommended in Brazil either when patients are considered likely to be compliant or can undergo supervised treatment. The similar effectiveness between these two regimens is encouraging, reinforcing the idea that cure rate is a function of the total primaquine dose rather than treatment length [16, 17].

The importance of addressing the effectiveness of primaquine in real life for radical cure of *P. vivax* infections was highlighted recently [18]. This equal effectiveness of the short-course regimen has important practical implications because a patient friendly primaquine regimen is desirable and regimens of shorter duration are more likely to be adhered to [19]. The evidences on which this short-course primaquine regimen in Brazil is based

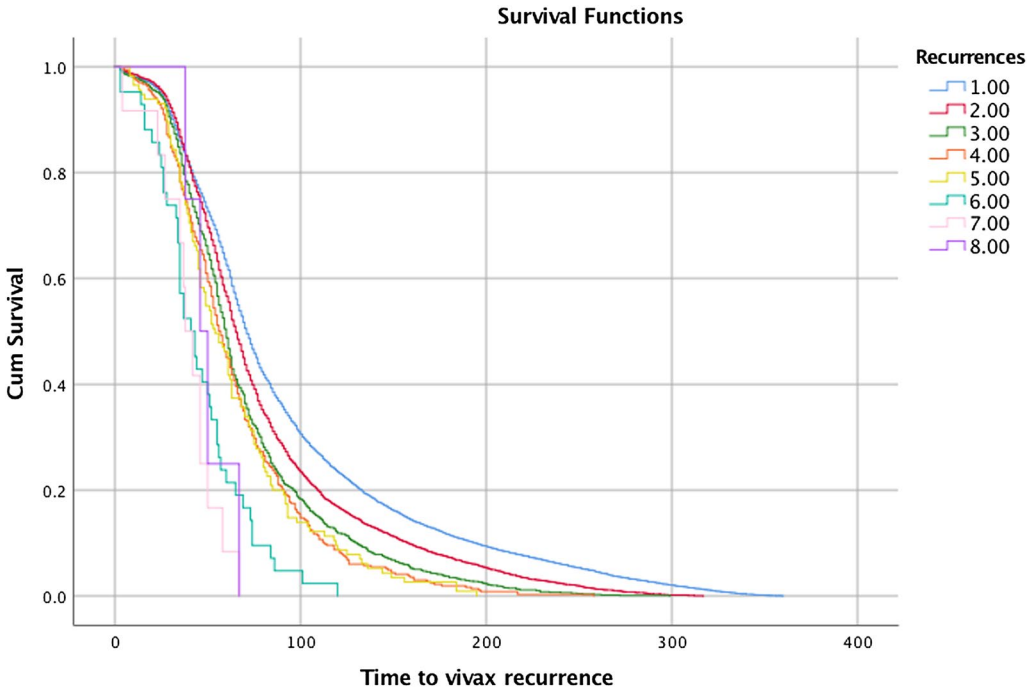

Fig. 2 Kaplan Meier graph sequential episodes of vivax malaria

Table 3 Factors related to *P. vivax* recurrence

Predictor factor	Categories	Generalized Estimation Equation (GEE) model		Prentice, Williams and Peterson (PWP) survival model
		N (%) N = 151,784	RR (95% CI), p value	HR (95% CI), p value
Age categorical	Older than 36 years	68,173 (44.9)	0.62 (0.57–0.67), p < 0.0001	0.75 (0.70; 0.81), p < 0.0001
	15–35 years	46,380 (30.6)	0.71 (0.65–0.77), p < 0.0001	0.78 (0.72;0.84), p < 0.0001
	12–14 years	8337 (5.5)	0.77 (0.7–0.85), p < 0.0001	0.81 (0.74;0.88), p < 0.0001
	9–11 years	7604 (5)	0.82 (0.75–0.91), p < 0.0001	0.87 (0.80;0.96), p = 0.003
	4–8 years	11,982 (7.9)	0.8 (0.75–0.86), p < 0.0001	0.83 (0.78;0.88), p < 0.0001
	≤ 3	9308 (6.1)	REF	REF
Hypnozoite treatment	No primaquine	2667 (1.8)	1.43 (1.29–1.58), p < 0.0001	1.53 (1.39;1.69), p < 0.0001
	Primaquine 3–4.2 mg/kg	149,117 (98.2)	REF	REF
Gender	Male	90,914 (59.9)	1.11 (1.07–1.14), p < 0.0001	1.08 (1.05;1.11), p < 0.0001
	Female	60,870 (40.1)	REF	REF
Ethnic background	Not informed	1377 (0.9)	1.60 (1.4–1.82), p < 0.0001	1.19 (1.02;1.38), p = 0.03
	Native indigenous	25,705 (16.9)	0.78 (0.73–0.84), p < 0.0001	0.89 (0.84;0.95), p < 0.0001
	Mixed background	106,819 (70.4)	1.03 (0.97–1.09), p = 0.34	0.95 (0.87;1.04), p = 0.25
	Asian background	1527 (1)	0.96 (0.8–1.15), p = 0.68	0.94 (0.80;1.11), p = 0.49
	African background	5908 (3.9)	0.94 (0.86–1.04), p = 0.23	0.95 (0.87;1.04), p = 0.25
	White	10,448 (6.9)	REF	REF
Economic activity	Road dam construction	467 (0.3)	1.01 (0.8–1.27), p = 0.94	0.92 (0.74;1.13), p = 0.42
	Fishing hunting	3106 (2)	0.72 (0.63–0.82), p < 0.0001	0.73 (0.65;0.82), p < 0.0001
	Vegetal extraction	1191 (0.8)	0.59 (0.48–0.73), p < 0.0001	0.70 (0.58;0.85), p < 0.0001
	Mining	7046 (4.6)	0.81 (0.74–0.89), p < 0.0001	0.86 (0.79;0.93), p < 0.0001
	Tourism	3182 (2.1)	0.79 (0.71–0.89), p < 0.0001	0.76 (0.68;0.84), p < 0.0001
	Agriculture livestock	32,919 (21.7)	0.77 (0.73–0.82), p < 0.0001	0.79 (0.75;0.83), p < 0.0001
	Others	89,132 (58.7)	0.85 (0.81–0.9), p < 0.0001	0.91 (0.87;0.95), p < 0.0001
	Domestic	14,741 (9.7)	REF	REF
Years in school	Complete graduation	1341 (0.9)	1.14 (0.97–1.35), p = 0.12	1.04 (0.89;1.22), p = 0.63
	Complete high school	13,647 (9)	1.14 (1.06–1.23), p < 0.0001	1.07 (1.00;1.15), p = 0.04
	Incomplete high school	12,070 (8)	1.15 (1.07–1.24), p < 0.0001	1.09 (1.02;1.17), p = 0.02
	Complete fundamental studies	9745 (6.4)	1.11 (1.03–1.2), p = 0.01	1.05 (0.97;1.13), p = 0.24
	Not informed	17,596 (11.6)	1.33 (1.22–1.44), p < 0.0001	1.27 (1.17;1.37), p < 0.0001
	Incomplete fundamental studies	81,190 (53.5)	1.12 (1.06–1.18), p < 0.0001	1.06 (1.01;1.12), p = 0.02
	Illiterate	16,195 (10.7)	REF	REF

REF reference group

on are a limited number of clinical trials [20, 21], programmatic experience, and historical evidence suggesting that the efficacy of primaquine is more a function of the cumulative dose than the duration of treatment [17]. However, the evidence has been deemed insufficient to support international guidelines. An ongoing systematic review [22] and the results of this current analysis may increase this body of evidence.

Children under 3 years old had an increased risk of recurrence compared to all other age categories, with a trend towards increasingly lower risk with increasing age. There are several potential explanations for this in addition to differences in acquired immunity: The doses of chloroquine and primaquine may be inadequate at this young age [23] or the absence of child-friendly formulations may result in poor adherence and reduced effectiveness. Herein it was demonstrated a reduced risk of recurrence in the native indigenous population. Similarly, it was also showed that the risk was lowest in the illiterate population. Both may reflect higher exposure rates early in life and a more rapid acquisition of protective immunity in these groups [24].

Previous falciparum infection was not found to caused more rapid recurrence of *P. vivax*, in contrast to

suggestions from others in the literature [24]. It is not clear if this is a function of a long duration of post-treatment prophylactic effect of ACT to suppress *P. falciparum* infections.

It was found that the time to recurrence appears to reduce with each subsequent episode of vivax recurrence. Although these observations are in contrast with the current understanding of hypnozoite activation which implies a gradual lengthening, rather than shortening with each successive relapse, these results are consistent with previous observations in Brazil [25]. The underlying assumption related to the gradual lengthening hypothesis is that with multiclonal infections the earliest active and more rapidly multiplying parasite became patent first [24]. These observations relied on artificial infection and animal models. Although this study cannot differentiate with relapse, reinfections and recrudescence, it does not provide strong support for a gradual lengthening of relapse intervals. Further research to address the issue is needed.

The observation that the addition of primaquine was already effective in preventing recurrence in the first 28 days is interesting. It implies that the addition of primaquine to chloroquine either reduced recrudescence or early relapses and may support the synergic effect of primaquine and chloroquine in the blood stage [26–29]. This trend is also seen in the following period until the 60th day. A small (RR = 1.14) effect was also seen beyond 60 days, but it is not clear if this reflects a long-lasting anti-relapse effect of primaquine [4] or a higher exposure risk in the non-primaquine recipients resulting in more new infections and thus a higher rate of recurrence.

The study design is an important limitation. This is a 1-year retrospective open cohort, meaning that patients infected before the study start yet recurring during the study were considered as initial events. Second, the only way that the effectiveness of short course of primaquine could be evaluated was to use the pregnant population as the reference group because this group does not receive primaquine during pregnancy. However, this is a biased population and the results must be interpreted with this in mind. Potential confounders include changes in mosquito biting frequency during pregnancy, altered personal protection and healthcare-seeking behaviour, altered immunological responses, and changes in distribution of drugs, although chloroquine pharmacokinetics parameters in pregnant women does not appear to be very different from the rest of the population [30]. Despite these limitations, our results do suggest that the current national treatment guidelines in Brazil are effective in reducing relapse.

Additionally, every data matching strategy has its limitations and they can result in different number of recurrences depending on the reliability of the data entry. A unique identifier for the patients would allow a better evaluation of the sensitivity and specificity of the strategy and increase the use of the database as routine. Besides the data quality concerns, microscopy does not allow differentiation between relapse, recrudescence and reinfection of the *P. vivax* episodes and the aggregate recurrence was used in the analysis. Routine genotyping to differentiate these is currently not feasible at scale under programmatic conditions. Efforts to differentiate relapse and reinfection in this study by restringing the analysis to infants born during the study and, therefore, free of hypnozoites, was not successful as the sample of newborns was too small. Multi-year cohorts may address this issue.

Conclusions

Overall, this study confirmed that under real-life conditions, short-course primaquine was equally as effective as the 14-day regimen, which is the standard in many other countries. ACT-primaquine combinations were also more effective than chloroquine alone or chloroquine-primaquine combinations in delaying recurrence. The concomitant use of primaquine and chloroquine either reduced recrudescence or very early relapse and may support their synergic effect in the blood stage. Finally, the study demonstrates the feasibility of using record linkage to identify potential *P. vivax* recurrences, associated risk factors and impact of treatment using routine surveillance data. These routine data could also potentially provide a baseline for evaluation of further malaria control interventions that address the malaria control in Brazil.

Additional files

Additional file 1. Venn Diagram of record linkage.

Additional file 2. SIVEP form.

Additional file 3. Data flowchart.

Abbreviations

ACT: artemisinin-based combination therapy; FIOCRUZ: Oswaldo Cruz Foundation; GEE: Generalized Estimation Equation models; HR: Hazard Ratio; PWP: Prentice–Williams–Peterson models; RDT: rapid diagnostic tests; RR: relative risk; SIVEP-Malária: Brazilian National Malaria Surveillance System.

Authors' contributions

AD, DL, PM, and FK participated in the study concept and design of the study. AS and AD performed the record linkage. JCA and AD performed the analysis of the data. AD, FK, CJF, PM, JCA and DL participated in interpretation of the data and critical revisions of the manuscript. All authors read and approved the final manuscript.

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Competing interests
The author declares following competing interests and financial disclosure: André Daher is a public servant of the Institute of Drug Technology (Farmanguinhos), Oswaldo Cruz Foundation (Fiocruz), Ministry of Health. Farmanguinhos is one of the study sponsors. Farmanguinhos is a public federal pharmaceutical industry, it does not sell medicine on the market. The Brazilian Ministry of Health exclusively drives its drug production. These disclosures do not alter our adherence policies on sharing data and materials. There are no restrictions on the sharing of data and/or materials.

Availability of data and materials
Not applicable.

Consent for publication
This manuscript does not contain any individualized data. The confidentiality of the patients' records has been observed according ethical regulations.

Ethics approval and consent to participate
The study protocol was reviewed and approved by the Ethics Committee at National Institute of Infectious Disease, Oswaldo Cruz Foundation (No. 1.591.434 CAAE 56245716.1.0000.5262) and complied with procedures of the Health Surveillance Secretary (SVS)—Ministry of Health of Brazil to access datasets containing personal information. The investigators ensured confidentiality of all records.

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Chapter IV

Introduction Chapter IV

The importance of the vivax ACT trial to Brazil

The study *Efficacy and safety of Artemisinin-based Combination Therapy and chloroquine with concomitant primaquine to treat Plasmodium vivax malaria in Brazil: an open label randomized clinical trial* (Daher et al., 2018a) was conducted to evaluate potential new treatment regimens that could be used to replace the current chloroquine based regimen recommended as public health policy in Brazil.

The study was designed with the support of the National Malaria Control Programme in an effort to improve the efficacy, tolerability and adherence of vivax treatment. This effort included: (i) the development of a new coated formulation of chloroquine to mask the bitter taste of the drug, potentially improving the tolerability (Pereira et al., 2016b), (ii) a new formulation of primaquine that lead to drug exposure comparable to the FDA reference product (FDA, 2019), (iii) a co-blisters of chloroquine and primaquine (7 days regimen) according to four weight bands, as co-packaging may improve treatment compliance (Orton and Barnish, 2005) and improve effectiveness (malERA, 2017b), and finally (iv) Artemisinin-based Combination Therapies (ACTs) + primaquine to treat vivax.

There is a convincing rationale for a simpler drug treatment for both *P. falciparum* and *P. vivax* malarias, and ACTs are the most obvious candidates for this purpose. The benefits of a unified treatment for both malaria species have been discussed for almost

ten years (Douglas et al., 2010) including in our paper (Daher et al., 2018a), namely: (i) efficacious treatment in co-endemic areas with unreliable diagnosis per species (Douglas et al., 2010); (ii) or high incidence of mixed infections; (iii) prevention of *P. vivax* relapse following *P. falciparum* infections (White, 2011, Commons et al., 2019); (iv) simplified drug stock and supply management; (v) maintenance of ACT production in industrial scale if falciparum treatment purchases fade out; (vi) availability of more than one vivax treatment avoiding selective pressure; (vii) a child friendly formulation where liquid chloroquine is not available.

However, although it is the development of chloroquine-resistant *P. vivax* that has been the final rationale for adoption of this approach (World Health Organization., 2010a), these others advantages ought to be taken in account. They may justify update of treatment guidelines before the end of the drug lifespan, sparing the emergence of chloroquine resistance. Here, we present the efficacy and safety results of three vivax treatment regimens using either an ACT or chloroquine with concomitant use of primaquine in Brazil.

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RESEARCH

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Efficacy and safety of artemisinin-based combination therapy and chloroquine with concomitant primaquine to treat *Plasmodium vivax* malaria in Brazil: an open label randomized clinical trial

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Abstract

Background: There is general international agreement that the importance of vivax malaria has been neglected, and there is a need for new treatment approaches in an effort to progress towards control and elimination in Latin America. This open label randomized clinical trial evaluated the efficacy and safety of three treatment regimens using either one of two fixed dose artemisinin-based combinations or chloroquine in combination with a short course of primaquine (7–9 days: total dose 3–4.2 mg/kg) in Brazil. The primary objective was establishing whether cure rates above 90% could be achieved in each arm.

Results: A total of 264 patients were followed up to day 63. The cure rate of all three treatment arms was greater than 90% at 28 and 42 days. Cure rates were below 90% in all three treatment groups at day 63, although the 95% confidence interval included 90% for all three treatments. Most of the adverse events were mild in all treatment arms. Only one of the three serious adverse events was related to the treatment and significant drops in haemoglobin were rare.

Conclusion: This study demonstrated the efficacy and safety of all three regimens that were tested with 42-day cure rates that meet World Health Organization criteria. The efficacy and safety of artemisinin-based combination therapy regimens in this population offers the opportunity to treat all species of malaria with the same regimen, simplifying protocols for malaria control programmes and potentially contributing to elimination of both vivax and falciparum malaria.

Trial registration RBR-79s56s

Keywords: Malaria, *Plasmodium vivax*, Antimalarial treatment, Chloroquine, Mefloquine, Artesunate, Lumefantrine, Artemether, Primaquine, Artemisinin-based combination therapy, ACT, Clinical trial

Background

Malaria control and elimination activities have mainly focused upon *Plasmodium falciparum* as the leading cause of malaria mortality and morbidity worldwide. Nevertheless, *Plasmodium vivax* remains a major public health problem [1]. In 2010, 2.48 billion people worldwide were living in areas at risk for vivax infection. Brazil is the largest endemic area in the Americas; where stable transmission and a dispersed population [2, 3] pose major challenges for malaria control [4, 5]. In 2014, 143,552 malaria cases were reported [6].

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Over the last 30 years, the proportion of malaria cases due to vivax in Brazil has increased from 50%, in 1988 to 84% in 2014. *Plasmodium vivax* now causes 65% of hospitalizations due to malaria in the Brazilian Amazon region, and 13 out of 38 malaria deaths in Brazil in 2014 were due to *P. vivax* [6]. Control and management of this disease is therefore important, particularly in light of the increasing recognition that *vivax* infection is not as benign as first thought and that vivax can both cause severe disease [7–9], and have negative impacts on prosperity, longevity, school performance, pregnancy and the economy [10–12]. Optimizing the treatment of vivax malaria is critical to improve vivax control.

