Studies to provide recommendations on the pre-elimination of Plasmodium vivax in Lao PDR

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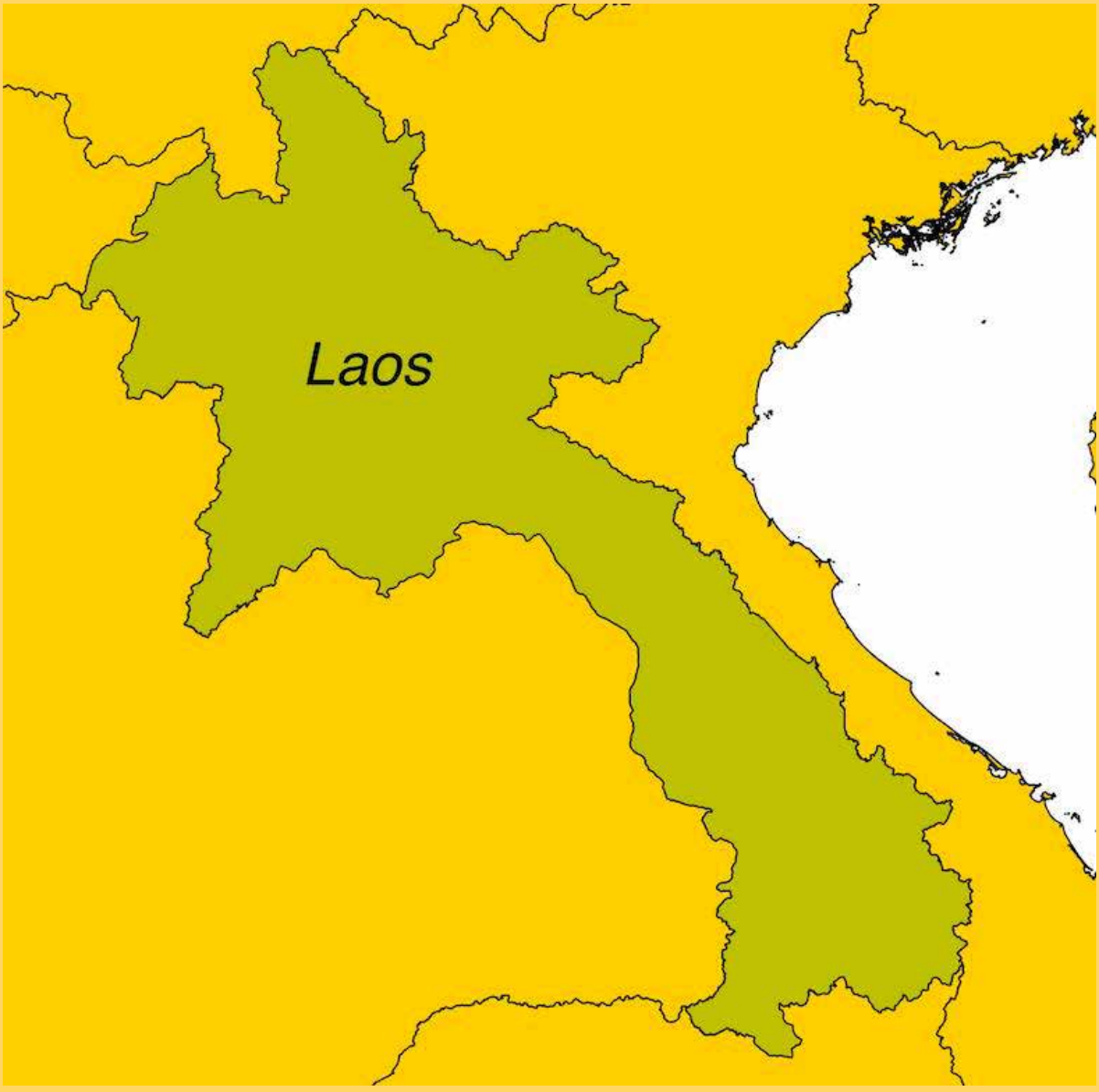

Koukeo Phommasone

Studies to provide recommendations on the pre-elimination of *Plasmodium vivax* in Lao PDR

ACADEMISCH PROEFSCHRIFT

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Chapter 1-Introduction and Outline of the thesis

Introduction

Malaria

Malaria is a mosquitos-borne infectious disease caused by a parasitic single-celled microorganism belonging to the *Plasmodium* genus. Humans are infected through the bites of infected female *Anopheles*. There are five known *Plasmodium* species that can cause malaria in humans: *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* and *Plasmodium knowlesi*. The first two species are the most common globally. While *P. falciparum* is responsible for more deaths, *P. vivax* is the most widely spread of all malaria species and able to cause relapses from liver-dormant parasites called hypnozoites. Although *P. vivax* is considered as benign, it can cause severe malaria and death [1].

Malaria epidemiology globally

Malaria remains a major public health problem in malaria endemic countries despite a global reduction in the burden of the disease over the last two decades. It causes not only marked morbidity and mortality, it also negatively impacts on economic development [2]. The number of clinical malaria cases dropped by 18% from 262 million cases in 2000 to 214 million in 2015, and the number of malaria deaths reduced by 48% (from 839000 to 438000) during the same period [3]. According to the World Malaria Report, 11 countries have received certification from the World Health Organization as malaria-free countries since 2000 (Figure 1).

Malaria control has been stepped up since the inauguration of the Roll Back Malaria Initiative in 1998 by the World Health Organization with the financial and technical support of UNICEF, the United Nations Development Programme, the World Bank and the World Health Organization (World Health Report 1999). However, over the last few years, progress has slowed down compared to rapid decline between 2010 and 2015 (Figure 2). In 2017, WHO estimated that 219 million people were infected, about 5 million more compared to those in 2015. The majority of clinical cases were from sub-Saharan Africa. Only a small proportion (5%) were from Southeast Asia.

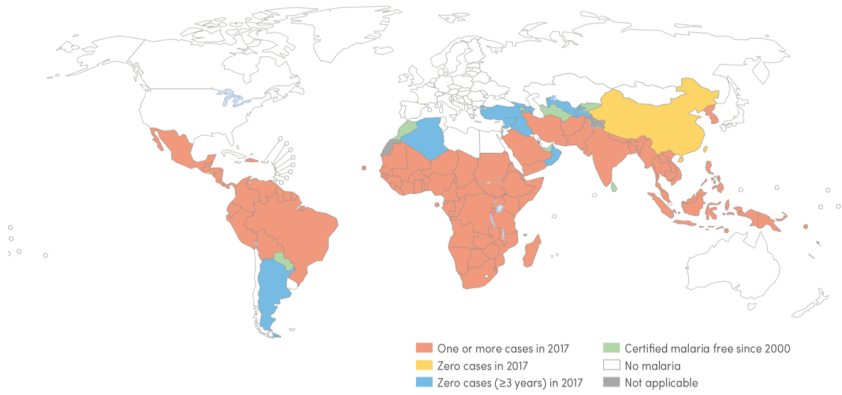

Figure 1. Countries with indigenous cases of malaria in 2000 and their status by 2017.

Source: WHO malaria report 2018.

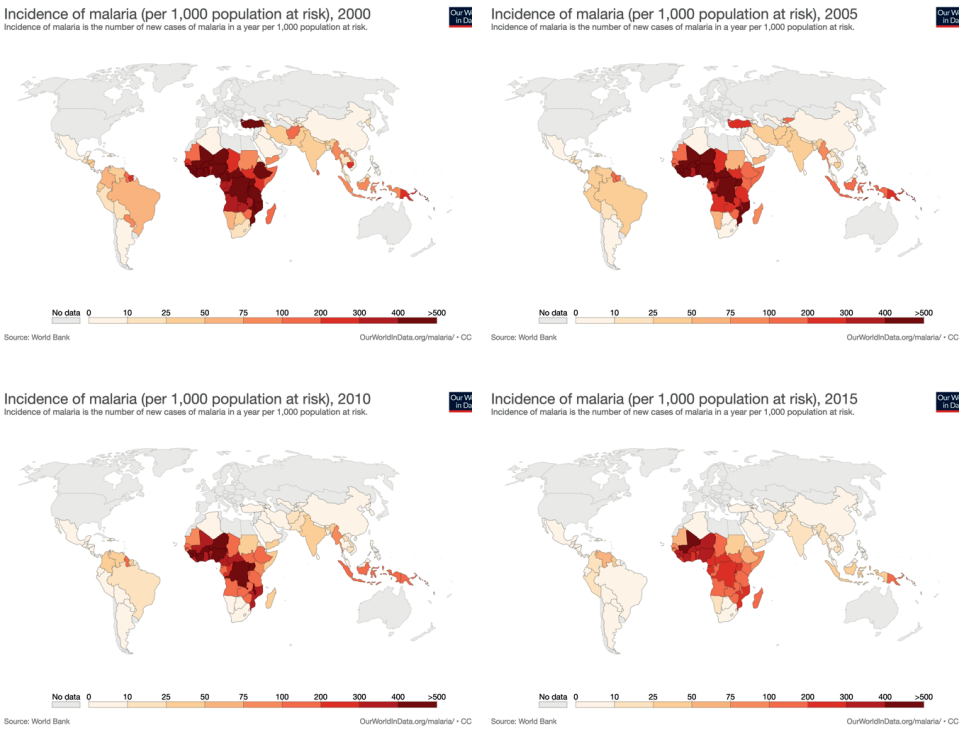

Figure 2. Reduction in incidence of malaria from 2000 to 2015.

Source: World Bank.

Malaria epidemiology in the Greater Mekong Subregion (GMS)

The GMS comprises of six countries: Cambodia, Myanmar, Thailand, Lao People's Democratic Republic, Vietnam and China (Yunnan province only). The malaria trend in this region follows the global trend with a declining incidence. However, malaria epidemiology is heterogenous and complex between and within the countries. Most malaria transmission is found in forested and isolated areas, and along international borders, which poses a challenge for malaria control and elimination. Migration and mobilization of people facilitates the introduction of malaria into areas that are close to malaria elimination.

The GMS has been known as a hub of emergent antimalarial-resistant *P. falciparum* for decades. The parasites here were first resistant to chloroquine in 1950s, then to sulfadoxine, pyrimethamine, then quinine (the first report of quinine-resistant *P. falciparum* was from South America), mefloquine, and recently to artemisinin and piperaquine. *P. falciparum* resistant to artemisinin poses a great threat to global malaria control and elimination if it spreads to Africa where malaria is most prevalent [4, 5]. Concerning antimalarial resistance in *P. vivax*, chloroquine resistant *P. vivax* did not originate from the GMS. The first chloroquine resistance in *P. vivax* was reported in Papua New Guinea and then spread to other parts of the world [6].

Malaria control strategies: Past and Present

Historically, the first Global Malaria Eradication Program was implemented from 1955 until 1968 in malaria endemic countries including three countries in Africa. The program resulted in malaria elimination in all developed countries in Europe, North America, some of the former Soviet Republics, and some island nations through the concerted effort of the World Health Organization [7]. The program consisted of the use of the insecticide Dichloro-diphenyltrichloroethane known as DDT, larvicides, and antimalarial drugs (mainly chloroquine) for treating malaria patients. This program was less successful, and considered as infeasible, in many developing countries in the tropics and subtropics where malaria was highly endemic and the health infrastructure was poor. Then the program was stopped in 1969 after the collapse of financial support together with the emergence of chloroquine and DDT resistance as well as resistance to other residual insecticides, resulting in an increase of malaria worldwide in the 1970s and 1980s [8]. The malaria problem had increased due to insufficient financial and human resources, climate changes, changes in land use, and migration of people, and political and civil unrest in some countries [9]. In response to this and with new drugs and tools which were becoming available, a ministerial conference was held in 1992 in Amsterdam

leading to the endorsement of the Global Malaria Control Strategy which was subsequently confirmed by the World Health Assembly in 1993. This strategy was mainly developed to address the malaria situation in Africa, and consisted of four basic technical elements: to provide early diagnosis and prompt treatment; to plan and implement selective and sustainable preventive measures including vector control; to detect, contain, or prevent epidemics; and to strengthen local capacities [10]. This strategy promoted decentralization of health services to the rural poor areas and adaptation to local condition and local needs, which differed from the one used during the eradication era [9].

The current Global Malaria Control programme uses a range of effective tools, which include insecticide treated nets (ITNs), indoor residual spraying (IRS), user-friendly packaging of drugs, and access to early diagnosis with rapid diagnostic tests leading to prompt treatment with the most efficacious therapy-ACT. Successful malaria control depends largely on treatment with efficacious anti-malarial drugs. The artemisinins were discovered by Chinese scientists in the 1970s. They were widely used as monotherapy from the 1990s until 2000s. The use of artemisinins alone results in high rates of recrudescence. Therefore, WHO discouraged its use as monotherapy and recommended to use artemisinins with other long-acting drugs as so-called artemisinin combination therapies (ACTs) in 2001. Artemisinin or its derivatives (artesunate, artemether, dihydroartemisinin) are known for their rapid but short action against circulating and sequestered parasites. Therefore, the combination of artemisinins with other long action antimalarials is essential. Although the ACTs were used in some countries in Southeast Asia in the 1990s, they were globally adopted in 2006 [11]. Since then, ACTs have become the first-line recommended treatment for uncomplicated *P. falciparum malaria* in the malaria endemic world.

ACTs have played a vital role in malaria control in malaria endemic countries. Maintaining the efficacy of ACT in the treatment of malaria is a global health priority. The efficacy of the programme is now affected by insecticide resistance, changes in vector feeding and biting behaviour, and outdoor malaria transmission. Despite the widespread resistance to pyrethroids (the only chemical class of insecticide) that is used on ITNs, there is no evidence yet that this affects the effectiveness of ITNs [12]. To manage resistance, the combination of permethrin (a pyrethroid) and pyriproxyfen, a long-lasting chemical, such as piperonyl butoxide (PBO) increases efficacy and overcomes resistance [13, 14]. There are about 12 different insecticides in four chemical classes available for IRS that can serve as alternatives in case of emerging resistance, amongst them DDT that played a major role in the historical malaria elimination

efforts. The efficacy of these two main vector controls strategies (ITN and IRS) depend on high coverage and utilization, but they cannot prevent outdoor malaria transmission. Therefore, there is a need to use additional vector control that targets vectors at different life cycle stages. One of these approaches is larviciding, but knowledge gaps remain in its application. Knowing vector behaviour like endophagia and exophagia (indoor or outdoor feeding), endophilia and exophilia (indoor or outdoor resting) and anthropophilia and zoophilia (human or bovine blood feeding) in endemic areas is important for choosing the right intervention. Apart from vector control, the gains made have been attributed to prompt diagnosis and treatment with ACTs, although ACTs are now under threat with widespread artemisinin resistant *P. falciparum* throughout the GMS.

Given the recent success in malaria control and past experience on malaria eradication in the 1950s and 1960s, the hope to eliminate malaria from countries and regions where the intensity of malaria transmission is low or moderate with well-established healthcare infrastructures is deemed feasible by experts [15]. In response to the spread of multidrug resistant *P. falciparum* in the GMS, it is essential to contain the spread of the antimalarial parasites and have strategies to eliminate malaria. The Strategy for Malaria Elimination was established for 2015-2030 with the ultimate goal to eliminate *P. falciparum* by 2025 and all malaria by 2030 in 35 countries. Elimination of malaria means the interruption of local transmission (reduction to zero incidence of indigenous cases) of a specified malaria parasite in a geographical area as a result of deliberate activities. The Global Technical Strategy for Malaria 2016-2030 comprises of 3 pillars: Pillar 1: ensure that everybody has access to prompt diagnosis, treatment and prevention; Pillar 2: accelerate efforts towards elimination and attainment of malaria free status; Pillar 3: transform malaria surveillance into a core intervention.