In Brazil, the current treatment recommended to treat vivax is chloroquine with concomitant use of 7 days of primaquine. The primaquine total dose ranges from 3 to 4.2 mg/kg [13]. Recent trials have not demonstrated chloroquine resistant vivax in Brazil [14–16], although there are still concerns about chloroquine resistance emergence [17]. The availability of other options, particularly ones that may be useful for the treatment of both vivax and falciparum malaria, would help to avoid selective pressure over a single therapeutic regimen. This study was designed to evaluate new approaches for the acute treatment of vivax in preparation for plans for both vivax elimination and updated treatment guidelines.

This randomized clinical trial evaluated the efficacy and safety of three vivax treatment regimens using either an ACT or chloroquine with concomitant use of primaquine in Brazil. The primary objective was to establish whether cure rates above 90% could be achieved in each arm [18].

Methods

Study population

Patients with uncomplicated vivax malaria were included in the study, after giving informed consent, if they met the following inclusion criteria: age between 18 and 70 years; weight between 50 and 90 kg, *P. vivax* mono-infection confirmed by microscopy, asexual parasite count > 250/μL, axillary temperature ≥ 37.5 °C or a history of fever during the past 48 h, and haemoglobin > 7.0 g/dL. Exclusion criteria were: malaria treatment in the previous 63 days; signs of severe malaria; concurrent other febrile conditions or chronic disease (such as severe cardiac, hepatic or renal disorders or HIV); the use of any medication known to interfere with anti-malarial pharmacokinetics; previous history of intolerance to any study drug; known glucose-6-phosphate deficiency; pregnancy confirmed by urinary human chorionic gonadotropin (hCG) testing; and breastfeeding.

Study design and drug administration procedures

This prospective, randomized, open label, three-arm efficacy study of uncomplicated vivax malaria was conducted according to World Health Organization (WHO) methods for surveillance of anti-malarial drug efficacy [18] at two centres in the Amazon Region of Brazil: the Tropical Medicine Research Centre (CEPEM) in Rondônia and Tropical Medicine Foundation Dr Heitor Vieira Dourado (FMT-HVD) in Manaus.

A 90% cure rate or greater is considered by the WHO to be sufficient evidence of efficacy to support the choice of a specific regimen by National Malaria Control Programmes in their treatment guidelines [18]. The sample size was calculated with an expected failure rate of 5%. 88 patients were included in each study arm to achieve a precision of 5% and allowing for 20% loss to follow-up, leading to a total of 264 patients.

A randomization list using blocks of six and allocation rate 1:1:1 was generated by software (Etcetera, version 2.72). Sequentially numbered (0–176 CEPEM and 177–264 FMT) opaque sealed envelopes were provided to the local clinical coordinators and used to randomize patients. Differences in dosing schedules and the difficulty of dummy blinding meant that neither patients nor healthcare workers were blinded, but microscopists were not aware of treatment allocation. The statistician was blind to the treatment allocation until the database had been locked.

Eligible patients were allocated to one of the following three treatment groups: (a) chloroquine (CQ); (b) fixed dose combination of artesunate and mefloquine (ASMQ) and; (c) fixed dose combination of artemether and lumefantrine (AL). All three arms received the same primaquine regimen.

Group A received chloroquine (Farmanguinhos—Fiocruz, Batch Numbers 12080940 and 14060467) 600 mg on day 1, and 450 mg on days 2 and 3. This is the Brazilian Ministry of Health current recommendation for uncomplicated malaria vivax treatment [13].

Group B received two tablets daily for 3 days of a fixed dose combination of 100 mg + 200 mg artesunate and mefloquine (ASMQ) tablets (Farmanguinhos—Fiocruz Batch Numbers 11100680 and 13040348) in a total of six tablets.

Group C received four tablets twice a day for 3 days of a fixed dose combination of 20 mg + 120 mg artemether and lumefantrine (AL) tablets (Coartem®—Novartis, Batch Numbers F2618 and K30711) in a total of 24 AL tablets.

All groups also received two tablets of 15 mg primaquine (Pq) (Farmanguinhos—Fiocruz, Batch numbers 12010038 and 13030282) for 7, 8 or 9 days (according to three weight ranges; ≥ 50–69; 70–79; 80–90 kg,

respectively), as recommended by National Malaria Control Programme treatment guidelines [13]. Patients received a total primaquine dose between 3.0 and 4.2 mg/kg.

The first 3 days' treatment were supervised for Group A and B. For Group C, only the first daily AL dose was directly observed. Patients in the AL arm were asked about their adherence to the previous second daily dose after the morning supervised dose. For all groups, the first dose was administered after diagnosis, and all subsequent supervised doses were taken between 8 and 10 a.m. If the patient vomited within 30 min after a dose, the same dose was administered again. At D7 follow-up visit, patients were inquired about their treatment adherence to unsupervised primaquine on days 4 through 7. The use of other treatments was recorded at every visit.

Glucose-6-phosphate dehydrogenase (G6PD) deficiency screening was not performed: this is not routinely performed in Brazil and it is not required by the national treatment guidelines. At enrolment, patients were asked about adverse events during previous primaquine use.

Ethics statement

The clinical study protocol and informed consent form were reviewed and approved by the Ethics Committee at CEPEM (No. 31/11 CEP/CEPEM 0018.0.046.000-11 CAAE—SISNEP and Plataforma Brasil No. 74869 CEP/CEPEM No. 05462612.7.0000.0011 CAAE). In April 2014, an amendment (No. 644.709 CEP/CEPEM) approved the inclusion of the second study site FMT-HVD, in Manaus. The Brazilian National Council on Ethics in Research (CONEP), Ministry of Health, accredits the CEPEM Ethics Committee. The study is registered at the Brazilian Register of Clinical Trials (RBR-79s56 s U1111-1132-8050), a primary repository of WHO. The clinical study was conducted in accordance with the Helsinki Declaration (Edinburgh, 2000), Good Clinical Practice [19, 20] and the Brazilian National Health Council (CNS) resolution 466/2011. During the study, monitoring visits were conducted to ensure GCP adherence. Written informed consent was obtained for every subject prior to enrolment. If the study subject was illiterate, an impartial third party witnessed the informed consent process. All subjects were informed of the nature and possible associated risks of the trial and that they were free to withdraw their consent to participate at any time. The investigators and study staff ensured confidentiality of all records.

Efficacy and safety evaluations

Patients were assessed on the day of enrolment and on days 1, 2, 3, 7, 14, 21, 28, 42 and 63 days after study inclusion. The scheduled study procedures comprised a full history, physical examination, and urinalysis at enrolment and assessment for clinical signs and adverse events (AE) at every follow-up visit. Blood samples were collected for parasite counts at 0, 3, 7, 14, 21, 28, 42 and 63 days, and haemoglobin was measured at 0, 14, 28, 42 and 63 days. An additional blood smear was also collected whenever treatment failed. Data was double entered using an electronic clinical record form (OpenClinica Community, version 3.1.3.1). Analysis was performed using R (version 3.2.5).

The primary efficacy endpoint was the proportion of the population with an adequate clinical and parasitological response (ACPR) at 63 days. Early treatment failure or late treatment failure were classified in line with standard WHO methodology [18]. The primary analysis was per protocol (PP); an intention-to-treat (ITT) analysis was also performed. The PP population excluded any participant with a protocol violation. Patients who missed the 28 or 42-day visit, but who had an ACPR at the subsequent follow visit was considered as a success at the previous visit. In the ITT population, protocol violations or losses to follow-up were considered as parasitological and clinical failure.

The secondary efficacy endpoints included the success rate at day 3 (72 h after first drug administration) as well as gametocyte clearance, fever clearance, and the cumulative success rate at days 28, 42 and 63.

The cumulative success rate by day 63, i.e. the probability of remaining parasite-free at day 63, was calculated using a Kaplan–Meier survival curve. Categorical variables were summarized using frequencies and percentages, while for quantitative variables, means, standard deviations (SD) and maximum–minimum values were used. Parasite counts were presented using geometric means.

The study was not designed to compare outcomes between treatment arms. However, exploratory analyses were conducted to explore the impact of the treatment arm on the safety and efficacy outcomes. The proportions of categorical variables were compared using Pearson's Chi squared test with Yates' continuity correction at significance level of 5%. Nonparametric tests of Wilcoxon and Kruskal–Wallis were used for continuous variables. Additionally, generalized linear models (binominal and Poisson distribution, respectively) were used to estimate the effect of predictors (baseline characteristics and the use of other medications) on treatment success at day 63 and the numbers of AEs that were possibly, probably or highly probably related to the treatment.

A safety analysis was conducted in the ITT population describing frequency, causality, and severity of AEs in each treatment arm. AEs reports were subdivided into: serious AEs, AEs leading to treatment suspension, and AEs which were described as possibly, probably or highly

probably related. The mean haemoglobin results at baseline and follow-up are also presented.

Patients were encouraged to seek unscheduled assessments if any AE was suspected. All clinical or laboratory abnormalities were categorized as Grade I to IV according to the Common Terminology Criteria for Adverse Events (CTCAE) of the National Cancer Institute [21]. Any suspected serious AE (standard definitions) was reported to the sponsor and the Ethical Review Committee. Recognized drug-related events were recorded as an AE, even if it could be related to malaria.

Parasitological densities were estimated using Giemsa-stained blood slides at a magnification of 1000× using WHO recommended methods [18]. Two trained microscopists read slides independently. The final density was calculated as the mean of the two readings. A third microscopist examined slides if the two readers disagreed over whether there were parasites present, the species, or the parasite density (more than 50% difference). In such cases, the final density was considered as the average of the two closest counts. A slide was considered as negative only after examining 1000 leucocytes in microscopic fields. Gametocyte presence was also recorded.

Results

Baseline characteristics of the study population

A total of 2475 malaria thick smear positive patients were screened for inclusion in the two trial sites (CEPEM and FMT-HVD) from August 2012 to February 2015. 264 (10.7%) were randomized (1:1:1) to one of three different treatment arms: CQ + Pq; ASMQ + Pq and AL + Pq. The same number of patients was allocated to each arm. The main reasons for not including patients were unavailability for follow-up (19.9%), parasitaemia lower than 250/μL (15.5%) and malaria treatment within the past 63 days (15.2%). Figure 1 shows the CONSORT flow diagram, see Additional file 1 for reasons not to be included. Twenty-three patients did not complete the study; there was no difference in the proportions that discontinued between the arms. Only one protocol deviation occurred: the inclusion of a patient with a mixed infection, confirmed by PCR. The baseline characteristics of the patients were similar among the treatment groups (Table 1), and the ITT and PP populations.

There were 495 reports of concomitant medication use in the study. Analgesic and antipyretic were most frequently used (287 reports); there was no difference in use between the three arms. Use of anti-ulcer and antispasmodic drugs were more common in the AL arm; accounting for 40.5% (15/27) and 69.6% (16/23) of the patients that used these classes, respectively.

A table presenting the medication used (grouped by therapeutic class) in each study arm, and a figure illustrating the most commonly used medications used per study visit and treatment group are provided (see Additional file 2).

Treatment adherence was 100% for ASMQ and CQ, as the dose during the initial 3 days was supervised. Nineteen patients reported AL non-adherence to the second daily dose: 16 patients reported one missing dose, 2 patients reported two missing doses, and one patient missed doses on three occasions. However, only one patient with incomplete AL adherence presented a treatment failure at day 63. Patients were asked about Pq adherence at D7 visit. Only five patients reported incomplete treatment; two in each ASMQ + Pq and CQ + Pq arms and one in the AL + Pq arm. None of them presented treatment failure.

Efficacy evaluation

The primary objective was to demonstrate cure rate above 90% in each study arm. The cure rate was defined as the proportion of the population with an adequate clinical and parasitological response (ACPR) at day 63. Cure rates were below 90% in all three treatment groups at day 63 in the PP population, although the 95% CI did include 90% for all three drugs: 85% (95% CI [77–93%]) of the ASMQ group, 88% (95% CI [81–95%]) of the CQ group, and 84% (95% CI [76–92%]) of the AL group achieved an ACPR at day 63. The cure rate of all the three treatment arms was greater than 90% at 28 and 42 days in the PP population (Table 2).

Cure rates in the ITT analysis were slightly lower (see Additional file 3) predominantly reflecting the very conservative approach to missing data. Secondary efficacy endpoints included the success rate at day 3 (72 h after first drug administration) and the cumulative success rate at days 28, 42 and 63, calculated using a Kaplan–Meier survival curve. These results are summarized in Fig. 2.

Parasitological success rates at day 3 were 100% for all arms in both PP and ITT analyses, apart from the AL + Pq arm where two dropouts (one informed consent withdraw, and one lost to follow-up) meant that the ITT success rate at day 3 was 98% (95% CI [98–100]).

Fever clearance is an important surrogate for cure in malaria. Only one patient in the CQ + Pq arm and two in the AL + Pq arm had an axillary temperature higher than 37.5 °C at day 3. Four fever episodes were reported after day 7. One patient had fever at day 63 and was considered as a treatment failure. The other three patients completed 63 days follow up without evidence of parasites on microscopy. All patients cleared gametocytes by day 3. The five patients that had gametocytes present on day 2 had all been treated with CQ + Pq (Fig. 3).

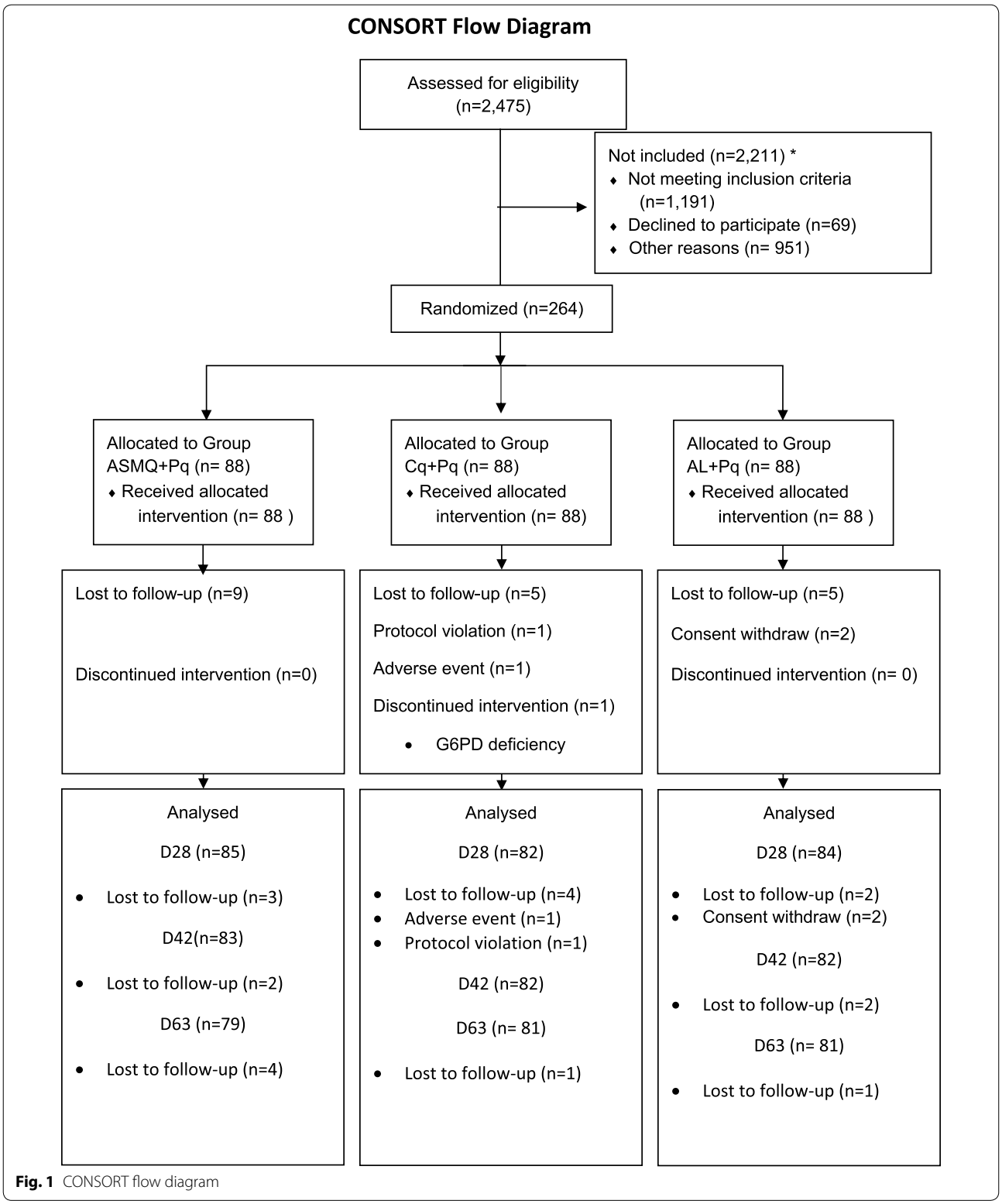


Fig. 1 CONSORT flow diagram

Safety results

Three serious adverse events (SAE) were reported. One patient in the CQ arm had treatment suspended because of haemolytic anaemia on day 3. Qualitative calorimetric test confirmed glucose-6-phosphate dehydrogenase (G6PD) deficiency. Another in the AL arm had a rise

Table 1 Baseline characteristics

Baseline characteristics	Treatment group		
	ASMQ + Pq	CQ + Pq	AL + Pq
N (%)	88 (33.3%)	88 (33.3%)	88 (33.3%)
Study site			
CEPEM	66 (75.0%)	66 (75.0%)	66 (75.0%)
FMT-HVD	22 (25.0%)	22 (25.0%)	22 (25.0%)
Gender			
Female	32 (36.4%)	22 (25.0%)	24 (27.3%)
Male	56 (63.6%)	66 (75.0%)	64 (72.7%)
Age (years)			
> 59	3 (3.4%)	4 (4.5%)	3 (3.4%)
18–39	45 (51.1%)	34 (38.6%)	49 (55.7%)
39–59	40 (45.4%)	50 (56.8%)	36 (40.9%)
Weight (kg)			
> 80	25 (28.4%)	22 (25.0%)	27 (30.7%)
50–65	27 (30.7%)	21 (23.9%)	23 (26.1%)
65–80	36 (40.9%)	45 (51.1%)	38 (43.2%)
Fever			
< 37.5	48 (54.5%)	54 (61.4%)	53 (60.2%)
> 37.5	40 (45.4%)	34 (38.6%)	35 (39.8%)
Weight (kg)	71.59 (10.99) [51–90]	72.7 (10.34) [53–90]	72.53 (10.59) [50–90]
Temperature	37.45 (1.38) [34.7–40.5]	37.31 (1.21) [35.0–39.9]	37.38 (1.22) [35.2–40.6]
Parasitaemia	2145 (2516.4) [258–13,340]	2155 (2908.5) [285–17,680]	2444 (3974.6) [270–20,080]
Age (years)	38.75 (10.9) [19.5–65.8]	41.88 (10.5) [19.7–64.9]	37.24 (11.8) [18.4–64.3]

Absolute number and percentage (%) were used to present categorical variables. Quantitative data presented using means, standard deviations (SD), and ranges [min–max]. Parasitaemia presented using geometric mean

in alanine aminotransferase (ALT) detected on day 12 after treatment. The third SAE reflected elective surgery and was not related to the trial. All patients recovered completely.