It seems that current interventions will not lead to malaria elimination in malaria endemic countries, as the strategy aims at treating only people with clinical malaria while those asymptomatic carriers who are even more prevalent than clinical cases will not seek care, and serve as malaria reservoirs [16]. To achieve malaria elimination, monitoring the asymptomatic malaria by early detection and treatment is crucial, especially among immigrant people from areas where malaria is prevalent. In countries with low malaria transmission, this strategy targeting the reservoir of malaria infection reduces the contact between infective human and vector resulting in reducing malaria transmission.

As mentioned earlier, malaria control and elimination programmes are now confronted with ACT resistant *P. falciparum* which is spreading throughout the GMS and high prevalence of infective asymptomatic reservoirs. Therefore, mass drug administration (MDA) has been reconsidered as a tool to accelerate malaria.

The other two challenges confronted by current malaria control programme

There has been mixed success and failure of past malaria control and elimination. Most of malaria control and studies have focused on *P. falciparum* infection with the ultimate goal to eliminate and eradicate it. Despite a lot of effort over the last two decades, the health burden of malaria is still unacceptable. In addition, the available knowledge, tools, and strategy which are mainly for *P. falciparum* control will not work everywhere and be sufficient either to control or eliminate other *Plasmodium* spp. [17]. In the context of malaria elimination, the programme should not ignore asymptomatic malaria and *P. vivax* as both will become more and more prevalent and predominant, and are harder to control than *P. falciparum*.

Asymptomatic malaria

With conventional microscopy or rapid diagnostic tests, people with malaria infection could be visualized as an iceberg which means only the top of it can be seen. Since the application of PCR particularly uPCR, asymptomatic malaria is recognized more, appears to be prevalent in endemic areas, and plays a potential role in malaria transmission. The prevalence of asymptomatic malaria along the border between eastern Myanmar and north-western Thailand and in Western Cambodia was about 28% during the surveys in 2013, and the majority of those were identified as *P. vivax* by using ultrasensitive polymerase chain reaction (uPCR) which is able to detect parasites at densities as low as 22 parasites/mL [18]. Although the contribution of low parasite densities in asymptomatic malaria carriers to malaria transmission is not clear, gametocytes are also present in those asymptomatic carriers [19] suggesting that they could play an important role in transmission. Vantaux and colleagues found that *P. falciparum* asymptomatic carriers were not infectious to mosquitoes unless they became symptomatic, but in contrast, about 4% of *P. vivax* asymptomatic carriers were infectious with infectivity persisting over several weeks [20] while others reported even much higher estimates up to 12% [21]. Despite a very low density of *P. vivax* in asymptomatic individuals, they were able to infect mosquitoes and maintain transmission [22]. Moreover, the time to spontaneous parasite clearance in *P. falciparum* asymptomatic carriers was much shorter compared to *P. vivax* asymptomatic carriers, within 4 months compared to at least 11 months, respectively [23]. In

addition, asymptomatic infection in pregnant women, can result in placental plasmodial infection or congenital plasmodial infection [24]. Blood transfusion can also be a cause of transmission of (asymptomatic) malaria infections, as has been seen in both malaria endemic and non-endemic countries [25]. Therefore, in the context of malaria elimination, asymptomatic malaria reservoirs should be targeted the same way as clinical cases and mass drug administration (MDA) is one of the interventions suggested for complete malaria elimination [26].

Plasmodium vivax

P. vivax, which is distributed widely geographically, is more resilient and capable of invading into areas with temperate climates that are not suitable for *P. falciparum*. *P. vivax* is the second most prevalent cause of malaria following *P. falciparum*. WHO estimated 13.8 million malaria cases due to *P. vivax* in 2015 [3]. Control and elimination of *Plasmodium falciparum* has received the most attention due to most of fatal cases being caused by this species. As a consequence, *Plasmodium falciparum* incidence has reduced at a faster rate leading to changes in malaria epidemiology with predominant *Plasmodium vivax* in Asia and America. The majority of vivax cases are in South and Southeast Asia where there is evidence of drug resistance [27]. Most of people in Africa are protected due to absence of Duffy antigen in their red blood cells, which makes parasites incapable of invading these cells [27]. The *P. vivax* malaria clinical spectrum ranges from benign to life-threatening disease and death, just as *P. falciparum* infections. Even though *P. vivax* rarely causes death, it is common that it contributes to death in patients with malnutrition, immune-compromise and bacterial infection [28]. It also poses a significant impact on the health and economy of infected people, and the health system due to its dormant liver stage parasites (the hypnozoites) that cause relapses at later stages. This latter phenomenon poses an extra challenge to elimination in the regions where *P. falciparum* and *P. vivax* co-exist. The frequency and relapse interval time vary in different parasite strains, the most frequent and short-time relapse are found in tropical strains [29]. Relapse of vivax malaria has important implications for the individual like anaemia resulting from repeated haemolysis, poor nutrition, poor cognitive performance, and school absenteeism [30]. Furthermore, the relapses can be a source of transmission at community level.

The mechanism of reactivation of hypnozoites is poorly understood. The recurrence rate depends on the inoculation rate, parasite strains, and antimalarials used. For chloroquine

sensitive *P. vivax*, the relapse after treatment with chloroquine occurs usually after 35 days and nearly 60% of infected patients relapse by day 60. The relapse starts occurring at day 17 after treatment with quinine and more than 60% of them relapse by day 35 [31]. Artesunate combined with mefloquine or piperaquine, which are slowly eliminated, reduce the risk of early *P. vivax* recurrence. Other factors like male sex, younger age, and the presence of mixed infection are likely to predispose to a recurrence [32]. It is also observed that treatment of *P. falciparum* cases is often followed by clinical case of vivax malaria with the recurrence rate up to 52% on Thailand-Myanmar border [33].

Malaria control and elimination programmes may meet little success if they do not target hypnozoites as a reservoir of relapses. Primaquine and tafenoquine, both 8-aminoquinolines, are the only licensed drugs for eradicating *P. vivax* hypnozoites resulting in preventing relapses of vivax malaria, but these drugs are not widely used due to potential fatal haemolytic anaemia in patients with G6PD deficiency. This explains the underutilization of primaquine in vivax malaria endemic areas. The utilization of primaquine can be improved by improving point of care diagnostics for G6PD which are inexpensive, heat stable and user-friendly. The World Health Organization currently recommends a 14-day course of primaquine for radical treatment of vivax malaria in patients without G6PD, while a weekly regimen for 8 weeks is recommended for patients with G6PD deficiency.

Mass Drug Administration approach and challenges

To achieve the goals set for malaria elimination by 2030 and also to contain the spread of artemisinin resistant *P. falciparum* in the Greater Mekong Subregion, strategies like mass screening and treatment (MSAT), focal screening and treatment (FSAT), and MDA are being evaluated. MSAT or FSAT involve screening of all individuals in a given geographical area and treating only those found positive for malaria. In 2003, these two strategies were considered appropriate in epidemic and complex emergency situations as actively searching for cases ensures prompt treatment [34], but the diagnostic tools like rapid diagnostic tests or microscopy have low sensitivity and fail to detect people with low parasite density. As a consequence, WHO does not recommend MSAT or FSAT as tools to interrupt malaria transmission [35]. According to the recommendation given by WHO's evidence review group, MDA can be used or considered to use in four conditions: 1) to eliminate *P. falciparum* in areas approaching interruption of transmission, where treatment is accessible, with effective vector control and surveillance, and minimal risk of reintroduction of infection; 2) to accelerate

malaria elimination and to contain the spread of multidrug resistance in areas of the Greater Mekong Subregion with good access to treatment, vector control and surveillance; 3) to reduce rapidly morbidity and mortality for epidemic control, along with the urgent introduction of other interventions; and 4) to reduce malaria morbidity and mortality in complex emergencies when the health system is unable to serve the affected communities [35].

MDA refers to the administration of treatment to all people in a given area, regardless of symptoms or any diagnostics (except those with contraindications to medicines used). MDA has been used to eliminate or to control many neglected tropical diseases including trachoma, lymphatic filariasis, schistosomiasis, onchocerciasis [36-40], and it was also a component of malaria elimination programmes in the 1950s. The earliest MDA programme for malaria was published in 1913 which was more than a century ago [41], and it has been used in many countries. Many of the MDA efforts for malaria were conducted more than 30 years ago [42]. All these efforts differed by the drugs used (regimen and dosage) and the number and timing of MDA rounds, making comparison of their effectiveness difficult. Although most of these MDA programmes claimed success, the majority of them actually reduced transmission only temporarily. A review done by Newby *et al*. identified 12 studies that met the definition of success with zero indigenous malaria cases for at least 6 months [41, 43, 44], while the majority of efforts completely failed if their main aim was to interrupt transmission [41]. A review of MDA strategies concluded that MDA with therapeutic doses might be effective if it is conducted in areas with low or moderate transmission [42], and that failure of MDA programmes conducted in the mid-twentieth century eradication era was due to using inadequate treatment doses. A subsequent review confirmed that MDA is likely to be successful if it is conducted in areas of low transmission together with other interventions, and identified other essential factors that play as a key role in the success of MDA, like directly observed therapy (DOT), high coverage, adequate drug regimen and conducive geographical settings [41]. However, a review of mass drug administration for malaria published in 2013 found only two cluster-randomized trials among 32 studies of MDA, one conducted in Tanzania and the other in the Gambia with different levels of endemicity [42]. This means that the remaining studies are probably at high risk of bias for selection or baseline imbalances. A multi-country cluster-randomized control trial conducted in four Southeast-Asian countries including Lao PDR between 2013 and 2017, aimed to evaluate the efficacy of targeted malaria elimination (TME) with the goal of eliminating artemisinin resistant *P. falciparum* with three monthly rounds of dihydroartemisinin-piperaquine (DP) and single low dose primaquine. This

study also assessed the feasibility, safety, and acceptability of TME. This study would fill the knowledge gap in MDA and serves to provide evidence for future scale up and policy implementation.

Community engagement

Participation of the community in MDA is one of the keys to success, and community engagement is an essential component in disease control by improving or facilitating participation [45]. A community is a complex group of people. Just because people share the same geographic areas does not mean they share the same interests or culture including perception, beliefs, knowledge and goals. Therefore, community and participation are defined differently depending on the cultural, structural concept [46]. The benefits of community engagement in malaria control and elimination are yet to be fully understood resulting from low engagement activities in MDA conducted over the last century which therefore cannot provide evidence base for effective community participation. The vertical health projects in many countries failed due to lack of financial support to conduct community engagement activities, communities being resistant to the programmes, and a poor health infrastructure leading to poor enthusiasm for participation [45]. A systematic literature review on community engagement and coverage in mass anti-malarial administration by Adhikari *et al*. shows that there were 51 MDA related-articles published in 26 countries between 1931 and 2011. Of those, 11 (22%) documented community engagement and population coverage, with the MDA-coverage being the highest when authorities and community were involved in community engagement activities [47]. In their review, the community engagement activities varied between studies from providing just information about the MDA and health education, to providing training to selected volunteers in the community. Of course, there is no universal model for effective community engagement [45]. In the areas where there is conflict of interest, the community engagement activities would fail if the community engagement programme fails to identify all stakeholders and the influential community members to get them involved in the activities. Similarly, in areas where malaria is not perceived as priority or risk by the community, community engagement with a multi-faceted, multi-level approach would be required as this perception might discourage and influence participation [48]. Actually, there are two main approaches that have been described in the literature. One of them is the vertical or top-down approach in which policy makers and professionals set the action plan and convince communities to actively participate in the intervention. The other is the horizontal or bottom-up approach that engages and supports communities in identifying and prioritizing their

own health concerns. Each has its disadvantages and advantages, but the combination of these two approaches is ideal for MDA implementation. While the former makes sure the intervention or implementation runs in a coordinated manner, the latter can guarantee long-term sustainability driven by the community [45]. The goal to eliminate malaria calls for strengthening health systems and reconsidering the use of mass drug administration to accelerate artemisinin resistant *P. falciparum*. Complete coverage of the population and 100% compliance to the treatment regimen are impossible to achieve but MDA is deemed potentially effective when participation coverage reaches at least 80% [49]. Therefore, the community should be sensitized in a clear manner, sensitization should start well before the start of the activity, and it should continue until the end the activity. Community engagement activities were also used to improve the uptake of MDA participation in the multi-country cluster-randomized trial in the Greater Mekong Subregion.

Aim and outline of this thesis

The aim of this thesis is to describe the impact of targeted malaria elimination with mass drug administration on *P. falciparum,* specifically in Laos, and its concomitant effect on *P. vivax* parasitaemia in Greater Mekong Subregion.

Main thesis research questions

The thesis aims to answer the following research question:

- What is the impact of MDA with dihydroartemisinin-piperaquine and single low dose primaquine on the prevalence and incidence of *P. falciparum*?
- What is the concomitant impact of MDA with the aim to eliminate *P. falciparum* on the prevalence and incidence of *P. vivax*?
- What is the effect of community engagement before and during the implementation of MDA on coverage and adherence, in a setting of low prevalence of malaria?

Scope and structure of this thesis

Chapter 1 – Introduction and outline of the thesis

Part I – The impact of mass drug administration on *P. falciparum* & *P. vivax*

Chapter 2 – The dynamic of asymptomatic *Plasmodium falciparum* infections following mass drug administrations with dihydroartemisinin-piperaquine plus a single low dose of primaquine in Savannakhet Province, Laos

Chapter 3 – Mass drug administrations with dihydroartemisinin-piperaquine and single low dose primaquine to eliminate *Plasmodium falciparum* have only a transient impact on *Plasmodium vivax*: findings from randomized controlled trials

Chapter 4 – The use of ultrasensitive quantitative-PCR to assess the impact of primaquine on asymptomatic relapse of *P. vivax* infections: a randomized, controlled trial in Lao PDR

Part II – Asymptomatic malaria

Chapter 5 – Asymptomatic *Plasmodium* infections in 18 villages of southern Savannakhet Province, Lao PDR (Laos)

Chapter 6 – Perceptions of asymptomatic malaria infection and their implications for malaria control and elimination in Laos

Part III – Community engagement

Chapter 7 – Factors associated with population coverage of targeted malaria elimination (TME) in Southern Savannakhet Province, Lao PDR

Part IV – Discussion of the main findings and perspectives

Chapter 8 – Discussion of the main findings and perspectives about how to eliminate all malarias.

Chapter 9 – Summary

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Part I-The impact of mass drug administration on *P. falciparum* & *P. vivax*

Chapter 2-The dynamic of asymptomatic *Plasmodium falciparum* infections following mass drug administrations with dihydroartemisinin-piperaquine plus a single low dose of primaquine in Savannakhet Province, Laos

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Malaria journal 2018, **17**(1):405.

Abstract

Background

The increase in multidrug resistant *Plasmodium falciparum* infections threatens the malaria elimination goals in countries within the Greater Mekong Sub-region. A multi-pronged approach assuring access to basic malaria control measures including insecticide treated bed nets and early diagnosis and treatment was followed by mass drug administrations (MDA) in southern Savannakhet Province, Laos. The main objective of this study was to evaluate the effectiveness and safety of mass drug administrations as well as their effects on the dynamic of asymptomatic *P. falciparum* infections in 4 malaria endemic villages.

Methods

Two villages were randomized to early MDA consisting of three rounds of a 3-day course of dihydroartemisinin - piperaquine with a single low dose of primaquine. In the other two villages MDA was deferred by one year. A total of 1,036 residents were enrolled in early MDA villages and 883 in control villages (deferred-MDA). Tri-monthly parasitaemia surveys using uPCR were conducted for a year in the four villages.