The safety analysis was conducted in the ITT population. A total of 1593 adverse events were reported. 1379 of them (86.5%) were classified as grade I and 208 events (13.1%) were classified as grade II. The distribution of adverse events by age and weight range, and treatment arm is shown in Table 3.

749 events (41.0%) were reported as possible, probable/likely or highly probably related to the treatment drug; 48.9% were grade I, 35.1% were grade II, none grade III and one SAE (grade IV). The distribution of all adverse events according with their intensity and causality are presented at Table 4. The AE distribution per causality and treatment group is shown in Additional file 4.

The distribution of all adverse events according with their intensity and causality per treatment group is provided (see Additional file 5).

All adverse events (1593) were grouped based on the main body system affected and treatment allocation (see Additional file 6). Non-specific complaints were most common (483), followed by gastrointestinal (450). As these symptoms could reflect the clinical illness or an AE, they were only considered as AE, if they were not present before dosing or they got worse after treatment. This overlap with symptoms may have led to an over estimation of AE. Tables listing all AE with a frequency higher than 3% per regimen allocation are provided (see Additional file 7).

The study treatment AE profile was very similar between study groups with the only category showing any difference being the occurrence of insomnia and abdominal pain ($p < 0.04$ and $p < 0.03$ respectively), using Pearson's Chi squared test.

Changes in haemoglobin (Hb) were a secondary outcome. There was a slight decrease in all groups at day 14 followed by recovery (Fig. 4). The median difference in haemoglobin at day 14 (Hb at day 14 − Hb at baseline/Hb at baseline) [22] in ASMQ + Pq, AL + Pq, and CQ + Pq were − 0.74 (95% CI − 1.13; − 0.36), − 0.49 (95% CI − 0.87; − 0.09), and − 0.58 (95% CI − 0.91; − 0.24), respectively. These were not statistically different. A drop of more than 20% from baseline was observed in 6 patients in each of the arms, ASMQ + Pq and AL + Pq and in 3 CQ + Pq patients. No patient had a drop of more than 30% from baseline. The mean and range haemoglobin at day 0, 14, 28, 42, and 63 is shown in Additional file 8. Unfortunately, haemoglobin was not

Table 2 Proportion of treatment success per treatment arm in PP population at day 28, 42 and 63

Visit day	Study treatment					
	ASMQ + Pq		CQ + Pq		AL + Pq	
	% (n)	95% CI	% (n)	95% CI	% (n)	95% CI
D28	100 (85)	–	100 (82)	–	96 (84)	92–100
D42	98 (83)	95–100	93 (82)	87–99	94 (82)	89–99
D63	85 (79)	77–93	88 (81)	81–95	84 (81)	76–92

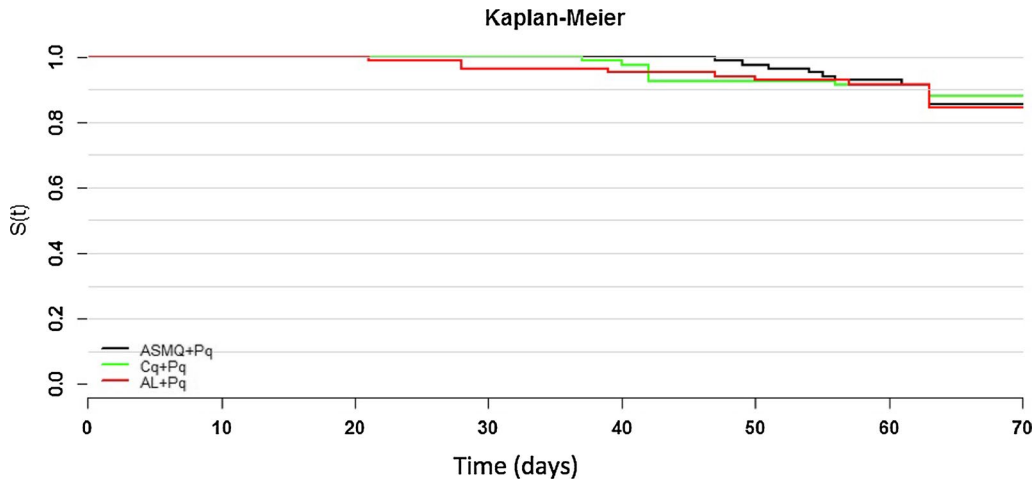
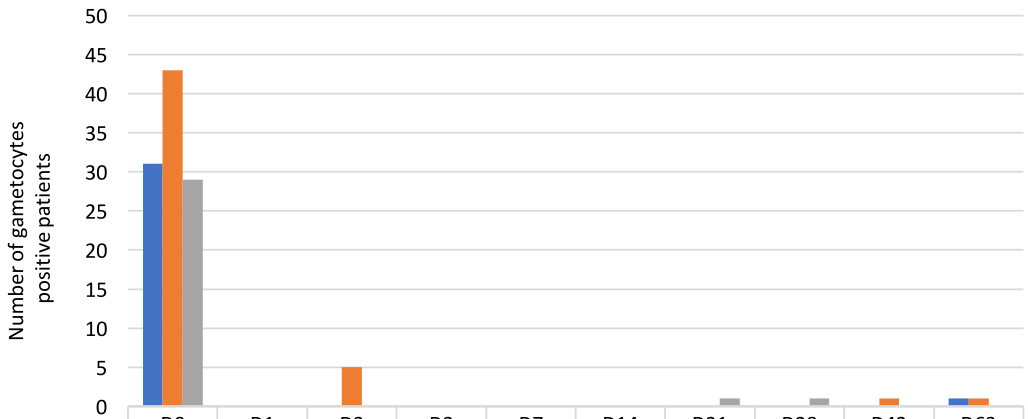


Fig. 2 Kaplan–Meier survival analysis of the three treatment arms



	D0	D1	D2	D3	D7	D14	D21	D28	D42	D63
ASMQ+Pq	31	0	0	0	0	0	0	0	0	1
Cq+Pq	43	0	5	0	0	0	0	0	1	1
AL+Pq	29	0	0	0	0	0	1	1	0	0

Fig. 3 Clearance of gametocytes

measured between 48 h and day 14; the lack of a day 7 measurement is a limitation of this study.

The effect of the study population's baseline characteristics and the use of concomitant medication on the frequency of AE in each treatment arm was assessed. In this generalized linear model (Poisson distribution), the dependent variable was the count of possible, probable/likely or highly probable AE related to the study treatments per person. Adverse events were less common in males (OR 0.78; 95% CI 0.60–0.99; $p = 0.04$), but use of antipyretic or antihypertensive was associated with high rates of adverse events (OR 1.64; 95% CI 1.29–2.10; $p < 0.01$ and OR 1.55; 95% CI 0.92–2.44; $p = 0.07$, respectively).

Exploratory analyses

This study was not powered to detect differences in cure rates between treatment regimens and no statistically significant difference could be detected between the treatment arms at any time point.

A generalized linear model (binominal distribution) was used to explore the influence of baseline variables and concomitant treatments upon failure at day 63, using a significance level of 5%. There was a strong association between the use of antipyretic medications

Table 3 Adverse events by age, weight range, and treatment arm

	Treatment group n (%)		
	ASMQ + Pq	CQ + Pq	AL + Pq
Age (years)			
> 59	14 (30.4)	21 (45.7)	11 (23.9)
18–39	190 (25.4)	255 (34.0)	304 (40.6)
39–59	199 (24.9)	367 (46.0)	232 (29.1)
Weight (kg)			
50–70	131 (20.2)	238 (36.8)	278 (43.0)
70–80	118 (25.8)	214 (46.7)	126 (27.5)
80–90	154 (31.6)	191 (39.1)	143 (29.3)

and treatment failure (OR 3.2; 95% CI 1.3–8.3; p = 0.01). A trend towards an association between the use of anti-ulcerative drugs and treatment success was also observed (OR 0.12; 95% CI 0.001–0.73; p = 0.06).

The effect of the treatment on the time to clearance of gametocytes was evaluated in the three arms. Patients in the ASMQ + Pq and AL + Pq arm had all cleared gametocytes by day 2. In the CQ + Pq arm, 5 out of 43 patients (11.6%) had gametocytes present of day 2. Gametocytes only reappeared in five of the 103 patients, making meaningful conclusions impossible.

Discussion

There has been recent international agreement that the importance of vivax malaria has been neglected [11, 23, 24], and that there is a need for new treatment approaches [25]. For most of the world, the first-line treatment recommendation of CQ + Pq has not changed since the 50s [11], despite the concerns about the emergence of CQ-resistance [17]. Standard treatment has been based upon the need to clear both the red cell forms and the hepatic forms and the relatively slow emergence of resistance of *P. vivax* to chloroquine may be due to the fact that standard CQ + Pq treatment has been an effective combination therapy [1]. In addition to clearing

Table 4 Distribution of all adverse events (1593) by intensity and causality

Grade	Causality n (%)					Total
	Doubtful	Unlikely	Possible	Probable/likely	Highly probable	
I	597 (37.5)	107 (6.7)	*538 (33.8)*	*118 (7.4)*	*19 (1.2)*	1379 (86.6)
II	86 (5.4)	49 (3.1)	*62 (3.9)*	*10 (0.6)*	*1 (0.1)*	208 (13.1)
IV	0 (0)	0 (0)	*0 (0)*	*1 (0.1)*	*0 (0)*	1 (0.1)
NA	3 (0.19)	2 (0.13)	*0 (0)*	*0 (0)*	*0 (0)*	5 (0.31)
Total	686 (43.1)	158 (9.9)	*600 (37.7)*	*129 (8.1)*	*20 (1.3)*	1593 (100)

Italic values indicate the relevant safety outcomes

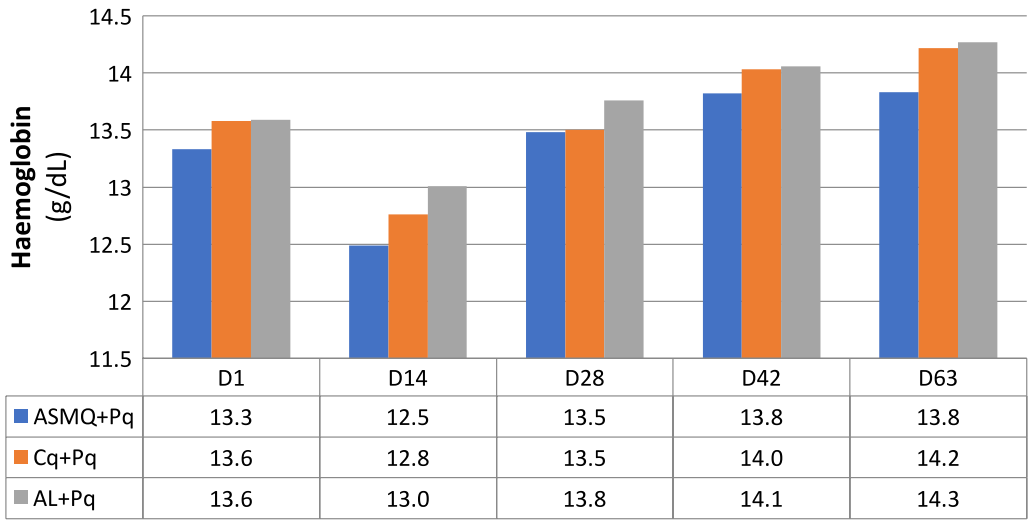


Fig. 4 Haemoglobin per regimen allocation at day 0, 14, 28, 42, and 63

hypnozoites, primaquine is active against blood stages of vivax [26] and chloroquine use also increases primaquine blood levels [27]; concomitant use of chloroquine and primaquine is more effective than sequential use [28].

ACT has been recommended by the WHO to treat vivax since 2010 [29], as it appears to have equivalent *P. vivax* schizonticidal activity to chloroquine [30] and it is recommended as the first-line treatment in areas of CQ-resistant *P. vivax* emerges. There are compelling arguments to look for a simpler and unified ACT treatment for both species of malaria. A single radical treatment would be useful in co-endemic areas where species diagnosis is difficult [31] or mixed infections occur that are misdiagnosed as *P. falciparum*. The use of ACT and primaquine for the treatment of *P. falciparum* infection also has the advantage of eradicating hypnozoites and preventing relapses from previous *P. vivax* infection that can happen following a *P. falciparum* infection [32]. There are also considerable logistic advantages for the use of ACT to treat both falciparum and vivax malaria with efficiencies of stock and supply management and an incentive to maintain ACT production as the requirement for falciparum treatment courses reduces.

In this study, all three treatment arms demonstrated cure rates > 90% in all treatment arms at day 42 but by day 63, cure rates had dropped below 90% (although all the 95% CI includes the 90% cure rate). It demonstrates the importance of a longer follow-up time in detecting failures, particularly in vivax when reinfection or relapse can occur [33]. This study did not detect any advantage from the use of an ACT with a longer half-life partner drugs, as has been shown previously; this may be due to the concomitant use of primaquine in our study [34]. Further studies with PCR analysis in an attempt to discriminate reinfection from relapse would be of interest.

Drug interactions and the safety profile are important factors in choosing the optimum first line treatment, in addition to pill burden and food restrictions. This trial evaluated the efficacy and safety of three vivax treatment regimens, and provides additional reassurance about the safety of ASMQ and AL when given with primaquine; information that is considered important by WHO as strategies for control and elimination of *P. vivax* malaria are developed [1]. Most of the AE with ACT regimens were mild to moderate (CTCAE grade I or II), and required minimal intervention. The frequencies of possibly, probably/likely or highly probably related AE did not substantially vary between the CQ + Pq regimen and the ACT + Pq regimens.

Despite this low level of adverse events, the ACT and primaquine combination still has significant drawbacks. Its use is limited in pregnancy, breastfeeding and G6DP-deficient population. The one SAE in this study was related to G6PD deficiency. These restrictions impose serious limitations to its use as an elimination tool in many parts of the world, although the primaquine regimen used in this study is the 7-day regimen currently recommended by the Brazilian National Malaria Control Programme. This regimen delivers a total dose from 3 to 4.2 mg/kg [13]. This is the WHO recommended total dose (although suggested over 14 days) and this regimen retains efficacy in some parts of the world [35–37]. The 7 day regimen has been suggested to improve adherence [13], although this has never been clearly demonstrated.

This study was not sufficiently powered to detect differences between the three arms and only followed up patients for 63 days, meaning that late relapses would not have been detected. The study population did not include children, limiting the conclusions of efficacy in this populations. The absence of PCR also meant that relapses could not be distinguished from reinfection; a critical issue in assessing vivax treatment. Nevertheless, this study demonstrates the efficacy and safety of two ACT and CQ in combination with 7 days of primaquine to treat uncomplicated vivax malaria in Brazil and demonstrated the feasibility and utility of a standardized treatment approach for all malaria cases.

Additional files

Additional file 1: Table S1. Reasons to not be included—Consort Diagram.

Additional file 2: Table S2. Distribution of use of concomitant medication (grouped in therapeutic class) in each study arm, parenthesis presents the percentage of the line. **Figure S1.** Distribution of most frequent medications used per study visit and treatment group.

Additional file 3: Table S3. Proportion of treatment success per treatment arm in ITT population (n = 88 per arm) at day 28, 42 and 63. **Table S4.** Proportion of treatment success per treatment arm in PP population at day 07, 14 and 21. **Table S5.** Proportion of treatment success per treatment arm in ITT population (n = 88 per arm) at day 07, 14 and 21.

Additional file 4: Table S6. Distribution of adverse events per causality and treatment group.

Additional file 5: Table S7. Distribution of adverse events per causality and intensity (grade) in the treatment group ASMQ + Pq. **Table S8.** Distribution of adverse events per causality and intensity (grade) in the treatment group CQ + Pq. **Table S9.** Distribution of adverse events per causality and intensity (grade) in the treatment group AL + Pq.

Additional file 6: Table S10. All adverse events (1593) per body system and treatment allocation.

Additional file 7: Table S11. Adverse events with frequency higher than 3% in the ASMQ + Pq arm (n total = 403). **Table S12.** Adverse events with frequency higher than 3% in the CQ + Pq arm (n total = 643). **Table S13.** Adverse events with frequency higher than 3% in the AL + Pq arm (n total = 547).

Additional file 8: Table S14. Haemoglobin mean per regimen allocation at day 0, 14, 28, 42, and 63.