Results

Eighty-four percent (872/1,036) of the residents participated in the MDAs, of whom 90% (781/872) completed 3 rounds of MDA (9 doses). In intervention villages, the prevalence of asymptomatic *P. falciparum* infections decreased by 85% after MDA from 4.8% (95%CI: 3.4-6.4) at baseline (Month 0 or M0) to 0.7% (95%CI: 0.3-1.6) at Month 12. In control villages there was a decrease of 33% in *P. falciparum* prevalence between M0: 17.5% (95%CI: 15.9-20.3) and M12: 11.6% (95%CI: 9.3-14.2). In bi-variate and multivariate analyses *P. falciparum* infections were significantly reduced with early MDA (adjusted incidence rate ratios (AIRR): 0.08, CI: 0.01 to 0.091) and completion of 3 MDA rounds (AIRR: 0.06; CI: 0.01 to 0.66). A quarter of participants (226 /872) reported adverse events of which 99% were mild.

Conclusion

The study found a significant reduction in *P. falciparum* prevalence and incidence following MDA. MDA was safe, well tolerated, feasible, achieved high population coverage and adherence. MDAs must be integrated in multi-pronged approaches such as vector control and preventive measures with a focus on specific risk groups such as mobile, migrant population and forest goers for a sustained period to eliminate the remaining parasite reservoirs.

Keywords: Asymptomatic parasitaemia, *P. falciparum*, elimination, MDA, Savannakhet, Laos.

Trial Registration: ClinicalTrials.gov Identifier: NCT01872702

Background

The emergence and spread of resistance to artemisinin and its partner drugs in the Greater Mekong Sub-region (GMS) currently available to treat *Plasmodium falciparum,* is a threat to the control and elimination of malaria [1]. Failure to contain and eliminate multi-drug resistant malaria from the GMS could result in a public health disaster [2].

The National Malaria Control Program of Laos relies on routine case detection and treatment in peripheral health centres [3]. Microscopy is available only in central, regional, provincial and district level health facilities and rapid diagnostic tests (RDTs) are the diagnostic mainstay [3]. Polymerase Chain Reaction (PCR) is not used for the routine diagnosis of malaria [4]. Artemisinin Combination Therapy (ACT) with coforumlated Artemether+Lumefantrine (AL) was introduced as a pilot intervention for early diagnosis and treatment (EDAT) in 2005 and scaled-up gradually to cover all health facilities across the country in 2008 [5]. AL remains the first line treatment in Laos [6].

A recent study had identified artemisinin resistant *P. falciparum* strains in Phouvong District of Attapeu [1] and in two districts of Champasak Province, southern Laos [5, 6]. In response to reports of multi-drug resistant *P. falciparum* malaria, among the multi-pronged approaches for containment and elimination, Laos has been intensifying the distribution of long lasting insecticide treated net (LLITN) and indoor residual spraying (IRS) [7].

As a part of the national strategic plan for malaria control and elimination, Laos has adopted the goal to eliminate *P. falciparum* malaria by 2030 [4, 5, 8]. There is an urgent need to find effective interventions to rapidly reduce the *P. falciparum* malaria reservoirs from the country.

The main objective of this study was to evaluate the feasibility, safety, acceptability and impact of a pilot implementation of targeted malaria-elimination (TME) on the dynamics of asymptomatic *P. falciparum* infection in Laos. TME aims to eliminate *P. falciparum* parasites by mass drug administration (MDA) with dihydroartemisinin-piperaquine (DHA-PP) plus a single low dose of primaquine (SLPQ) in villages where basic malaria control measures (early diagnosis, appropriate treatment, and universal access to long-lasting, insecticide-treated bednets) have been established but transmission persists. The dynamic of submicroscopic *P. falciparum* parasitaemia before MDA and during the tri-monthly follow-up period over a 12 months period was studied in order to provide a better understanding and objective evidence on its impact on the submicroscopic parasite reservoir. The findings from this study are

expected to guide the national malaria control strategies in Laos. This article describes the impact of MDA on *P. falciparum* infections; a second report will describe the impact on *P. vivax* infections.

Methods

Study site and design

This was a cluster-randomised, open, controlled clinical trial conducted between April 2016 and May 2017 in Nong District in the southern Savannakhet Province of Laos. In 2014, five of the 15 districts of Savannakhet Province were classified as strata 3 (high risk) where the Annual Parasite Incidence (API) was above 10 per 1,000 people at risk and Nong was the district with the second highest API (15 per 1,000 people) in the province [6]. The study was part of a multicentre trial conducted in Myanmar, Vietnam, Cambodia and Laos to evaluate the impact of mass DHA/piperaquine administrations on *P. falciparum* [9].

Community engagement and study procedures

Initial steps in community engagement entailed comprehensive workshops with central, provincial and district level authorities to explain the purpose of the project and its procedures. These workshops were attended by representatives of the government health authorities including the Lao Women's Union, Lao Youth Organization, education and culture departments, village heads and elders [7, 10, 11]. In parallel with engagement workshops, prevalence surveys were conducted in 20 villages to determine the prevalence of malaria [4]. Based on uPCR surveys the 4 villages with the highest *P. falciparum* prevalence and enthusiasm were chosen for this study: Phoun Mak My (PMM; population: 480), Tha Thay (TT; population: 526), Xuang Tai (XT; population: 371) and Oi Tan Tip (OTP; population: 512) [4] **(Figure 1).** Two villages, PMM and TT received MDA in the first year (intervention villages), and the remaining two villages XT and OTP received deferred MDA in the second year (control villages). The intervention, early versus deferred MDA was allocated by restricted randomization within two pairs of villages matched for geographical proximity and parasite prevalence. In the control villages the coverage and the impact of the deferred MDA on *P. falciparum* infections as well as adverse events were not evaluated.

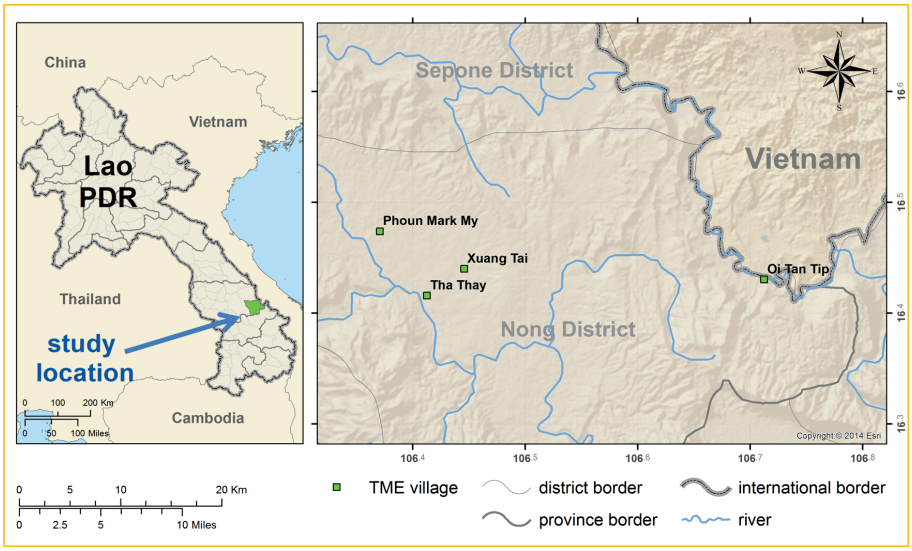

Figure 1: TME study sites in Savannakhet province of Laos

Intense community engagement activities were conducted in each village before and during the MDA as described in detail elsewhere [7, 10, 11]. All villagers who agreed to provide written informed consent (by parents or guardian in case of children) were invited to participate in the study except pregnant women, children aged less than 6 months, participants with a history of allergy or known contraindication to the study drugs and candidates who were in the opinion of the study clinician, too ill to participate. The baseline (M0) and tri-monthly follow-up surveys (M3, M6, M9 and M12) were conducted over a one-year period.

All the eligible participants were registered and provided with a unique study number. Sociodemographic characteristics of the participants and the recent clinical history were recorded in Open Data Kit (ODK®) application (www.opendatakit.org) using a smartphone. All participants were provided compensation for their travel expenses and loss of income consistent with the recommendations of the local ethical committee [10].

Mass Drug Administration

Following blood sampling each participant received (under direct observation) a full course of DHA-PP (7 mg/kg dihydroartemisinin and 55 mg/kg piperaquine) for 3 days plus a single low dose of PQ (0.25 mg/kg, given on day 1) on M0, M1, M2 [10]. A home visit was made by village volunteers together with TME doctors if participants developed an adverse event (AE)

following the MDA [10]. All adverse events or drug-related side effects were assessed, treated and documented.

Sample collection

During the quarterly blood surveys, a 3.0 mL blood sample was collected from participant age>5 years and 0.5 mL from children under 5 years [7, 10, 11]. The blood samples were collected in EDTA anticoagulated tubes and stored in ice packed cool boxes and transported to a centralized field laboratory in the district within 12 hours of blood collection. Upon return to the centralized laboratory, whole blood was separated and the red blood cell pellets were promptly frozen and stored at -30^0C for up to 7 days. Each sample was labelled with a barcode and negative controls were added to the sample pool. The samples were transported on dry ice to the molecular laboratory of the Mahidol Oxford Tropical Medicine Research Unit (MORU) in Bangkok, Thailand for uPCR analysis. All participants were diagnosed on sites for malaria using the SD Bioline Ag *P. falciparum*/Pan (Standard Diagnostics Inc.) rapid diagnostic test (RDT). Those with positive RDTs were treated with artemether/lumefantrine, as per the Lao national treatment guidelines. RDT tests were performed and interpreted by an experienced laboratory technician following the manufacturer's recommendation.

DNA extraction and PCR amplification

DNA was extracted from thawed packed red blood cells using an automated DNA extraction machine (QIAsymphony and DPS DNA midi kit; Qiagen, Germany). The DNA was dried, concentrated and then used as a template for PCR detection and quantification of *Plasmodium*. Quantitative PCR (uPCR) analysis was performed as described elsewhere [12]. Briefly, DNA of *Plasmodium* was detected and quantified using 18S rRNA-targeting primers. For *Plasmodium* positive samples, an attempt was made to identify the species using *P. falciparum* and *P. vivax* specific PCR primers as described [12].

Statistical analysis

The socio-demographic characteristics of the participants was described using frequency and percentage for the categorical variables while median and inter-quantile range (IQR) were used for the continuous variables except for parasite density which was analysed by using geometric mean and 95% confidence interval (CI). The Chi-squared test was used to test the statistical difference of normally distributed groups and the Wilcoxon rank-sum test to analyse was used

to test the statistical difference of the median or distribution between groups with skewed distribution.

The prevalence and 95% confidence intervals of *P. falciparum* infection for each 3-month interval was analysed using the number of people with *P. falciparum* or mixed infections as numerator and number of villagers for whom a uPCR results were available as denominator. The prevalence of *P. falciparum* infection at each time point after month 3 and over time was compared between early MDA and deferred-MDA villages using multilevel logistic regression models clustered by villages and adjusted for repeated observations.

The incidence rate (person-years) and 95% confidence intervals of *P. falciparum* infection for each 3-monthly period was estimated using the number of people found infected divided by their exposure time (person-year). The incidence rate for each time point after month 3 and over time was compared between early MDA and deferred-MDA villages using multilevel Poisson regression models clustered by villages and adjusted repeated observations.

The MDA coverage was estimated as the proportions of villagers who completed the MDA at month 0, 1, and 2 and for all 3 MDA rounds. Participation was categorized as “not received MDA”, “not completed MDA”, “completed 3 days MDA”, “taken any dose in 3 rounds”, and “completed all 3 rounds” using all villagers living at the time of the MDA in villages as denominator. The blood sampling coverage was estimated as number of people who gave blood sample in each 3-monthly survey with number of residents at the time of the survey in the early MDA and deferred-MDA villages.

A univariate analysis was performed to obtain the unadjusted estimates of incidence rate ratios (IRR) of infections and MDA status as well as associations with each of the baseline variables including sex, age, fever, bed net use and season. A multivariable analysis was used to obtain the adjusted estimates. The impact of the MDA was examined using a multilevel mixed effect Poisson model to obtain the IRR of *P. falciparum* infection with 95% confidence interval. Multilevel mixed effect modelling allowed adjusting for village, villager and repeated measurements specific random effects. In the multilevel model, level 1 was repeated measurements of villagers over time, level 2 was villagers, and level 3 was village. To assess the impact of the treatment and prophylactic effect of the antimalarial drugs a secondary analysis was conducted in which the first 3 months of surveillance in the intervention villages was omitted.

A p-value <0.05 was considered statistically significant. All analyses were performed using Stata, version 14 (StataCorp LLC, College Station, Texas). The seasonal variation in *P. falciparum* infections at each time point of both MDA and non-MDA villages was described using monthly rainfall data of Nong district provided by the Savannakhet Meteorological office.

Ethical consideration

The study protocol was approved by the National Ethic Committee for Health Research (013NIOPH/NECHR. 15th February, 2016), Ministry of Health, Laos, the Oxford Tropical Research Ethics Committee (OXTREC-1017-13. 9th April, 2015), and Ethical Committee of the Faculty of Tropical Medicine, Mahidol University (TMCD 00754. 8th Dec, 2016).

Results

Baseline characteristics

A total of 2,021 participants were registered and participated in the baseline survey in four selected villages, of which 52% (1060/2021) were from intervention (early MDA) villages and 48% (961/2021) from control (deferred MDA) villages **(Figure 2)**. The majority of participants in both early MDA (80%; 871/1036) and deferred-MDA villages (84%; 810/883) (p=0.046) reported that they had worked in the forest during the last three months. More than half of the population used a bed net in both early MDA (56%; 498/887) and deferred-MDA villages (62%; 506/811; p=0.030) villages **(Table 1)**.

Compliance with blood sampling, follow-up, and MDA coverage

The overall percentage of the villagers who participated in blood sampling at least once was 88% (948/1,073) in early MDA villages and 93% (875/936) in deferred-MDA (p<0.001). Among the 1,073 villagers, 36 (3.3%) were not eligible for the mass drug administration. The percentage of the participants who took at least one dose of MDA was 84% (872/1,036) and 90% (781/872) took the full nine doses in all three rounds in the early MDA villages. The percentage of the participants who had completed 3 days was 79.3% (822/1,036) at M0, 79.2% (821/1,036) at M1 and 81.3% (843/1,036) at M3**.** The percentage of the participants who had not completed any MDA was 2% (20/1,036) at M0, 1% (10/1,036) at M3 and 0.6% (6/1,036) at M3 **(Table 2).**