Abbreviations
ACT: artemisinin-based combination therapy; AE: adverse events; AL: fixed dose combination of 20 mg + 120 mg artemether and lumefantrine; AL + Pq: fixed dose combination artemether and lumefantrine with concomitant use of primaquine; ASMQ: fixed dose combination of 100 mg + 200 mg artesunate and mefloquine; ASMQ + Pq: fixed dose combination artesunate and mefloquine with concomitant use of primaquine; CEPEM: Tropical Medicine Research Centre of Rondônia; CI: confidence interval; CNS: Brazilian National Health Council; CONEP: Brazilian National Council on Ethics in Research; CQ: chloroquine; CQ + Pq: chloroquine with concomitant use of primaquine; CTCAE: Common Terminology Criteria for Adverse Events; FIOCRUZ: Oswaldo Cruz Foundation; FMT: Fundação de Medicina Tropical Doutor Heitor Vieira; G6PD: glucose-6-phosphate dehydrogenase; GCP: good clinical practice; hCG: human chorionic gonadotropin; ITT: intention-to-treat; PCR: polymerase chain reaction; PP: per protocol; SD: standard deviations; SEFAR: Laboratory of Pharmacokinetics; SISNEP: National System of Ethics in Research; UNIR: Federal University of Rondônia; WHO: World Health Organization.

Authors' contributions
AD, PM, and ACS participated in the study concept and design of the study. AD was the study coordinator. DP and MVL were the principal investigators. MAA, CTN, RR and IM conducted data collection and quality assurance. JCA performed data analysis. AD, DP, MVL, TCS, PM, ACS, MT and DL participated in interpretation of the data and critical revisions of the manuscript. All authors gave their final approval for publication of the manuscript. All authors read and approved the final manuscript.

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Competing interests
The authors declare following competing interests and financial disclosure: André Daher and Tereza Cristina dos Santos are employees of the Institute of Drug Technology (Farmanguinhos), Oswaldo Cruz Foundation (Fiocruz), a Brazilian governmental institution of Ministry of Health. Farmanguinhos is one of the study sponsors. Its team was involved in the study design, decision to publish, and preparation of the manuscript. Farmanguinhos does not sell medicine on the market. The Brazilian Ministry of Health exclusively drives its drug production. These disclosures do not alter our adherence to policies on sharing data and materials. There are no restrictions on the sharing of data and/or materials.

Consent for publication
This manuscript does not contain any individualized data. The confidentiality of the patients' records has been observed accordingly to ethical regulations.

Ethics approval and consent to participate
The Ethics Committee at the Tropical Medicine Research Centre of Rondônia (CEPEM No. 31/11 CEP/CEPEM e 0018.0.046.000-11 CAAE—SISNEP and Plataforma Brasil No. 74869 CEP/CEPEM e No. 05462612.7.0000.0011 CAAE) reviewed and approved the clinical study protocol, and informed consent from all participants was acquired before the study initiation, as declared in the ethics and regulatory statement. All signed informed consent forms will be kept for at least 5 years after the study end.

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Chapter V

Introduction Chapter III

Ancillary results of ACT vivax trial

There is an undeniable need for new treatment for vivax (Price et al., 2011) with higher acceptability and a better safety profile. The emphasis on tolerability of a new vivax treatment is critical, as this disease can present mild symptoms (in the partially immune population) and have a low lethality. In Brazil, the vivax mortality was 11/140000 patients in amazon region (Secretaria de Vigilância em Saúde, 2015), in 2014. Thus, serious adverse events related to any new treatments are unacceptable. For instance, this is the main reason for holding back the wide deployment of the single dose long half-life 8-aminoquinoline, tafenoquine.

ACTs with concomitant use of primaquine short course (0.5 mg/kg/day for 7-9 days) is the first choice to treat mixed malaria in Brazil (Ministério da Saúde do Brasil., 2010) and was prescribed to approximately one thousand patients over one year alone (Daher et al., 2019). Nevertheless, even in Brazil, there is a relative lack of data on the safety of ACTs for the radical cure of *P. vivax* (World Health Organization., 2015a), as programmatic use relies on passive pharmacovigilance to detect adverse events (AEs). Further evaluation on the safety of these regimens is opportune as they might became a therapeutic option to treat ≅171 thousand *P. vivax* cases per year (Secretaria de Vigilância em Saúde, 2019) in Brazil.

The study *Efficacy and safety of Artemisinin-based Combination Therapy and chloroquine with concomitant primaquine to treat Plasmodium vivax malaria in Brazil:*

an open label randomized clinical trial (Daher et al., 2018a) was the first time that fixed dose combinations of artesunate plus mefloquine and artemether plus lumefantrine were evaluated with concomitant use of primaquine during 7-9 days for the radical treatment of *P. vivax* in clinical trial conditions in Brazil. These conditions allowed a careful record of efficacy and safety outcomes. This current analysis links this high quality clinical data with new laboratory results that characterize the parasites, the patients' drug metabolism and their pharmacokinetics parameters.

The characterization of the parasites was performed by genotyping three microsatellite loci and two polymorphic loci of Merozoite surface antigen-1. This allowed classifying recurrence as related relapses or reinfections/unrelated hypnozoite activation. The patients' drug metabolism was assessed by genotyping and classifying the activity of cytochrome P450 enzymes CYP2D6 (Bennett et al., 2013) (Baird et al., 2018) and CYP2C8 (Gil and Gil Berglund, 2007), as both are known to participate in the metabolism of the primaquine and chloroquine, respectively. The pharmacokinetic parameters (Area Under the Curve (3-63d) and the terminal elimination half-life) of mefloquine, chloroquine and lumefantrine were measured in whole blood using a validated high performance liquid chromatography-tandem mass spectrometry method.

Initially, we explore the influence of pharmacokinetics upon the safety of these regimens, as higher drug exposures could be associated with a higher frequency of adverse events. Throughout this trial, 1593 adverse events were recorded from 251 patients allocated in three treatment arms during 63 days follow up. Only one serious adverse event was related to the treatment: a patient in the chloroquine arm presented haemolytic anaemia on day 3 due to confirmed glucose-6-phosphate dehydrogenase (G6PD) deficiency. Most

of the events (86.6%) were grade I (institute, 2010) highlighting the commitment of the research team in defining the treatments' safety profiles.

The adverse events classified as possible, likely and highly probable related to the test drugs were grouped by body system. The influence of the pharmacokinetic parameters, gender and weight in the frequency of AEs were assessed. The single statistically significant association was the relative risk of having pruritus and the chloroquine half-life (RR 1.09, 95% CI 1.03-1.14, p= 0.001). Although this association is well known, the result was an indicative that this assessment could detect the relation of AEs and drug exposure.

The study also explored the influence of pharmacokinetics upon the therapeutic success of these regimens, as the drug exposure under the therapeutic threshold could potentially explain the relapses and recrudescence; lower parasite clearance by day-3; or shorter time to failure. No influence of pharmacokinetics upon the efficacy outcomes could be detected. Nevertheless, these evaluations were conducted in small samples meaning that a true association may not be stablished due to lack of power.

Finally, the most interesting results were the influence of characterization of parasites and patients' drug metabolism upon the therapeutic failures. Relapses are a major contribution to the global *P. vivax* burden and a central target to improve the efficacy of the radical treatment. The characterization of parasites often demonstrates that relapses are due to heterologous activation of hypnozoites (de Araujo et al., 2012, Imwong et al., 2007), although, in this study most of the relapses were due to homologous parasites, excluding re-infection. The study also shown that impaired primaquine metabolism (reduced

activity of the enzyme CYP2D6) was higher in population with homologous relapses (26%) than in the general population (≅ 11%) (Gaedigk et al., 2017). These results highlighted the importance of primaquine in the efficacy of radical treatment of vivax, as they linked impaired bioavailability of primaquine active metabolites to high failures rates, although with no statistical significance (RR= 1.23, CI 95% 0.88-1.72, p=0.40).

The results also suggest that the efficacy of the radical treatment may not be critically related to the long half-life drugs in the doses administered in the study. Therefore, the Brazilian effort to improve the efficacy of the vivax treatment can benefit from a new formulation of primaquine that ensure drug exposure comparable to the FDA reference product (FDA, 2019). A new primaquine formulation has been developed, and it may increase the bioavailability of the drug and its active metabolites, even in those patients with impaired CYP2D6 activity. The ongoing primaquine bioequivalence study might lay the basis needed to further explore the influence of primaquine exposure upon the therapeutic success of these regimens.

Overall, these results provided further reassurance about the safety of the combined use of ACTs and short course primaquine to treat malaria vivax. Despite the encouraging results, the study has several limitations, specially a small sample size. Lastly, the need for strengthening the pharmacovigilance system and conducting phase IV studies to ensure safety of thousands of patients when deploying a new treatment in remote areas must be reiterated.

Pharmacokinetics/ Pharmacodynamics of Chloroquine and Artemisinin Based Combination Therapies with Primaquine, *Submitted Malaria Journal* 2019.

Pharmacokinetics/ Pharmacodynamics of chloroquine and artemisinin-based combination therapies with primaquine

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Abstract

Background

Activation of hypnozoites of *Plasmodium vivax* malaria causes multiple clinical relapses, which contribute to the *P. vivax* burden and continuing transmission. Artemisinin-based combination therapies (ACT) are effective against blood-stage *P. vivax* but require co-administration with primaquine to achieve radical cure. The therapeutic efficacy of primaquine depends on the generation of a therapeutically active metabolite via cytochrome P450 2D6 (CYP2D6). Impaired CYP2D6 metabolism has been associated with primaquine treatment failure. This study investigated the association between impaired *CYP2D6* genotypes, drug-exposure to the long-acting ACT component (schizonticidal drugs) and tolerance and efficacy.

Methods

Adult patients with acute *P. vivax* malaria were enrolled in a recently completed trial and treated with artesunate-mefloquine; chloroquine; or artemether-lumefantrine. All received concomitant primaquine (0.5 mg/kg/day for 7–9 days). The association between efficacy and safety and drug exposure was explored using Area-Under-the-Curve (AUC) and half-life ($t_{1/2}$) estimates obtained by non-compartmental analysis of the long half-life drugs. Parasite recurrences by day-63 were categorized as related relapses or reinfections/unrelated hypnozoite activation by genotyping three microsatellite loci and two polymorphic loci of Merozoite surface antigen-1. The *CYP2D6* genotype was identified with Taqman Assays by Real-Time PCR to 9 polymorphisms (eight SNPs and one deletion). Impaired CYP2D6 activity was inferred using the Activity Score System.

Results

Most recurrences in the ASMQ (67%), CQ (80%) and AL (85%) groups were considered related relapses. 8/9 (88.9%) of the patients with impaired CYP2D6 activity relapsed with related parasite compared to 18/25 (72%) with normal activity (RR= 1.23, 0.88;1.72, p=0.40). There were no associations between the measured PK parameters and recurrence. Patients with longer chloroquine half-lives had more pruritus (RR=1.09, 1.03;1.14, p=0.001). Higher CQ AUCs were associated with reduced falls in haemoglobin by day 14 (Coef -0.02, -0.005;-0.03, p=0.01). All regimens were well tolerated.

Conclusion

Genotyping of *P. vivax* showed that activation of related (homologous) hypnozoites was the most frequent cause of recurrence. The high proportion of the impaired CYP2D6 activity among patients with recurrent infections suggests that slow primaquine metabolism might influence related relapse rates in Brazil among patients receiving primaquine for radical cure, although confirmatory studies are needed. There was no association between drug exposure of the long-acting ACT component (schizonticidal drugs) and risk of related relapse. ACTs were well tolerated. These results provide further re-assurance about the safety and efficacy of ACTs when combined with short course primaquine to treat uncomplicated malaria vivax in Brazil.

Trial registration RBR-79s56s (http://www.ensaiosclinicos.gov.br/rg/RBR-79s56s/)

Keywords

Malaria, *Plasmodium vivax,* antimalarial treatment, chloroquine, mefloquine, lumefantrine, primaquine, artemisinin-based combination therapies**,** ACT, pharmacokinetics, clinical trial.

Background

The biological features of *Plasmodium vivax* malaria provide major challenges for pre-elimination and elimination programmes in areas co-endemic for *P. falciparum* and *P. vivax* [1] [2] [3]. These features include the early appearance of gametocytes, a high proportion of asymptomatic or chronic carriers [4] and the parasite latent form, hypnozoites, that produces relapses. Relapses make a major contribution to the global of the *P. vivax* burden [5]. It was estimated that relapses constituted 76–90% and 79% of total infections in a Papua New Guinea and Thailand respectively [6]. To eliminate this reservoir of latent infections, simple, effective radical treatment of vivax using 8-aminoquinolines such as primaquine or the recently approved analogue tafenoquine is required.

In most countries with endemic *P. vivax,* the preferred first-line radical treatment for *P. vivax* remains chloroquine, combined with 7 to 14-day primaquine regimens, and this has barely changed since the 50's [7]. Chloroquine is no longer recommended for the case-management of *P. falciparum* malaria due to the spread of parasite resistance [8] resulting in the use of different treatment regimens for *P. vivax* and *P. falciparum* in areas where these species are co-endemic. This is programmatically complicated. The use of a single regimen to treat all species of malaria would simplify malaria treatment guidelines [9]. Artemisinin-based combination therapies (ACT) are emerging as the best option in this context, particularly in settings where there are concerns about chloroquine-resistant *P. vivax* [10]. ACTs are effective against the blood stage of *P. vivax* [11] but must be co-administered with primaquine to eliminate *P. vivax* hypnozoites [10, 12]. Short course primaquine regimens are preferable as they have been proven to have an efficacy not inferior to the standard 14 days' regimens [13-16]. However, there is a relative lack of

data on the safety, pharmacodynamics and pharmacokinetics of ACTs when provided in combination with daily primaquine regimens for the radical cure of *P. vivax* [17]. Safety is a major concern when deploying new treatments, but in the case, there are also concerns that primaquine / ACTs drug interactions may reduce the overall regimen efficacy, by inhibiting CYP2D6 or reducing synergistic effect of the current regimen. There are uncertainties about the best partner ACT, as drugs with a longer half-life may prevent early relapses.

Characterization of parasites and patients' drug metabolism is needed to ascertain the pharmacodynamics, pharmacokinetics and therapeutic success of these regimens. The main malaria clinical trials outcomes are parasitological clearance and recurrence rate [21]. Vivax recurrence includes (i) recrudescence of parasites that have been previously cleared and microscopically undetectable; (ii) re-infection from another mosquito bite; (iii) relapses, i.e. activation of hypnozoites, genetically related (homologous) or unrelated (heterologous). The primaquine metabolite that is active against human hypnozoite is unknown [22], but the metabolism of primaquine to its active metabolite is dependent on the cytochrome P450 enzyme CYP2D6 [22-24]. Low *CYP2D6* activity results in slow metabolism of primaquine to the active metabolite. CYP2D6 activity may be a proxy of primaquine's active metabolite exposure and a risk factor for relapse among primaquine recipients [25-27]. Similarly, the cytochrome P450 enzymes CYP2C8 was investigated as it is known to participate in the metabolism of chloroquine [28].

A previous randomized clinical trial in Brazil of the treatment of uncomplicated *P. vivax* malaria compared the safety and efficacy of the fixed-dose ACTs artemether-lumefantrine and artesunate-mefloquine against the standard treatment with chloroquine, all three in combination with short course primaquine (0.5 mg/kg/day for 7–9 days) [29]. This current study investigated the pharmacokinetics of the long-acting ACT component

(schizonticidal drugs) with concomitant primaquine upon the safety and efficacy of these three treatment regimens and the influence of genetic variability of parasite and host, including the frequency of mutations in CYP2D6 gene over relapse rate.

Methodology

Overview study design

The patients included in the current analysis were enrolled in a larger clinical trial previously published and designed in accordance with WHO guidelines [21]. The trial was designed to evaluate the safety and efficacy of the schizonticidal drugs with concomitant use of primaquine for vivax cure. The details of the trial design and methods have been reported elsewhere [29]; In brief, patients were eligible if they had acute uncomplicated malaria due to *P. vivax* mono-infection confirmed by microscopy with fever or a history of fever in the previous 48 hours, were aged 18 to 70 years old, weighed between 50 and 90 kg, and had parasite densities >250/μL and haemoglobin levels >7.0 g/dL. G6DP deficiency was not an exclusion criterion. They were randomly allocated to three treatment groups: a) artesunate-mefloquine (100+200 mg QD for 3 days) (ASMQ); b) chloroquine (CQ) (600 mg on day 1, and 450 mg on days 2 and 3), and c) artemether-lumefantrine (20+1 20 mg BID for 3 days) (AL). All three arms received the same concomitant primaquine regimen (7–9 days: 0.5 mg/kg/day).

Patients were assessed on the day of enrolment and days 1, 2, 3, 7, 14, 21, 28, 42 and 63. The main end-points were either treatment failure or adequate clinical and parasitological response. Blood samples were collected for parasite counts at every scheduled visit, on any day of treatment failure and for drug levels on days 0, 3, 7, 14, 21, 28, 42 and 63.

Samples (100 μL) were transferred to Whatman (USA) ET 31 CHR E 3MM filter papers for later pharmacokinetic analysis and parasite genotyping [30].

Parasitological densities were estimated using Giemsa-stained blood slides at a magnification of 1,000x using WHO-recommended methods [21]. Adverse events (AE) were assessed at each follow-up visit, and patients were encouraged to return to the clinic if they were ill in between scheduled visits. All AEs, including laboratory abnormalities, were categorized by body system.

Pharmacokinetics/ Pharmacodynamics

Whole blood concentrations of mefloquine (MQ), chloroquine (CQ) and lumefantrine (LMF) were measured using a validated HPLC-MS/MS method in accordance with Brazilian [31] and international regulatory requirements for bioanalytical methods [32]. The pharmacokinetics assays were conducted at the Equivalence and Pharmacokinetics Service (SEFAR)/Oswaldo Cruz Foundation, which is accredited by the Brazilian regulatory agency, Agência Nacional de Vigilância Sanitária (ANVISA). Non-compartmental analysis was performed for chloroquine, lumefantrine and mefloquine using the Pmetrics® [33] package to estimate two main parameters; the overall Area Under the Curve (AUC) (3-63d) and the terminal elimination half-life ($t_{1/2}$). The terminal elimination half-life was only calculated for subjects with five or more available samples. These two parameters were used as proxy indicators for overall drug exposure for correlation analyses with drug efficacy and safety profiles.