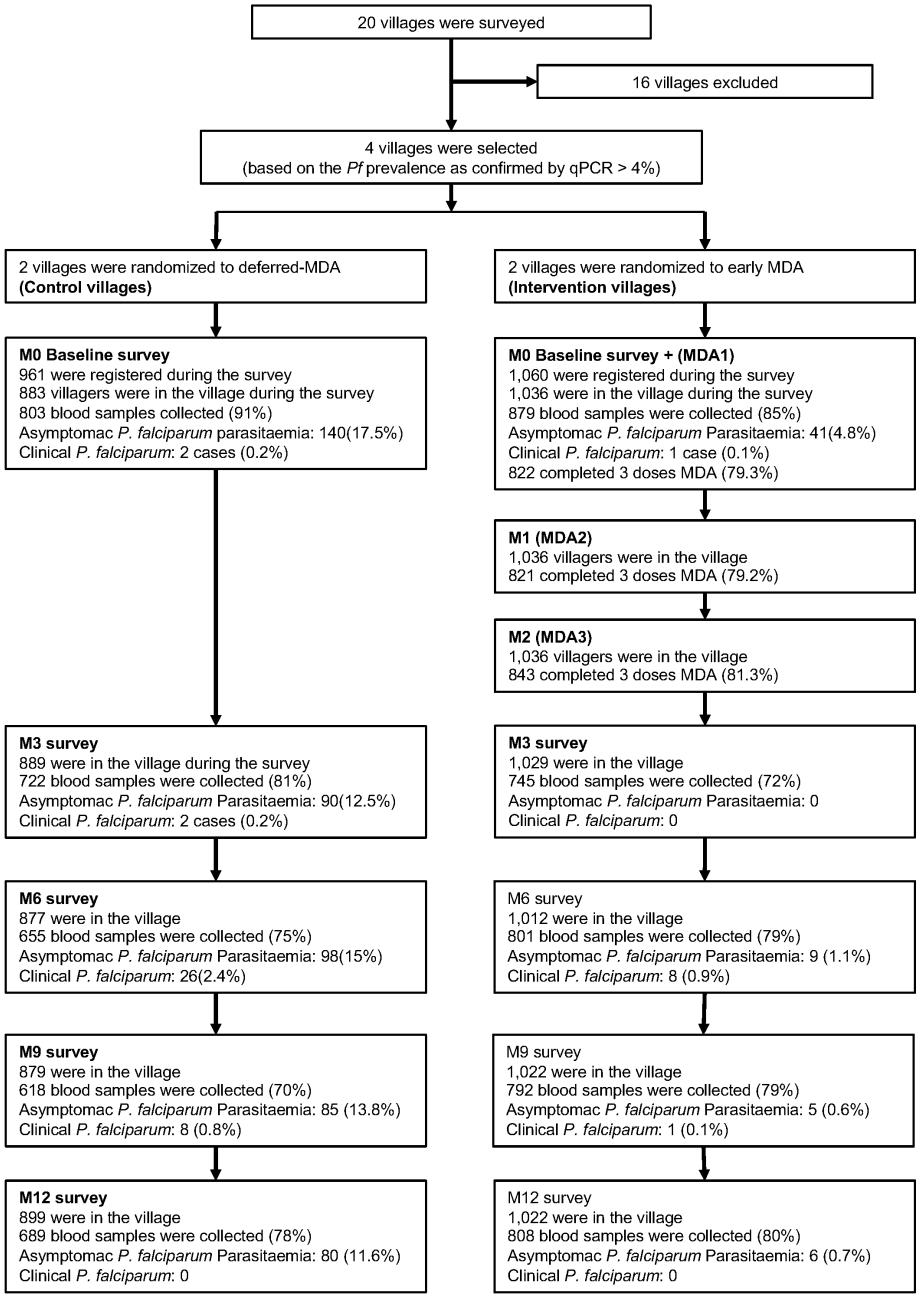

Fig. 2 Study overview

Table 1. Baseline characteristics and socio-demographic of the study participants.

Characteristics	Early MDA-Intervention Villages (N=1,060)	Deferred-MDA Control Villages (N=961)	p-value*
Gender, n (%)			0.981
Male	537 (51)	462 (51)	
Female	523 (49)	499 (49)	
Age, median (IQR)	17 (8-32)	15 (6-30)	<0.001
Age Group (years), n (%)			0.644
<10	345 (33)	319 (35)	
10 - 19	241 (23)	204 (22)	
20 - 39	289 (27)	243 (27)	
40 or More	185 (17)	145 (16)	
Occupation, n (%)			0.005
Farmer	579 (55)	461 (51)	
Child	198(19)	231 (25)	
Student	231 (22)	181 (20)	
Others	52 (5)	38 (4)	
Type of resident, n (%)			0.783
Permanent	1,042 (98)	898 (99)	
Temporary	18 (2)	13 (1)	
Villagers in village during the survey, n (%)	**1,036(98)**	**883(97)**	
Stay overnight in forest, n (%)	**(n=871)**	**(n=810)**	0.046
No	766 (88)	732 (90)	
Yes, < 2 weeks ago	78 (9)	67 (8)	
Yes, >=2 weeks	27 (3)	11 (1)	
Weight (kg) median(IQR)	**(n=888)**	**(n=811)**	0.075
	40(19-49)	38(18-46)	
Height (cm), median (IQR)	**(n=886)**	**(n=811)**	<0.001
	144 (118-153)	143 (115-152)	
Temperature (c), median (IQR)	**(n=888)**	**(n=811)**	<0.001
	36.9 (36.7-37.1)	36.9 (36.7-37.0)	
Fever on registration date, (T>=37.5C°)	43(5)	27(3)	0.117
Fever**	120 (14)	146 (18)	0.011
Bed net use, n (%)	**(n=887)**	**(n=811)**	0.030
Regular	498 (56)	506 (62)	
Irregular	322 (36)	249 (31)	
Never use	67 (8)	56 (7)	
Asymptomatic Pf prevalence, n (%)	**(n=859)**	**(n=802)**	<0.001
	41 (4.8)	140 (17.4)	
Pf Clinical cases, n (%)	1 (0.1%)	2 (0.2%)	
Pf density*, geometric mean (95% CI)**	10,864 (1,676-20,795)	61,426 (42,542-88,693)	<0.001

*N (%) Tested using Chi-square test or Fisher's exact test (f)

Median (IQR) tested using Wilcoxon rank-sum test

**History of fever combined with Temp>=37.5

*** test in log scale using Student's test

The effects of MDA on the prevalence and incidence of asymptomatic *P. falciparum* in the early MDA villages as confirmed by uPCR

In early MDA villages, the prevalence (95% CI) of asymptomatic *P. falciparum* parasitaemia at baseline (M0) was 4.8% (3.4-6.4; **Figure 3**). The prevalence of *P. falciparum* infections during tri-monthly follow-up was 0% (0- 0.5) at M3, 1.1% (0.5-2.1) at M6, 0.6% (0.2-1.5) at M9 and 0.7% (0.3-1.6) at M12. In deferred-MDA villages, *P. falciparum* prevalence was 17.5% (15.9-20.3) at baseline. The prevalence of *P. falciparum* parasitaemia was significant declining overtime in early MDA villages (AOR=0.82; CI: 0.74-0.86, p<0.001) but this was not significant in deferred-MDA villages (AOR: 0.97; CI: 0.94-1.01, p=0.101) (**Table 3**).

The incidence rate (95% CI) of *P. falciparum* infections per 1000 person-years in early MDA village was 0/1000 (0-20) at M3, increased to 46/1000 (21-87) at M6, 26/1000 (8-61) at M9 and remained 30 (11-66) at M12 (**Figure 4**). The incidence rate in the deferred villages was 510/1000 (410-627) at M3, 617/1000 (501-752) at M6, 563/1000 (450-696) at M9 and 478/1000 (379-595) at M12 (AIRR: 0.08; CI: 0.01-0.88, p=0.039; **Table 3** and **Figure 4**).

The incidence rate was significantly lower in people who had completed 3 MDA rounds than in people who had completed less than three rounds of MDA (AIRR: 0.06, 95% CI 0.01 to 1.70; p=0.022) (**Table 4**). If the infections that occurred during the first three months of surveillance in the intervention villages is omitted the AIRR is 0.11 (95% CI 0.01 to 1.16).