The correlation between drug exposure and treatment failure was evaluated using the Mann-Whitney test. The effect of pharmacokinetic parameters on the frequency of adverse events likely or probably related to the test drug was expressed as the relative risks (RR) obtained from random effects Generalized Estimation Equation (GEE) log-

binomial regression models. Linear regression was used to test the effects of PK parameters on the fall in haemoglobin (Hb) concentrations by day 14 relative to enrolment values [34]. The effects of these pharmacokinetic parameters on treatment failure (both early and later failures) were evaluated as odds ratios (OR) from binomial logit link regression models, using Generalized Linear Models (GLM). Time to failure was tested using standard Cox regression. The proportion of clonal variability at recurrence was compared between treatment arms using Fisher's exact test. Two-sided p-values of <0.05 were considered statistically significant.

DNA extraction and Genotyping of the Parasites microsatellites and polymorphic blocks of MSP-1 and patients´ CYP2D6 and CYP2C8

DNA was extracted from dried blood using QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) following the instructions of the manufacturer. Three microsatellite loci (MS2, MS6, MS7) and two polymorphic loci (blocks 2 and 10) of Merozoite surface antigen 1 (MSP-1) were amplified using specific primers and conditions as previously described [35, 36]. The exact length and relative abundance (fluorescence levels) of each PCR product were determined in the DNA automatic sequencer (ABI 3730, Applied Biosystems, Thermo Fischer Scientific, Waltham, MA, USA) with fluorescein labelled forward primers and an internal size standard (GeneScan 500 LIZ, Applied Biosystems). The predominant allele for each locus was identified as the highest peak of fluorescence in the electropherogram using GeneMapper 4.1 software (Applied Biosystems). The multiplicity of parasite variants was estimated measuring extra peaks in the electropherogram with fluorescence above the cut off (150 arbitrary fluorescence units) and at least one third the high of the main peak. Parasite recurrences within 63 days were categorized as 'related', including totally identical (homologues) if all five polymorphic loci (MS2; MS6; MS7; MSP1B2; MSP1B10) were identical and 'similar' if 80% of their

alleles were identical; and otherwise as unrelated (heterologous) (eTable 1). Number of alleles and heterozigosity expected were calculated in Arlequim software v. 3.5.2.2 (http://cmpg.unibe.ch/software/arlequin35/).

The cytochrome P450 enzymes CYP2D6 [24] [22] and CYP2C8 [28] are known to participate in the metabolism of the primaquine and chloroquine, respectively. Two SNPs (G416A[rs11572080] and A805T [rs11572103]) were genotyped in the *CYP2C8* gene. In the *CYP2D6* gene eight SNPs were genotyped; (G-1584C [rs1080985], C100T [rs1065852], C1023T [rs28371706], G1846A [rs3892097], C2850T [rs16947], G2988A [rs28371725], G3183A [rs59421388] and G4180C [rs1135840]) and one deletion (2615-2617delAAG [rs5030656]) were genotyped. The copy number was also determined. All SNPs genotyping were performed by Real-time PCR using specific hydrolysis probe [37] in ViiA 7 Real-time PCR system (Applied Biosystems). Haplotypes and *CYP2D6* star alleles were inferred using the Phase software (version 2.1). *CYP2D6* gene copy number was determined with Hs00010001_cn assay (Applied Biosystem) in Real-time PCR [38]. Each allele got one value that was used to calculate the CYP2D6 activity score (AS). Patient were categorised based on their AS score into normal metabolizer fast (gNM-F) (AS=1.5 or 2.0), normal metabolizer slow (gNM-S) (AS=1), intermediate metabolizer (gIM) (AS=0.5), poor metabolizer (gPM) (AS=0), and ultra metabolizer (gUM) (more than 2 copies of the normal allele) (AS≥2.0) [35]. Impaired CYP2D6 activity was defined as AS scores less than 1.5.

Results

Population

These study is based on a subset of samples from the original trial. Only 2/3 of samples (1400 samples of 175 patients) were evaluated in Pharmacokinetics/ Pharmacodynamics analysis due to logistical issues, however the baseline characteristics of this subset were similar across the three arms (Table 1). Characterization of parasites and drug metabolism was conducted in all 35 patients with parasite recurrence within 63 days.

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY POPULATION

	ASMQ	CQ	AL
N of patients	60	58	57
Male n (%)	35 (58)	46 (79)	39 (68)
Weight in kg	70 (11)	73 (10)	73 (10)
Haemoglobin in g/dL	13.4 (1.8)	14.0 (3)	13.6 (2)
Parasitaemia/uL*	2145.56 [258-13335]	2155.78 [285-17685]	2444.71 [270-19815]
Age in years	39.0 (11)	42.1 (10.5)	38.7 (11)

Data represent n (%) and means and (SD).

*Parasitaemia was expressed by geometric mean and [min-max].

Characterization of parasites and drug metabolism in the population with treatment failure.

The frequency of 'related' (homologous and similar) and unrelated (heterologous) parasites among 35 patients with parasite recurrence within 63 days by study arm is shown in Table . Overall, 67%, 80% and 85% of the recurrent malaria in the ASMQ, CQ

and AL groups respectively were considered related relapses. Among the recurrences the pooled percentage of related relapses across the three arms was 77.1%.

TABLE 2. CLINICAL OUTCOMES AGGREGATED BY DRUG TREATMENT (%).

Clinical outcome (%)	PCR results	ASMQ		CQ		AL		overall	
Homologous relapse	homologous	6	66.7 %	6	80.0 %	8	84.6 %	20	77.1 %
	similar	2		2		3		7	
Reinfections or Heterologous hypnozoites' activation	heterologous	4	33.3 %	2	20.0 %	2	15.4 %	8	22.9 %

Genetic analysis of the parasite populations comparing all recurrences also demonstrated that 77.1% (27/35) of patients presented with a single clonal infection at the initial infection and at the recurrence. The number of parasite variants between initial infection and recurrence remained the same in 23 (65.7%) of the 35 patients with recurrent infections and increased in 10 (28.5%) and decreased in 2 (5.7%). There was no difference in multiplicity of parasite clones at recurrence between treatment arms (p=0.51).

CYP2D6

TABLE 3. INFERRED PHENOTYPE OF CYP2D6 BASED ON GENOTYPING BY TREATMENT ARM (%).

CYP2D6 metabolism	AS	Phenotype	ASMQ		CQ		AL*	
Normal	1.5 or 2	gNM-F	5	50.0%	9	90.0%	9	81.8%
	≥ 2	gUM	1		0		0	
Impaired	1	gNM-S	5	50.0%	0	10.0%	2	18.2%
	0.5	gIM	0		1		0	

	0	gPM	1	0	0

AS = Activity Score; gNM-F = normal-fast metabolizer; gUM = ultrarapid metabolizer; gNM-S = normal-slow metabolizer; gIM = intermediate metabolizer and gPM = poor metabolizer.
*CYP2D6 phenotype of two patients (ID 33 and 231) could not be defined.
* *CYP2D6* genotype of two patients (ID 33 and 231) could not be defined

Eight of nine (88.9%) patients classified to have reduced enzymatic activity for primaquine metabolism based on their CYP2D6 genotypes relapsed with related parasites (RR= 1.23 95%CI (0.88-1.72) p=0.40) (Table). Eighteen out of 25 (72%) normal metabolisers had related relapses.

CYP2C8

The frequency of two polymorphisms in *CYP2C8* gene in the population who failed is presented in Table. Out of the ten patients in the CQ arm with parasite recurrence, one had the SNP G416A genotype indicative of reduced enzyme activity of *CYP2C8*. The AUC and half-life for chloroquine of this patient were 91.9 μg/mL.h and 11.32 days respectively, compared with 102.3 μg/mL.h and 19.3 days in the nine CYP2C8 non-mutated genotypes in the CQ arm. These results for the patient with the mutated genotype fall within the 95% CI for the overall population (Table).

TABLE 4. INFERRED PHENOTYPE OF CYP2C8 BASED ON GENOTYPING BY DRUG TREATMENT GROUP.

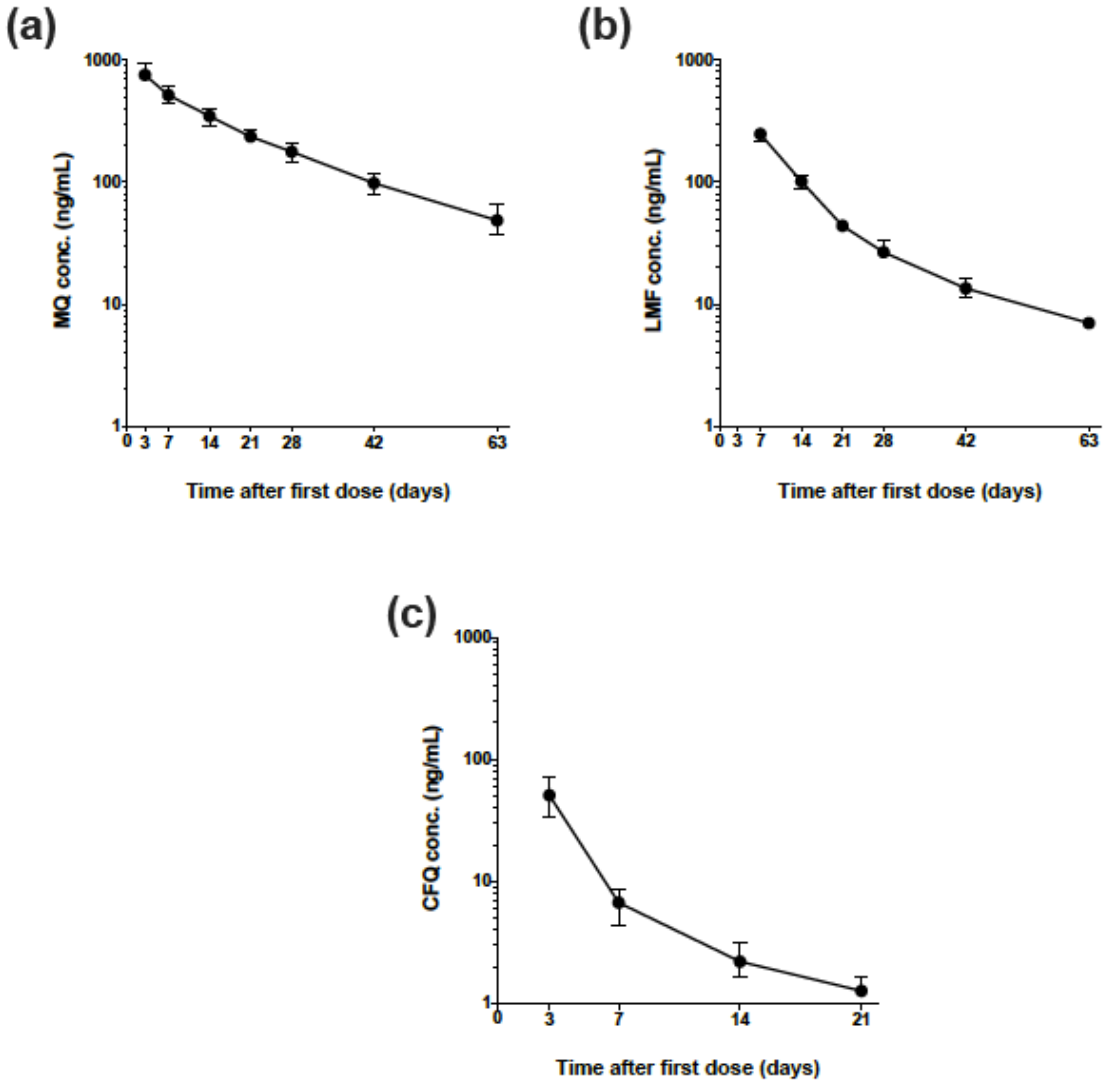
CYP2C8 (SNP)	Genotype	**MQ**	**CQ**	**LMF**
G416A	Normal	7	9	13
	Mutant*	5	1	0
A805T	Normal	12	10	13
	Mutant	0	0	0

*All mutants are heterozygotes.

Pharmacokinetics/Pharmacodynamics

$AUC_{0d\text{-}63d}$ values were calculated for each patient in each arm; the terminal elimination half-life ($t_{1/2}$) could only be calculated for those receiving chloroquine or mefloquine. **Figure 1** shows the terminal PK profile generated for mefloquine, chloroquine and lumefantrine.

FIGURE 1 PHARMACOKINETIC PROFILE OF MEFLOQUINE (MQ), LUMEFANTRINE (LMF) AND CHLOROQUINE (CQ). PROFILES WERE GENERATED FROM 58-60 SUBJECTS FOR EACH DRUG. DATA SHOWS MEDIAN WITH 95 CONFIDENCE INTERVAL (CI).



Drug exposure (defined by AUC3d-63d or elimination half-life) under the therapeutic threshold could potentially explain the relapses and recrudescence, but not re-infections. The relationship of drug exposure (defined by AUC3d-63d or elimination half-life) to recurrence was investigated by comparing the PK parameters in the cured population with those with confirmed related relapse (homologous + similar). There were no statistically significant differences in AUC3d-63d or elimination half-life for MQ, CQ or LMF between patients without recurrent infections by day-63 and those with relapses in univariate (Table). Weight and gender were also not associated with parasite recurrence by day 63 (eTable 3); parasite clearance by day-3 (eTable 4); or time to failure (eTable 5).

TABLE 5. MEDIAN OF PHARMACOKINETIC PARAMETERS FOR MEFLOQUINE, CHLOROQUINE AND LUMEFANTRINE IN PATIENTS WITH HOMOLOGOUS PARASITES BY TREATMENT OUTCOME.

		Mefloquine			Chloroquine			Lumefantrine		
		Cure	Relapse	p-value	Cure	Relapse	p-value	Cure	Relapse	p-value
N of patients		53	6	-	52	7	-	52	7	-
AUC (0d-63d) (µg/mL.h)	Per outcome	338.6	302.0	0.67	105.9	95.8	1.0	7.3	3.73	0.96
	overall	338.6 [256.0-408.0]		-	103.8 [83.0-126.3]		-	6.7 [2.7-12.0]		-
Half-life (days)	Per outcome	17.7	25.6	0.58	18.5	19.3	0.42	NA	NA	-
	overall	17.8 [15.0-24.8]		-	18.7 [15.4-27.8]		-	NA		-

Overall values are median with 25-75 percentiles in square brackets and N=59. NA= Non-Applicable

Higher drug exposures could be associated with a higher frequency of adverse events. The influence of AUC, half-life and weight on the frequency of adverse events likely and probably related to the treatment per body systems (n ≥30) are presented in eTable7. The relative risk of having pruritus increased as the half-life of chloroquine increased (RR

1.09, 95% CI 1.03-1.14, p= 0.001). Conversely, the influence of drug exposure on the haemoglobin drop (defined as Hb at day 14 − Hb at baseline/Hb at baseline) shown that the higher the AUC of CQ the lower the reduction in the haemoglobin decline (Coef -0.02, 95% CI -0.03;-0.00, p=0.01) (eTable 6).

Discussion

This study showed by genotyping polymorphic loci of *P. vivax* that relapse due to related parasites was the most frequent cause of recurrence by day-63 in all three treatment arms of our previous trial [20] where 13-16% of infections recurred by day-63. The current study also showed that the treatment failure rate in patients with reduced inferred CYP2D6 activity (26%) was higher than in the general population (≅ 11%) [39]. Moreover, eight out of nine recurrences among patients with low CYP2D6 activity were related relapses compared to 18 out of 25 with inferred normal CYP2D6 activity. Low CYP2D6 activity and presumptive low exposure to the active metabolite of primaquine or disruption of the PQ-enzyme interactions might influence related relapse rates in Brazil, although further studies are need to elucidate this effect. Likewise, the impaired activity of CYP2C8 may result in a lower exposure to desethylchloroquine, the chloroquine active metabolite. The PK parameters and clinical outcomes of the single patient with impaired CYP2C8 in chloroquine arm did not differ from the overall population.

Other causes of low exposure to primaquine are also potential risk factors for relapses, such as low adherence or impaired bioavailability. Similarly, others factors which affect the success of anti-infective therapeutics may influence the responses to the radical treatment, such as differences in the biology of the parasite, the immune status of the patients and the density of latent hypnozoites [3].

This was the first time it was evaluated that some of the ACT combinations, namely ASMQ, were evaluated for the cure of *P. vivax* in clinical trial conditions, and thus in combination with daily primaquine (0.5 mg/kg/day for 7-9 days) regimens. The correlation between drug exposure to the long-acting components of the ACTs and the risk of recurrence and adverse events was also assessed. Potential interactions between primaquine and the ACTs and also, the safety of these regimens have not been extensively assessed [17]. Drug interactions such as lumefantrine inhibiting CYP2D6 [40] may reduce the overall regimen efficacy; on the other hand, drugs with a longer half-life may prevent early relapses. This study could not demonstrate a significant associations between the PK parameters of the long half-life drugs and the risk of recurrence, or the risk of relapse due to either homologous and heterologous relapses or time to recurrence (eTable3, eTable4, and eTable5, respectively), although the numbers of relapses were small. Patients with longer chloroquine elimination half-life estimates were more likely to report pruritus. Transient, mild to moderate pruritus is a well-known adverse effect of chloroquine [41] and a threat to treatment adherence. A smaller drop in haemoglobin by day 14 was associated with higher CQ exposure (AUC), which may reflect better therapeutic efficacy achieved with higher concentrations of CQ [42].

This study has several limitations. The blood sampling schedule was designed to evaluate the blood levels of the long half-life drugs; the pharmacokinetic data allowed the prediction of the drug exposures up to 63 days post-treatment with up to eight sample points available for each patient. It did not allow a proper modelling of primaquine levels and limited the calculation of the elimination half-life of lumefantrine. The absence of desethylchloroquine blood levels measurement is another study limitation. A trial designed with six month follow up would have been able to evaluate relapses with more accuracy, as the median time to vivax recurrence in Brazil is 71 days [15]. The

characterization of parasites and drug metabolism were conducted only in 35 patients with treatment failure limiting the comparisons. Genotyping vivax parasites to infer relapse frequencies also presents limitations. Genotyping in vivax does not allow differentiation between new infections (reinfections) or activation of unrelated (heterologous) hypnozoites. In this study, only recurrences with homologous and similar parasites were considered relapses. However, relapses are often heterologous activation of hypnozoites [36, 43]. The recrudescence of submicroscopic parasite population [44] is also a biologically plausible explanation for homologous parasites in two samples. Future use of more sensitive parasite detection strategies, such as ultrasensitive PCR of all consecutive samples of the patients who failed could elucidate these results.

Conclusion

The genotyping of polymorphic loci of *P. vivax* showed that relapse due to genetically related parasites was the most frequent cause of recurrence in all three treatment arms. The high proportion of *CYP2D6* genetic polymorphisms among patients with recurrent infections suggests that impaired primaquine metabolism might influence the related relapse rates in Brazil among patients receiving primaquine for radical cure, further studies are needed to confirm this finding. The three ACT regimens were very effective, and there was no association between drug exposure levels of the long-acting components of the ACTs and risk of relapse. The ACTs were well tolerated overall. These results provided further reassurance about the safety of the combined use of ACTs and short course primaquine (0.5 mg/kg/day for 7-9 days) to treat uncomplicated malaria vivax in Brazil.

Manuscript information

Abbreviations

ACT: Artemisinin-based combination therapy; AE: Adverse events; AL: Fixed dose combination of 20 + 120 mg artemether and lumefantrine with concomitant use of primaquine; ANVISA: National Regulatory Agency; ASMQ: Fixed dose combination of 100 + 200 mg artesunate and mefloquine with concomitant use of primaquine; CI: Confidence interval; CNS: Brazilian National Health Council; CQ: chloroquine with concomitant use of primaquine; G6PD: Glucose-6-phosphate dehydrogenase; HPLC-MS/MS: High performance liquid chromatography-tandem mass spectrometry; PCR: Polymerase chain reaction; SD: Standard deviations; SEFAR: Equivalence and Pharmacokinetics Service; WHO: World Health Organization.

Ethics approval and consent to participate

The Ethics Committee at the Tropical Medicine Research Centre of Rondonia (CEPEM Nº 31/11 CEP/CEPEM e 0018.0.046.000-11 CAAE – SISNEP and Plataforma Brasil Nº 74869 CEP/CEPEM e Nº 05462612.7.0000.0011 CAAE) reviewed and approved the clinical study protocol and informed consent from all participants was acquired before the study initiation, as declared in the ethics and regulatory statement. All signed informed consent forms are kept for at least five years after the study end.

Consent for publication

This manuscript does not contain any individualized data. The confidentiality of the patients' records has been observed according to ethical regulations.

Availability of data and materials

The evaluation of pharmacokinetics' parameters, gender and weight as predictors of failures per treatment drug (LGM, binomial logit link); evaluation of pharmacokinetics' parameters and weight as predictors of D3 failures per treatment drug (LGM, binomial logit link); and evaluation of pharmacokinetics' parameters, gender and weight as predictors of time to failures per treatment drug (Cox regression, binomial logit link) are provided in the supporting file.

Competing interests

The authors declare following competing interests and financial disclosure: André Daher is an employee of the Institute of Drug Technology (Farmanguinhos), Oswaldo Cruz Foundation (Fiocruz), a Brazilian governmental institution of Ministry of Health. Farmanguinhos is one of the study sponsors. He was involved in the study design, decision to publish, and preparation of the manuscript. Farmanguinhos does not sell medicine on the market. The Brazilian Ministry of Health exclusively drives its drug production. These disclosures do not alter our adherence policies on sharing data and materials. There are no restrictions on the sharing of data and/or materials.

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Authors' contributions

AD was the study coordinator. DP and MVL were the principal investigators. MAAA, CTN, collected the clinical data. JCA and AD performed the analysis of the data. LBF, DMDS, DPP performed the pharmacokinetics analysis. GA performed the analysis of the pharmacokinetics data. CFAB, ACRS, TNS and DFR performed the PCR analysis. AD, JCA, DGL, FOtK, CFAB participated in interpretation of the data and critical revisions of the manuscript. All authors gave their final approval for publication of the manuscript.

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Conclusion

Vivax malaria is a neglected disease. Recent reversal of the trend towards declining incidence emphasizes the importance of sustainable control strategies to move towards elimination. Although a more holistic approach will be needed to eliminate this poverty related disease, there is an irrefutable and urgent need for new treatments. Optimizing the current therapeutic regimens is the first step to meet this demand.

This thesis presents evidences for the safety and efficacy of a patient friendly regimen namely Artemisinin based Combination Therapies (ACT) with concomitant use of short course primaquine.

Short course primaquine regimen (0.5 mg/kg/day 7 days) using the same total dose recommended worldwide has been the standard of care in Brazil for some time, but until now the evidence has been regarded insufficient to support the shortening of 14 days that is seen in the World Health Organization (WHO) treatment recommendation. A systematic review including 1211 patients was conducted to compare the efficacy of both regimens and concluded that was little or no difference in *P. vivax* recurrence rates at 6 months when using the same total dose (210 mg) over 7 days as compared to 14 days (chapter 2), two new major randomized clinical trials in the south-east Asia have also confirmed similar efficacy with short course regimens (Taylor, 2018).

My work has led to similar conclusions. The evaluation of 154,970 patients' data recorded in the Brazilian national surveillance system demonstrated no difference in time to recurrence or recurrence frequency between patients treated with 14-day or 7–9 day

primaquine regimens (HR = 1.02, 95%CI 0.96–1.09) and (RR = 0.97, 95%CI 0.90–1.04), respectively.

The data presented here increased the body of evidence to support the recommendation that primaquine short course regimens can be adopted into WHO treatment guideline. However, shorter course primaquine may improve adherence and the effectiveness of vivax treatment worldwide, there is still room for further improvements in treatment. One potential approach is to replace chloroquine by ACTs.

Previous work suggested that ACTs have a similar efficacy in the treatment of vivax as standard treatment with chloroquine. Several studies support this, including a systematic review, and in clinical practice, ACTs have been extensively used as the first line treatment for mixed infection or chloroquine-resistant vivax. This thesis further investigated the efficacy of ACTs in a randomized clinical trial with two ACTs and chloroquine, all with concomitant use of primaquine short course in Brazil. All three regimens had 42 day cure rates above 90%. The cohort study also suggested that *Plasmodium vivax* recurrence took longer in patients taking ACT than chloroquine (both with primaquine) HR = 1.27 (0.97–1.66, p = 0.08).

Finally, we explored the influence of drug exposure, and both parasite and host characteristics upon the clinical outcomes. The evaluation of the pharmacokinetics and pharmacodynamics interactions suggested that exposure to the long half-life drugs were not associated with any of the efficacy outcomes, including treatment failure within 63 days follow up and time to failure. However, in this study there was a high frequency of failures due to homologous parasite relapses, meaning that the hypnozoites may have not

been cleared by primaquine. There was also a trend towards an association between impaired CYP2D6 enzyme activity and relapse with homologous parasites, in line with the evidence that the efficacy of primaquine depends on the generation of a therapeutically active metabolite via cytochrome P450 2D6 (CYP2D6).

One approach to overcoming the issue of decreased primaquine exposure in impaired CYP2D6 populations would be to increase the primaquine dose. This approach has been adopted in the latest clinical trials in the south-east Asia. Our systematic review compared the recurrence rate at 6 months follow up of the WHO high standard (0.5 mg/kg/day) versus standard (0.25 mg/kg/day) 14 days' treatment and showed no difference (RR 0.82, 95% CI 0.47-1.43), but as only two studies (639 participants) were available.

The potential limitation of increasing primaquine exposure is the risk of adverse events (AE). In our review, 8 out of 380 participants in the high-dose 14-day group discontinued treatment due to adverse events, compared to 2 out of 398 in the standard 14-day group (RR 4.19, 95% CI 0.90 -19.60). No serious adverse events were reported in either study arms, but none of the studies included the population with G6DP deficiency, which is the most frequent enzyme deficiency in humans, and causes haemolytic anaemia.

The other major safety issue was the relative lack of information about the safety of the concomitant use of ACT and multi-day primaquine. We endeavoured to addressed this in the randomized clinical trial and the subsequent pharmacokinetic and pharmacodynamics study. The only serious adverse event related to the treatment was haemolysis in a G6DP deficient patient treated with chloroquine and primaquine. The AEs were reported meticulously and 86.5% were grade I, i.e. they do not need any medical intervention.

Drug exposure was not related to increasing the risk of AE, except that an increased half-life of chloroquine was associated with higher risk of having pruritus (RR 1.09, 95% CI 1.03-1.14, p= 0.001), providing further assurance that exposure of both ACT and multi-day primaquine regimen remain within the safety margins when co-administered.

Once the similarity of the safety, efficacy and effectiveness profiles of the standard chloroquine plus primaquine and these new approaches to treat vivax were established, there are other factors rather than safety, efficacy and effectiveness that may drive the update of the treatment guidelines were identified.

There are several advantages of ACT plus primaquine as a unified treatment for both *P. vivax and P. falciparum* species. This approach (i) surpass the need of species identification in co-endemic areas ; (ii) treats mixed infections; (iii) prevent *P. vivax* relapse following *P. falciparum* infections; (iv) simplifies drug stock and supply management; (v) sustains of ACT production in industrial scale if falciparum treatment purchases fade out; (vi) avoids selective pressure, making more than one vivax treatment available; (vii) has small pill burden, depending on the ACT ; (vii) and finally, it provides a children friendly formulation where liquid chloroquine is not available, as in Brazil.

The effectiveness study identified that the younger patients are the most susceptible to recurrences. There are several potential explanations for this in addition to differences in acquired immunity, but the inadequate doses and the absence of child-friendly formulations may play a major role in poor adherence and reduced effectiveness. The ACTs tested here are available in dispersible tablets, and if administered with a new

primaquine tablet (5mg) could address this neglected population reducing the overall reservoir in the population in risk.

Finally, the deduplication methodology of the data of the national surveillance system based on bloom filters can be used as a way of recording patients' follow up, as it links successive observations of the same individual over a period of time. This use of routine health information systems allows evaluating the impact of large scale interventions in public health, building capacity and strengthening a sustainable surveillance systems. Future prospective research designs using this methodology may demonstrate causal inferences of the proposed treatment optimizations upon the recurrence incidence providing evidence to the policy change, even in a low endemicity setting, as Brazil. This study gives a basis to future studies to evaluate the effectiveness of the deployment of these approaches and of new ones nationwide in Brazil.

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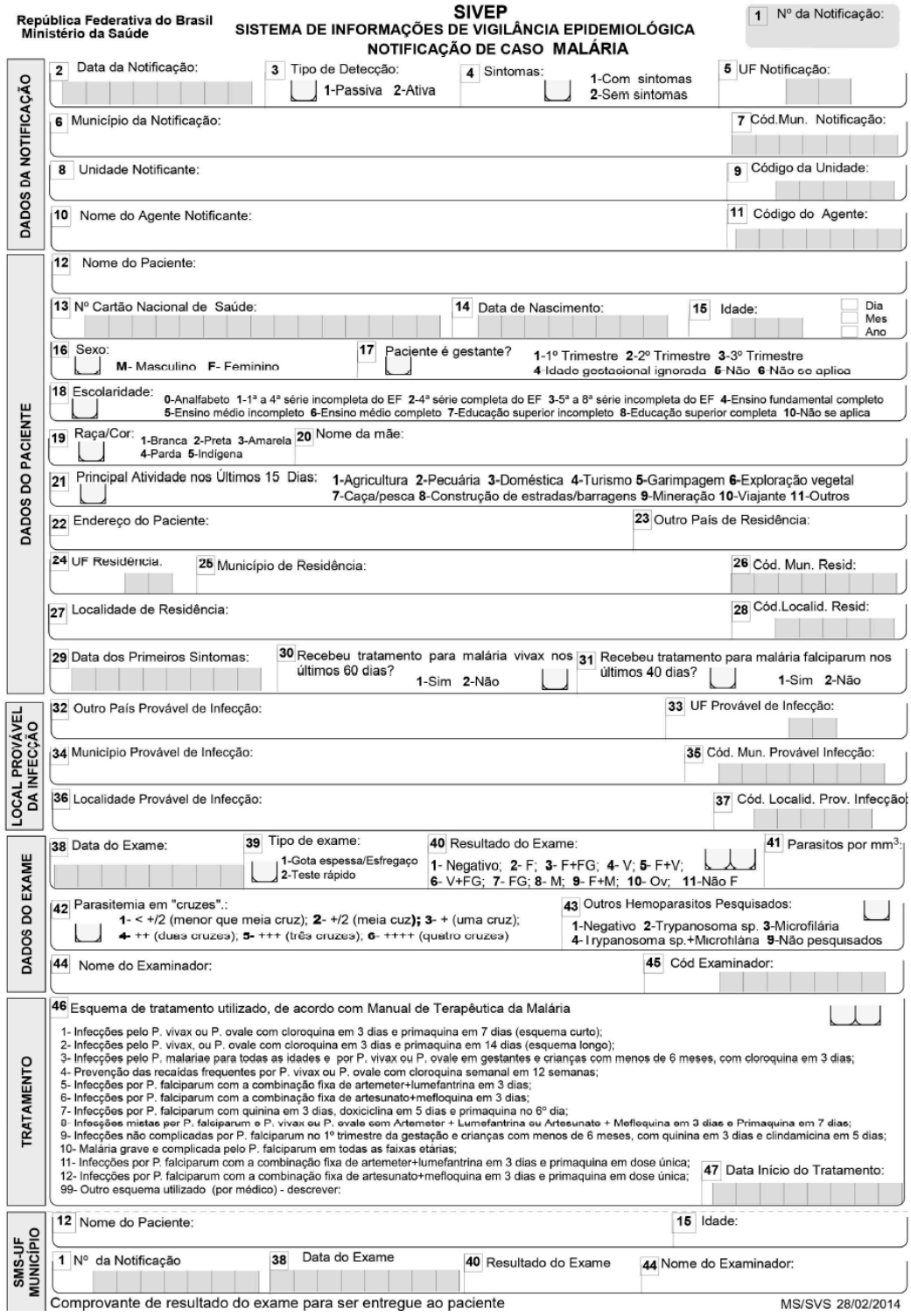
Appendices

Additional material: Evaluation of Plasmodium vivax malaria recurrence in Brazil. *Malar J,* 2018.

Additional material 2. National System of Malaria Surveillance form (SIVEP-malaria)



República Federativa do Brasil
Ministério da Saúde

SIVEP
SISTEMA DE INFORMAÇÕES DE VIGILÂNCIA EPIDEMIOLÓGICA
NOTIFICAÇÃO DE CASO MALÁRIA

1 Nº da Notificação:

DADOS DA NOTIFICAÇÃO

2 Data da Notificação:
3 Tipo de Detecção: 1-Passiva 2-Ativa
4 Sintomas: 1-Com sintomas 2-Sem sintomas
5 UF Notificação:
6 Município da Notificação:
7 Cód.Mun. Notificação:
8 Unidade Notificante:
9 Código da Unidade:
10 Nome do Agente Notificante:
11 Código do Agente:

DADOS DO PACIENTE

12 Nome do Paciente:
13 Nº Cartão Nacional de Saúde:
14 Data de Nascimento:
15 Idade: Dia / Mes / Ano
16 Sexo: M- Masculino F- Feminino
17 Paciente é gestante? 1-1º Trimestre 2-2º Trimestre 3-3º Trimestre 4-Idade gestacional ignorada 5-Não 6-Não se aplica
18 Escolaridade: 0-Analfabeto 1-1ª a 4ª série incompleta do EF 2-4ª série completa do EF 3-5ª a 8ª série incompleta do EF 4-Ensino fundamental completo 5-Ensino médio incompleto 6-Ensino médio completo 7-Educação superior incompleto 8-Educação superior completa 10-Não se aplica
19 Raça/Cor: 1-Branca 2-Preta 3-Amarela 4-Parda 5-Indígena
20 Nome da mãe:
21 Principal Atividade nos Últimos 15 Dias: 1-Agricultura 2-Pecuária 3-Doméstica 4-Turismo 5-Garimpagem 6-Exploração vegetal 7-Caça/pesca 8-Construção de estradas/barragens 9-Mineração 10-Viajante 11-Outros
22 Endereço do Paciente:
23 Outro País de Residência:
24 UF Residência.
25 Município de Residência:
26 Cód. Mun. Resid:
27 Localidade de Residência:
28 Cód.Localid. Resid:
29 Data dos Primeiros Sintomas:
30 Recebeu tratamento para malária vivax nos últimos 60 dias? 1-Sim 2-Não
31 Recebeu tratamento para malária falciparum nos últimos 40 dias? 1-Sim 2-Não

LOCAL PROVÁVEL DA INFECÇÃO

32 Outro País Provável de Infecção:
33 UF Provável de Infecção:
34 Município Provável de Infecção:
35 Cód. Mun. Provável Infecção:
36 Localidade Provável de Infecção:
37 Cód. Localid. Prov. Infecção:

DADOS DO EXAME

38 Data do Exame:
39 Tipo de exame: 1-Gota espessa/Esfregaço 2-Teste rápido
40 Resultado do Exame: 1- Negativo; 2- F; 3- F+FG; 4- V; 5- F+V; 6- V+FG; 7- FG; 8- M; 9- F+M; 10- Ov; 11-Não F
41 Parasitos por mm^3:
42 Parasitemia em "cruzes".: 1- < +/2 (menor que meia cruz); 2- +/2 (meia cuz); 3- + (uma cruz); 4- ++ (duas cruzes); 5- +++ (três cruzes); 6- ++++ (quatro cruzes)
43 Outros Hemoparasitos Pesquisados: 1-Negativo 2-Trypanosoma sp. 3-Microfilária 4- Trypanosoma sp.+Microfilária 9-Não pesquisados
44 Nome do Examinador:
45 Cód Examinador:

TRATAMENTO

46 Esquema de tratamento utilizado, de acordo com Manual de Terapêutica da Malária

1- Infecções pelo P. vivax ou P. ovale com cloroquina em 3 dias e primaquina em 7 dias (esquema curto);
2- Infecções pelo P. vivax, ou P. ovale com cloroquina em 3 dias e primaquina em 14 dias (esquema longo);
3- Infecções pelo P. malariae para todas as idades e por P. vivax ou P. ovale em gestantes e crianças com menos de 6 meses, com cloroquina em 3 dias;
4- Prevenção das recaídas frequentes por P. vivax ou P. ovale com cloroquina semanal em 12 semanas;
5- Infecções por P. falciparum com a combinação fixa de artemeter+lumefantrina em 3 dias;
6- Infecções por P. falciparum com a combinação fixa de artesunato+mefloquina em 3 dias;
7- Infecções por P. falciparum com quinina em 3 dias, doxiciclina em 5 dias e primaquina no 6º dia;
8- Infecções mistas por P. falciparum e P. vivax ou P. ovale com Artemeter + Lumefantrina ou Artesunato + Mefloquina em 3 dias e Primaquina em 7 dias;
9- Infecções não complicadas por P. falciparum no 1º trimestre da gestação e crianças com menos de 6 meses, com quinina em 3 dias e clindamicina em 5 dias;
10- Malária grave e complicada pelo P. falciparum em todas as faixas etárias;
11- Infecções por P. falciparum com a combinação fixa de artemeter+lumefantrina em 3 dias e primaquina em dose única;
12- Infecções por P. falciparum com a combinação fixa de artesunato+mefloquina em 3 dias e primaquina em dose única;
99- Outro esquema utilizado (por médico) - descrever:

47 Data Início do Tratamento:

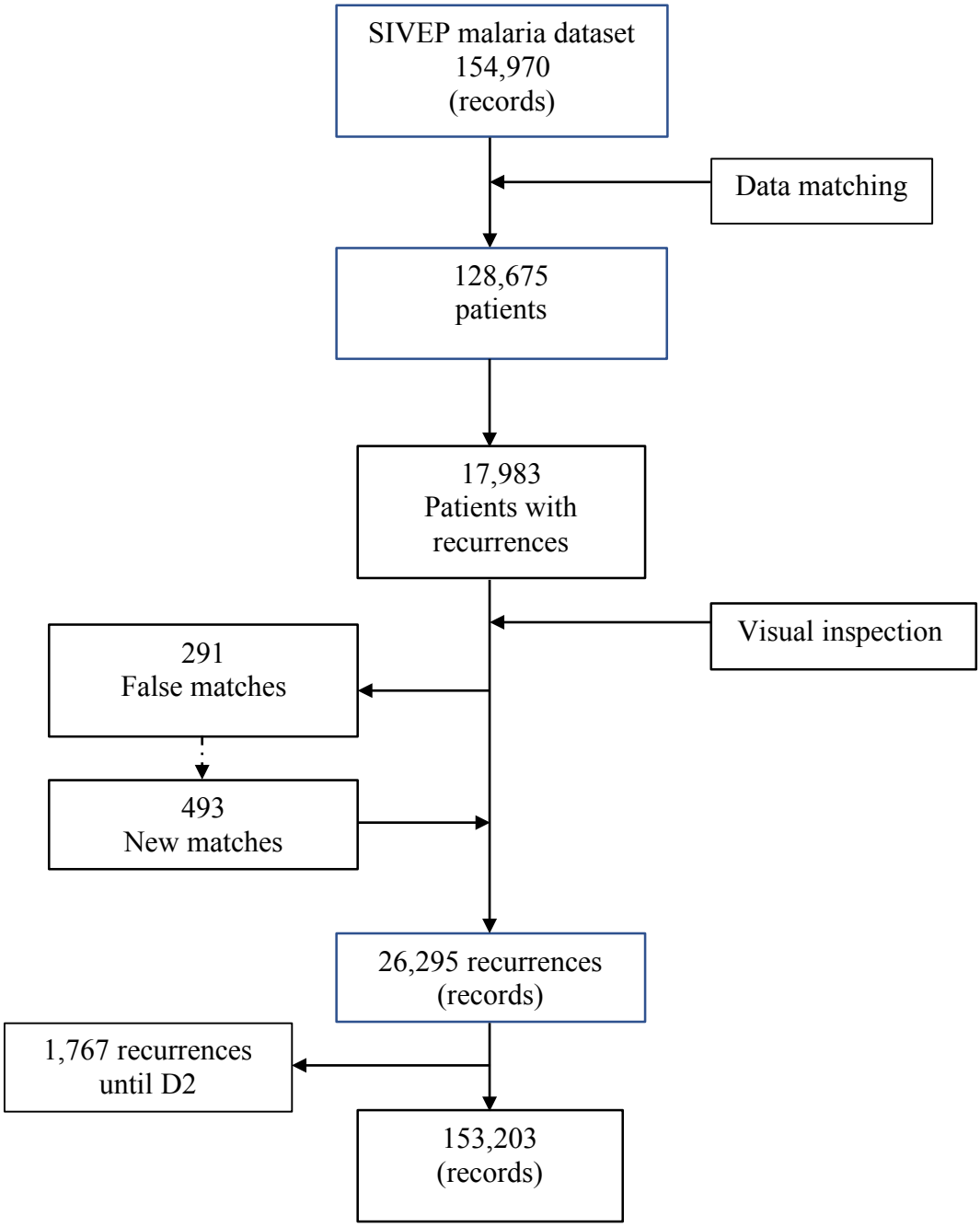
SMS-UF MUNICÍPIO

12 Nome do Paciente:
15 Idade:
1 Nº da Notificação
38 Data do Exame
40 Resultado do Exame
44 Nome do Examinador:

Comprovante de resultado do exame para ser entregue ao paciente

MS/SVS 28/02/2014

Additional material 3. Data flowchart



Additional material: Efficacy and safety of Artemisinin-based Combination Therapy and chloroquine with concomitant primaquine to treat Plasmodium vivax malaria in Brazil: An open label randomized clinical trial. *Malaria Journal,* 2017

Additional material 1.

Table S 1 Reasons to not be included- Consort Diagram

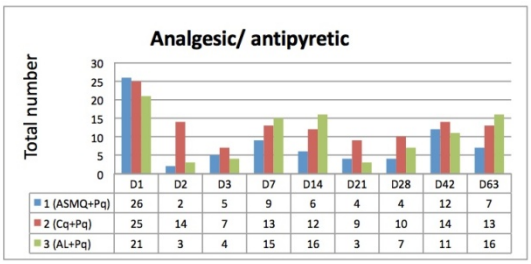
Reasons to not be included	**numbers of patients**	**(%)**
Unavailability for follow-up	492	(19.9%)
Parasitaemia lower than 250/μL	394	(15.9%)
Malaria treatment within the past 63 days	376	(15.2%)
Residency in rural area	187	(7.6%)
Weight range, i.e., <50kg or > 90kg	139	(5.6%)
Age range, i.e., < 18 or > 70 years old	122	(4.9%)
Falciparum malaria or mixed infection	113	(4.6%)
Other	89	(3.6%)
Declined to participate	69	(2.8%)
Pregnancy	44	(1.8%)
Haemoglobin < 7 g/dL	03	(0.1%)

Additional material 2.

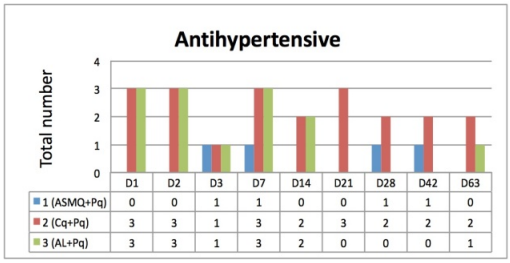
Table S 2 Distribution of use of concomitant medication (grouped in therapeutic class) in each study arm, parenthesis presents the percentage of the line.

Therapeutic class of concomitant medication	study arm N (%)		
	ASMQ+Pq	Cq+Pq	AL+Pq
Total	115(23.23)	189(38.18)	191(38.59)
analgesic/ antipyretic	74(25.78)	117(40.77)	96(33.45)
antispasmodic	5(17.24)	3(10.34)	21(72.41)
antifungal	1(33.33)	1(33.33)	1(33.33)
antiemetic	7(29.17)	3(12.5)	14(58.33)
antihistaminic	2(33.33)	4(66.67)	0(0)
antipsychotic	0(0)	1(100)	0(0)
antiulcer	5(14.29)	10(28.57)	20(57.14)
antibiotic	4(19.05)	7(33.33)	10(47.62)
antidepressant	0(0)	1(50)	1(50)
antihypertensive	4(10.53)	21(55.26)	13(34.21)
anti-vertigo	0(0)	1(50)	1(50)
antiviral (topic)	1(50)	0(0)	1(50)
anti-dyslipidaemia	1(50)	1(50)	0(0)
expectorant	0(0)	5(100)	0(0)
hepatic-protector	4(36.36)	3(27.27)	4(36.36)
hydration	1(100)	0(0)	0(0)
hypoglycaemic drug	0(0)	6(54.55)	5(45.45)
traditional drugs	2(40)	1(20)	2(40)
vitamins	4(40)	4(40)	2(20)

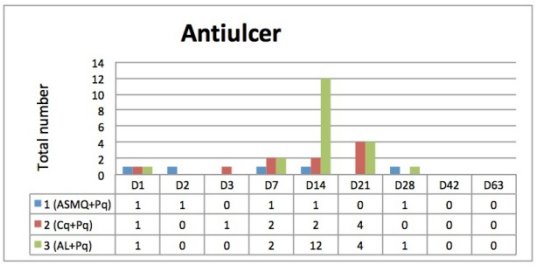
Figure S 1. Distribution of most frequent medications used per study visit and treatment group



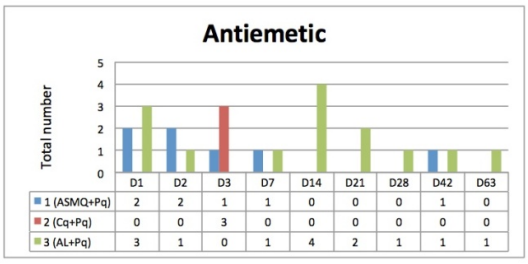
	D1	D2	D3	D7	D14	D21	D28	D42	D63
1 (ASMQ+Pq)	26	2	5	9	6	4	4	12	7
2 (Cq+Pq)	25	14	7	13	12	9	10	14	13
3 (AL+Pq)	21	3	4	15	16	3	7	11	16



	D1	D2	D3	D7	D14	D21	D28	D42	D63
1 (ASMQ+Pq)	0	0	1	1	0	0	1	1	0
2 (Cq+Pq)	3	3	1	3	2	3	2	2	2
3 (AL+Pq)	3	3	1	3	2	0	0	0	1



	D1	D2	D3	D7	D14	D21	D28	D42	D63
1 (ASMQ+Pq)	1	1	0	1	1	0	1	0	0
2 (Cq+Pq)	1	0	1	2	2	4	0	0	0
3 (AL+Pq)	1	0	0	2	12	4	1	0	0



	D1	D2	D3	D7	D14	D21	D28	D42	D63
1 (ASMQ+Pq)	2	2	1	1	0	0	0	1	0
2 (Cq+Pq)	0	0	3	0	0	0	0	0	0
3 (AL+Pq)	3	1	0	1	4	2	1	1	1

Additional material 3.

Table S 3. Proportion of treatment success per treatment arm in ITT population (n = 88 per arm) at day 28, 42 and 63

Visit day	Study treatment					
	ASMQ+Pq		CQ+Pq		AL+Pq	
	%	IC 95%	%	IC 95%	%	IC 95%
D28	97%	[91-100]	93%	[88-98]	92%	[86-98]
D42	92%	[86-98]	86%	[79-94]	88%	[81-94]
D63	76%	[67-85]	81%	[72-89]	77%	[69-86]

Table S 4. Proportion of treatment success per treatment arm in PP population at day 07, 14 and 21.

Visit day	**Study treatment**					
	ASMQ+Pq		**Cq+Pq**		**AL+Pq**	
	% (n)	**IC 95%**	**% (n)**	**IC 95%**	**% (n)**	**IC 95%**
D07	100% (87)	-	100% (85)	-	100% (86)	-
D14	100% (87)	-	100% (84)	-	100% (84)	-
D21	100% (86)	-	100% (84)	-	99% (84)	[97-101]

Table S 5. Proportion of treatment success per treatment arm in ITT population (n=88 per arm) at day 07, 14 and 21.

Visit day	Study treatment					
	ASMQ+Pq		CQ+Pq		AL+Pq	
	%	IC 95%	%	IC 95%	%	IC 95%
D07	99%	[97-101]	98%	[95-101]	98%	[95-101]
D14	99%	[97-101]	97%	[93-101]	98%	[95-101]
D21	98%	[95-101]	95%	[91-100]	94%	[89-99]

Additional material 4.

Table S 6. Distribution of adverse events per causality and treatment group.

Causality	Treatment group n (%)			
	ASMQ+Pq	CQ+Pq	AL+Pq	Total
Doubtful	160 (23.3)	283 (41.2)	243 (35.4)	686
Unlikely	35 (22.1)	70 (44.3)	53 (33.5)	158
Possible	163 (27.2)	238 (39.7)	199 (33.2)	600
Probable/ Likely	36 (27.9)	43 (33.3)	50 (38.8)	129
Highly Probable	9 (45)	9 (45)	2 (10)	20

Additional material 5.

Table S 7. Distribution of adverse events per causality and intensity (grade) in the treatment group ASMQ + Pq.

Grade	Causality (N,%) ASMQ+Pq					
	Doubtful	**Unlikely**	**Possible**	**Probable/ Likely**	**Highly Probable**	**Total**
1	141(34.99)	27(6.7)	151(37.47)	34(8.44)	8(1.99)	361(89.58)
2	18(4.47)	8(1.99)	12(2.98)	2(0.5)	1(0.25)	41(10.17)
4	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
NC*	1(0.25)	0(0)	0(0)	0(0)	0(0)	1(0.25)
Total	160(39.7)	35(8.68)	163(40.45)	36(8.93)	9(2.23)	403(100)

* Non-classified

Table S 8. Distribution of adverse events per causality and intensity (grade) in the treatment group CQ + Pq

Grade	Causality (N,%) CQ+Pq					
	Doubtful	**Unlikely**	**Possible**	**Probable/ Likely**	**Highly Probable**	**Total**
1	254 (39.5)	52 (8.09)	212 (32.97)	37 (5.75)	9 (1.4)	564 (87.71) 76
2	28(4.35)	17(2.64)	26(4.04)	5(0.78)	0(0)	(11.82)
4	0(0)	0(0)	0(0)	1(0.16)	0(0)	1(0.16)
NC*	1(0.16)	1(0.16)	0(0)	0(0)	0(0)	2(0.31)
Total	283 (44.01)	70 (10.89)	238 (37.01)	43 (6.69)	9 (1.4)	643 (100)

Table S 9. Distribution of adverse events per causality and intensity (grade) in the treatment group AL + Pq.

Grade	Causality (N,%) AL+Pq					
	Doubtful	**Unlikely**	**Possible**	**Probable/ Likely**	**Highly Probable**	**Total**
1	202(36.93)	28(5.12)	175 (31.99)	47(8.59)	2(0.37)	454(83)
2	40(7.31)	24(4.39)	24(4.39)	3(0.55)	0(0)	91 (16.64)
4	1(0.18)	1(0.18)	0(0)	0(0)	0(0)	2(0.37)
Total	243(44.42)	53(9.69)	199 (36.38)	50(9.14)	2(0.37)	547(100)

Additional material 6.

Table S 10. All adverse events (1,593) per body system and treatment allocation

Variable		Treatment allocation n (%) ASMQ+Pq	CQ+Pq	AL+Pq	Total
Total		403(25.3)	643(40.36)	547(34.34)	1593
Body System					
	Cardiovascular	4(28.57)	3(21.43)	7(50)	14
	Dermatological	28(25)	55(49.11)	29(25.89)	112
	Digestive	110(24.44)	158(35.11)	182(40.44)	450
	General Status	115(23.81)	203(42.03)	165(34.16)	483
	Excretory	1(10)	7(70)	2(20)	10
	Musculoskeletal	28(25)	43(38.39)	41(36.61)	112
	Central nervous System	77(31.3)	107(43.5)	62(25.2)	246
	Peripheral nervous System	3(50)	1(16.67)	2(33.33)	6
	Reproductive female	2(66.67)	0(0)	1(33.33)	3
	Respiratory	34(22.08)	66(42.86)	54(35.06)	154
	Trauma	1(33.33)	0(0)	2(66.67)	3

Additional material 7.

Table S 11. Adverse events with frequency higher than 3% in the ASMQ+Pq arm (n total=403)

ASMQ+Pq	
Symptoms	**n(%)**
headache	51(12.66)
insomnia	39(9.68)
nauseas	36(8.93)
asthenia	31(7.69)
dizziness	28(6.95)
dyspnoea	27(6.7)
pruritus	24(5.96)
abdominal pain	22(5.46)
muscle articular pain	21(5.21)
vomitus	16(3.97)

Table S 12. Adverse events with frequency higher than 3% in the CQ+Pq arm (n total=643)

Cq+Pq	
Symptoms	**n(%)**
headache	84(13.06)
insomnia	62(9.64)
pruritus	53(8.24)
abdominal pain	52(8.09)
asthenia	49(7.62)
nauseas	42(6.53)
anorexia	39(6.07)
dyspnoea	38(5.91)
muscle articular pain	32(4.98)
cough	22(3.42)

Table S 13. Adverse events with frequency higher than 3% in the AL+Pq arm (n total=547)

AL+Pq	
Symptoms	n**(%)**
headache	62(11.33)
abdominal pain	57(10.42)
asthenia	54(9.87)
nauseas	40(7.31)
dyspnoea	33(6.03)
vomitus	33(6.03)
insomnia	32(5.85)
pruritus	26(4.75)
muscle articular pain	25(4.57)
epigastric pain	24(4.39)
cough	21(3.84)
anorexia	19(3.47)
dizziness	17(3.11)

Additional material 8.

Table S 14. Haemoglobin mean per regimen allocation at day 0, 14, 28, 42, and 63

Days	**Regimen allocation**					
	ASMQ+Pq		**CQ+Pq**		**AL+Pq**	
	Mean (SD)	Range (min-max)	Mean (SD)	Range (min-max)	Mean (SD)	Range (min-max)
D1	13.33 (1.66)	(8.6-17.3)	13.58 (2.6)	(8.7-32.6)	13.59 (2.01)	(9-18.2)
D14	12.49 (1.46)	(9.9-16.2)	12.76 (1.33)	(10.3-16.4)	13.01 (1.42)	(8.4-16.3)
D28	13.48 (1.27)	(10.9-16)	13.5 (1.17)	(10.7-16.3)	13.76 (1.2)	(10.1-16.2)
D42	13.82 (1.18)	(11.5-16.4)	14.03 (1.45)	(10.3-18.8)	14.06 (1.3)	(10.4-17.1)
D63	13.83 (1.33)	(11.1-16.4)	14.22 (1.41)	(10.9-17.6)	14.27 (1.15)	(11.3-16.7)

Additional material: Pharmacokinetics/ Pharmacodynamics of chloroquine and Artemisinin-based Combination Therapies with primaquine

Additional material 1.

Table S 15. Genotyping of Plasmodium vivax polymorphic loci from patients during initial infection and recurrence

ID sample#	MS2	MS6	MS7	MSP1B2	MSP1B10
11 d0	294 bp	233 bp	349 bp	410 bp	290 bp
11 d42	294 bp	233 bp	349 bp	410 bp	290 bp
20 d0	298 bp	209 bp	349 bp	426 bp	255 bp
20 d63	298 bp	209 bp	349 bp	426 bp	255 bp
24 d7	342 bp	199 bp	385 bp	NA	255 bp
24 d63	298 bp	199 bp	349 bp	410 bp	223 bp
33 d0	298 bp	209 bp	349 bp	426 bp	255 bp
33 d62	298 bp	209 bp	385 bp	426 bp	255 bp
35 d0	292 bp	209 bp	355 bp	426 bp	290 bp
35 d62	300 bp	257 bp	358 bp	426 bp	255 bp
43 d0	294 bp	233 bp	349 bp	410 bp	290 bp
43 d51	294 pb	233 bp	349 bp	410 bp	290 bp
50 d0	312 bp	209 bp	361 bp	397 bp	242 bp
50 d41	302 bp	209 bp	355 bp	410 bp	237 bp
53 d0	298 bp	209 bp	361 bp	397 bp	233 bp
53 d49	298 bp	209 bp	361 bp	397 bp	233 bp
60 d0	298 bp	209 bp	361 bp	397 bp	242bp
60 d63	292 bp	199 bp*	388 bp	397 bp	255 bp*
67 d0	294 bp	230 bp	349 bp	410 bp	290 bp
67 d54	294 bp	230 bp	349 bp	410 bp	290 bp
69 d0	298 bp	209 bp	349 bp	414 bp	223 bp
69 d28	298 bp	209 bp	349 bp	414 bp	223 bp
73 d0	292 bp	209 bp	358 bp	397 bp	255 bp
73 d37	292 bp	209 bp	358 bp	397 bp	255 bp
102 d0	298 bp	209 bp	349 bp	410 bp	223 bp
102 d63	298 bp	209 bp	349 bp	410 bp	223 bp

103 d0	294 bp	230 bp	349 bp	410 bp	290 bp
103 d40	294 bp	230 bp	349 bp	410 bp	290 bp
111 d0	300 bp	209 bp	355 bp	426 bp	237 bp
111 d50	292 bp*	209 bp	349 bp	409 bp*	237 bp
119 d0	300 bp	209 bp	349 bp	408 bp	290 bp
119 d27	300 bp	209 bp	349 bp	408 bp	290 bp
126 d0	298 bp	209 bp	355 bp	410 bp	237 bp
126 d47	298 bp	209 bp	355 bp	426 bp*	237 bp
133 d0	298 bp	209 bp	355 bp	410 bp	237 bp
133 d62	298 bp	209 bp	355 bp	410 bp	237 bp
136 d0	298 bp	209 bp	355 bp	426 bp	237 bp
136 d63	298 bp	209 bp	355 bp	426 bp	237 bp
153 d0	298 bp	209 bp	358 bp	397 bp	242 bp
153 d63	292 bp	209 bp	358 bp	397 bp	242 bp
156 d0	300 bp	209 bp	349 bp	397 bp	255 bp
156 d63	300 bp	209 bp	349 bp	397 bp	255 bp
157 d0	298 bp	209 bp	361 bp	381 bp	242 bp
157 d63	298 bp	199 bp*	358 bp	NA	242 bp
160 d0	298 bp	230 bp	349 bp	426 bp	255 bp
160 d61	298 bp	230 bp	349 bp	426 bp	255 bp
162 d0	294 bp	209 bp	349 bp	397 bp	242 bp
162 d56	292 bp	209 bp	349 bp	397 bp	242 bp
163 d0	298 bp	209 bp	361 bp	405 bp	223 bp
163 d42	298 bp	209 bp	361 bp	405 bp	223 bp
166 d0	294 bp	230 bp	349 bp	392 bp	290 bp
166 d57	294 bp	230 bp	349 bp	410 bp	290 bp
182 d0	298 bp	224 bp	343 bp	426 bp	255 bp
182 d21	298 bp	224 bp	343 bp	426 bp	255 bp
198 d0	292 bp	209 bp	358 bp	397 bp	255 bp
198 d62	292 bp	209 bp	358 bp	397 bp	255 bp
207 d0	300 bp	209 bp	393 bp	410 bp	290 bp
207 d56	300 bp	209 bp	390 bp	410 bp	290 bp
214 d0	298 bp	209 bp	352 bp	426 bp	264 bp
214 d42	298 bp	209 bp	352 bp	426 bp	264 bp
216 d0	298 bp	199 bp	427 bp	397 bp	327 bp
216 d55	292 bp	209 bp	430 bp	414 bp	249 bp
231 d0	298 bp	209 bp	355 bp	410 bp	290 bp
231 d63	298 bp	209 bp	355 bp	410 bp	290 bp
246 d0	298 bp	209 bp	352 bp	410 bp	223 bp
246 d39	298 bp	209 bp	352 bp	410 bp	223 bp
250 d0	298 bp	209 bp	352 bp	426 bp	264 bp
250 d63	298 bp	233 bp	352 bp	426 bp	264 bp
251 d0	294 bp	209 bp	349 bp	410 bp	290 bp

251 d63	294 bp	199 bp	340 bp	410 bp	290 bp

Size of PCR products in base pairs (bp). #Number of patient and day of sample collection: d0 – diagnosis and treatment, dX – the day of recurrence. *Allele similar to initial infection present in lower intensity. NA – non-amplified.

Additional material 2.

Table S 16. Genotyping of CYP2C8 gene and CYP2D6 gene and predicted phenotype of CYP2D6 activity

ID patient	CYP2C8		CYP2D6														
	G1846A	G2988A	G-1584C	C100T	C1023T	C2850T	G3183A	G4180C	2615_2617 del AAG	Haplotype[a]	Allele 1	Allele 2	Copy number	AS[b] Allele 1	AS Allele 2	Total activity Score[c]	Phenotype[b]
11	GG	**GA**	**CG**	CC	CC	**TT**	GG	**CC**	AA	PV011: (4,7)	*2/*35	*41	2	1	0.5	1.5	gNM-F
20	GG	GG	CC	**TC**	CC	CC	GG	**GC**	AA	PV020: (2,6)	*1	*10/*10x2	2	1	0.5	1.5	gNM-F
24	GG	GG	**CG**	CC	**CT**	**TT**	GG	**CC**	AA	PV024: (4,5)	*2/*35	*17	2	1	0.5	1.5	gNM-F
33	**GA**	GG	CC	**TC**	**TT**	**TC**	GG	**CC**	AA	PV033: (5,10)	*17	NP	2	0.5	NP	NP	NP
35	GG	GG	CC	CC	CC	CC	GG	GG	AA	PV035: (2,2)	*1	*1	2	1	1	2	gNM-F
43	**GA**	GG	CC	**TC**	CC	CC	GG	**GC**	AA	PV043: (2,8)	*1	*4/*4x2	2	1	0	1	gNM-S
50	GG	GG	CC	CC	CC	CC	GG	GG	AA	PV050: (2,2)	*1	*1	2	1	1	2	gNM-F
53	GG	GG	CC	**TC**	CC	CC	GG	**GC**	AA	PV053: (2,6)	*1	*10	2	1	0.5	1.5	gNM-F
60	GG	GG	**GG**	CC	CC	**TT**	GG	**CC**	AA	PV060: (4,4)	*2/*35	*35xN/*2xN	3	1	2	3	gUM
67	**AA**	GG	CC	**TT**	CC	CC	GG	**CC**	AA	PV067: (8,8)	*4	*4	2	0	0	0	gPM
69	GG	GG	CC	CC	CC	CC	GG	GG	AA	PV069: (2,2)	*1	*1	2	1	1	2	gNM-F
73	GG	GG	CG	CC	CC	**TC**	GG	**GC**	AA	PV073: (2,4)	*1	*2/*35	2	1	1	2	gNM-F
102	GG	**GA**	CC	CC	CC	**TC**	GG	**GC**	AA	PV102: (2,7)	*1	*41	2	1	0.5	1.5	gNM-F
103	GG	GA	**CG**	CC	CC	**TT**	GG	**CC**	AA	PV103: (4,7)	*2/*35	*41	2	1	0.5	1.5	gNM-F
111	GG	GG	CC	CC	CC	CC	GG	GG	AA	PV111: (2,2)	*1	*1	2	1	1	2	gNM-F
119	GG	GG	CG	CC	**CT**	**TT**	GG	**CC**	AA	PV119: (4,5)	*2/*35	*17	2	1	0.5	1.5	gNM-F
126	**GA**	GG	CC	**TC**	CC	CC	GG	**GC**	AA	PV126: (2,8)	*1	*4	2	1	0	1	gNM-S

133	GG	GG	CC	CC	CC	CC	GG	GG	AA	PV133: (2,2)	*1	*1	2	1	1	2	gNM-F
136	**GA**	GG	CC	**TC**	CC	CC	GG	**GC**	AA	PV136: (2,8)	*1	*4	2	1	0	1	gNM-S
153	GG	GG	CC	CC	CC	CC	GG	GG	AA	PV153: (2,2)	*1	*1	2	1	1	2	gNM-F
156	GG	GG	**GG**	CC	CC	**TT**	GG	**CC**	AA	PV156: (4,4)	*2/*35	*2/*35	2	1	1	2	gNM-F
157	GG	GG	**GG**	CC	CC	**TT**	GG	**CC**	AA	PV157: (4,4)	*2/*35	*5	1	1	1	2	gNM-F
160	**GA**	GG	CC	**TC**	CC	CC	GG	**GC**	AA	PV160: (2,8)	*1/*1xN	*4/*4x2	NP	1	0	1	gNM-S
162	GG	GG	**CG**	CC	CC	**TC**	GG	**GC**	AA	PV162: (2,4)	*1	*2/*35	2	1	1	2	gNM-F
163	**GA**	**GA**	CC	**TC**	CC	**TC**	GG	**CC**	AA	PV163: (7,8)	*41	*4/*4x2	NP	0.5	0	0.5	gIM
166	GG	GG	**CG**	CC	CC	**TT**	GG	**CC**	AA	PV166: (1,4)	*2D	*2/*35	2	1	1	2	gNM-F
182	GG	**GA**	CC	CC	CC	**TC**	GG	**GC**	AA	PV182: (2,7)	*1	*41	2	1	0.5	1.5	gNM-F
198	GG	GG	CC	**TC**	CC	CC	GG	**GC**	**CA**	PV198: (3,6)	*9	*10	2	0.5	0.5	1	gNM-S
207	GG	GG	**CG**	CC	CC	**TC**	GG	**GC**	**CA**	PV207: (3,4)	*9	*2/*35	2	0.5	1	1.5	gNM-F
214	GG	GG	**GG**	CC	CC	**TT**	GG	**CC**	AA	PV214: (4,4)	*2/*35	*2/*35	2	1	1	2	gNM-F
216	**GA**	GG	CC	**TC**	CC	CC	GG	**GC**	AA	PV216: (2,8)	*1/*1xN	*4/*4x2	3	2	0	2	gNM-S
231	**GA**	GG	**GG**	**TC**	CC	**TC**	GG	**CC**	AA	PV231: (4,9)	*2/*35/*2xN	NP	3	1	NP	NP	NP
246	**GA**	GG	CC	**TC**	CC	CC	GG	**GC**	AA	PV246: (2,8)	*1	*4	2	1	0	1	gNM-S
250	GG	GG	CC	CC	CC	CC	GG	GG	AA	PV250: (2,2)	*1	*1	2	1	1	2	gNM-F
251	GG	**GA**	**CG**	CC	CC	**TT**	GG	**CC**	AA	PV251: (4,7)	*2/*35	*41	2	1	0.5	1.5	gNM-F

a *CYP2D6* haplotype inferred using Phase software. b Activity score and inferred phenotype of CYP2D6: AS = 1.5 or 2 – normal metabolizer fast (gNM-F); AS ≥ 2 - ultra metabolizer (gUM) (more than 2 copies of the normal allele); AS = 1 – normal metabolizer slow (gNM-S); AS = 0.5 – intermediate metabolizer (gIM); AS = 0 – poor metabolizer (gPM), according to Gaedigk et al. 2008 [20]. c Sum of AS attributed to allele 1 and 2 of *CYP2D6* gene. NP - not performed.

Additional material 3.

Table S 17. Evaluation of pharmacokinetics' parameters, gender and weight as predictors of failures per treatment drug (Generalized Linear Model, binomial logit link)

	MQ	**CQ**	**LMF**
n failures (%)	7 (8)	7 (13)	8 (14)
		OR (95% CI) p-value	
Female	0.73 (0.15-3.53), p=0.7	2.13 (0.09-50.66), p=0.64	0.35 (0.06-1.94), p=0.23
Half-life (days)	-	1.04 (0.87-1.23), p=0.69	-
Weight	0.94 (0.88-1.02), p=0.13	0.92 (0.84-1.01), p=0.09	0.93 (0.83-1.03), p=0.16
AUC (mcg)	1 (0.99-1.01), p=0.71	0.99 (0.96-1.02), p=0.63	0.97 (0.87-1.07), p=0.52

Additional material 4.

Table S 18. Evaluation of pharmacokinetics' parameters and weight as predictors of D3 failures per treatment drug (Generalized Linear Model, binomial logit link)

	MQ	CQ	LMF
n failures (%)	-	3 (6)	-
		OR (95% CI), p-value	
Weight	-	0.93 (0.84-1.03), p=0.18	-
AUC (mcg)	-	1.00 (0.99-1.02), p=0.70	-
Half-life (days)	-	1.05 (0.9-1.23), p=0.52	-

Only available to chloroquine, all males. ASMQ and AL 100% presented clearance at D3.

Additional material 5

Table S 19. Evaluation of pharmacokinetics' parameters, gender and weight as predictors of time to failures per treatment drug (Cox regression)

	MQ	CQ	LMF
n events (%)	7 (8)	7 (8)	8 (9)
		HR (95% CI), p-value	
Weight	0.95 (0.89-1.02), p=0.18	0.93 (0.85-1.03), p=0.15	0.93 (0.86-1.02), p=0.11
AUC (mcg)	1 (0.99-1.01), p=0.95	0.99 (0.96-1.02), p=0.65	0.96 (0.87-1.07), p=0.46
Half-life (days)	-	1.02 (0.89-1.17), p=0.8	-
Gender	0.74 (0.14-3.83), p=0.72	1.87 (0.2-17.84), p=0.59	0.38 (0.06-2.42), p=0.31

Additional material 6

Table S 20. Evaluation of pharmacokinetics' parameters and weight as predictors of the drop in haemoglobin at day 14 using ordinary least squares.*

	Mefloquine (N=52)	Chloroquine (N=52)	Lumefantrine (N=53)
		Coefficient (95%CI), p-value	
AUC (0d-63d) (µg/ml.h)	0.00 (-0.24-0.33), p=0.17	-0.02 (-0.03;-0.005), p=0.01	0.00 (-0.16-0.33), p=0.42
Half-life (days)	0.00 (0-0), p=0.87	-0.03 (-0.1-0.04), p=0.42	NA
Weight	0.00 (0-0), p=0.77	**	0.00 (0-0), p=0.37

*Hb at day 14 − Hb at baseline/Hb at baseline

**CQ AUC and weigh correlation is significant at the 0.01 level (2-tailed). Weight was excluded as a covariate. NA= Non-Applicable

Additional material 7

Table S 21. Evaluation of pharmacokinetics' parameters as predictors of frequent (n≥30) adverse event (possible and likely related to treatment) per system and drug using Generalized Estimation Equation log-binomial regression.

Treatment group		Adverse event (System) RR (95% CI), p-value			
		Dermatological (pruritus)	**Overall state**	**CNS**	**Digestive**
MQ	**AUC**	-	-	1 (1-1), p=0.49	1 (1-1), p=0.61
	Half-life	-	-	0.99 (0.95-1.04), p=0.71	1 (0.96-1.03), p=0.81
	Weigh	-	-	1.00 (0.97-1.03), p=0.93	1 (0.97-1.03), p=0.93
CQ*	**AUC**	1 (0.99-1.01), p=0.73	-	1.01 (1-1.02), p=0.07	0.99 (0.98-1), p=0.06
	Half-life	1.09 (1.03-1.14), p<0.01	-	1.02 (0.97-1.08), p=0.43	0.99 (0.95-1.04), p=0.73
LMF	**AUC**	-	0.96 (0.91-1.01), p=0.15	-	0.98 (0.95-1.01), p=0.24
	Weigh	-	1.01 (0.98-1.04), p=0.57	-	0.97 (0.94-1.01), p=0.11

*CQ AUC and weigh correlation is significant at the 0.01 level (2-tailed). Weight was excluded as a covariate