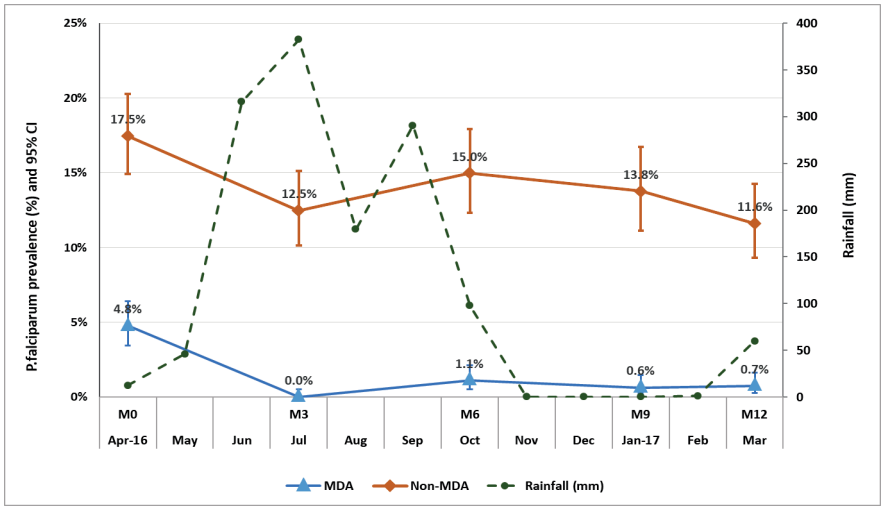

Figure 3. Seasonal variation of asymptomatic *P. falciparum* parasitaemia prevalence during tri-monthly follow-up survey in MDA and non-MDA villages.

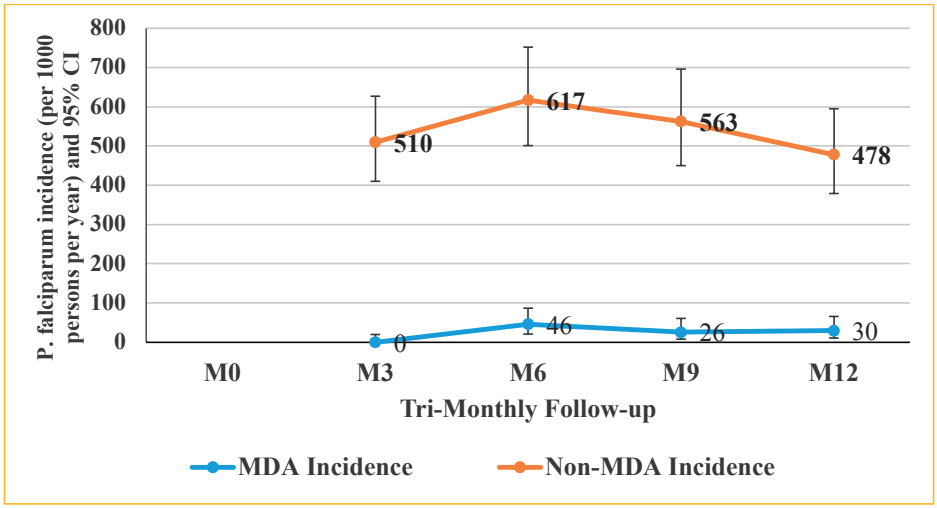

Figure 4. Comparison of asymptomatic *P. falciparum* parasitaemia incidence per 1000 person-years between intervention villages (MDA) and control villages (non-MDA) at tri-monthly basis during a period of one-year.

Note: The yellow line with triangle marker is the tri-monthly mean of asymptomatic *P. falciparum* parasitaemia Incidence (per 1000 person-years) 95% CI in non-MDA village. The blue line with triangle maker is the tri-monthly mean of asymptomatic *P. falciparum* parasitaemia Incidence 95% CI in MDA village.

The effects of MDA on the prevalence and incidence of clinical *P. falciparum* malaria as detected by RDT

Over the 12 months surveillance period 10 falciparum malaria cases, defined as fever and confirmed infection were diagnosed in early MDA villages and 38 in villages with deferred MDA (**Table 5**). The prevalence (95% CI) of falciparum malaria cases was 0.0% (0.0-0.4) at M3, 0.9% (0.4-1.7) at M6, 0.1% (0.0-0.6) at M9 and zero percent at M12 in the early MDA village. In the deferred-MDA village, the falciparum malaria prevalence was 0.2% (0.2-0.7) at M3, 2.4% (6-3.5) at M6, 0.8% (0.3-1.5) at M9 and zero percent at M12. The difference in falciparum malaria prevalence between early MDA and deferred-MDA villages was statistically significant at M6 and M9 (p=0.008 and p=0.035, respectively). The incidence per 1000 person-years of clinical *P. falciparum* malaria at M6 and M9 was significantly lower in the early MDA than in the deferred -MDA villages (p=0.011 and p=0.037, respectively) **(Table 5)**.

Seasonal variability of the prevalence of asymptomatic *P. falciparum* parasitaemia

The prevalence of asymptomatic *P. falciparum* parasitaemia increased in April (M0) (17.5% in deferred MDA and 4-8% in early MDA villages) and in October (M6) (15% in deferred MDA and 1.1% in early MDA villages). The falciparum prevalence increased in deferred MDA villages following the peak rainfall in July 2016 and slightly decreased during the dry season from November to February (**Figure 3)**.

Recurrent and persistent *P. falciparum* infections

The proportion of participants who had *P. falciparum* infections at M0 and were cured during follow-up at M3, M6, M9 and M12 was higher in early MDA village (40/41; 98%) than in deferred MDA village (39/140; 28%; p <0.001). In deferred MDA villages 26/140 (18.5%) participants were found to be infected with *P. falciparum* once, 32/140 (22.8%) twice, 22/140 (15.7%) three times and 21/140 (15%) four times. In the early MDA villages only one (2.5%) participant was infected with *P. falciparum* three times during tri-monthly follow-up (**Table 6**).

Adverse events

Adverse events were treated and followed-up in early MDA villages. 282 of 872 (32%) reported 295 adverse events following participation in the MDAs. The most frequent complaints were common cold (17.3%; 51/295), gastritis (7.5%; 22/295), diarrhoea (7.5%; 22/295), vomiting (6.8%; 20/295), dizziness (6.1%; 18/295), pruritus (6.1%; 18/295), watery stool (4.1%; 12/295), nausea (2.7%; 8/295), headache (2.7%; 8/295) and others (38%; 112/295). Most adverse events (98.6%; 291/295) were mild, 3 (1.0%) adverse events were moderate and 1(0.3%) adverse event was severe, a case of pneumonia was considered severe and required hospitalization **(Table 7).**

Table 2. Compliance of blood sampling, follow-up and MDA coverages.

Characteristics	Baseline	Follow-up time						Overall[b]
	M0 (MDA1)	M1 (MDA2)	M2 (MDA3)	M3	M6	M9	M12	
Number of villagers in village during the survey[a]								
Intervention village (early MDA)	1,036	1,036	1,036	1,029	1,012	1,022	1,022	
Control village (deferred MDA)	883			889	877	879	899	
Blood sampling coverage, n(%)								
Intervention village (early MDA)	879(85)			745(72)	801(79)	792(77)	808(79)	948(88%)
Control village (deferred MDA)	803 (91)			722(81)	655(75)	618(70)	689(77)	875(93%)
P-value								<0.001
MDA coverage in intervention village, n (%)								
Not received MDA	194(19)	205(19.8)	187(18.0)					
Not completed MDA	20 (2)	10(1)	6(0.6)					
Completed 3 days MDA	822 (79.3)	821(79.2)	843(81.3)					
People who took any dose in 3 rounds, n (%)			872(84)					
Completed 3 rounds (9 doses)			781(90)					

[a] Excluded people away; [b] At least participated once

Table 3. Prevalence and incidence of asymptomatic *P. falciparum* parasitaemia at baseline and during tri-monthly follow-up

Variables	Village Status	Baseline	Follow-up time				Over follow-up time
		M0	M3	M6	M9	M12	
Number of villagers*	Early MDA	859	745	801	792	808	
	Deferred-MDA		722	655	618	689	
Number of Pf infections	Early MDA	41	0	9	5	6	
	Deferred-MDA	140	90	98	85	80	
Pf prevalence, % and (95% CI)	Early MDA*	4.8(3.4-6.4)	0(0-0.5)	1.1(0.5-2.1)	0.6(0.2-1.5)	0.7(0.3-1.6)	
	Deferred-MDA	17.5(15.9-20.)	12.5(10.1-15.1)	15(12.3-17.9)	13.8(11.1-16.7)	11.6(9.3-14.2)	
**Comparison between group, AOR (95% CI, p-value) **				0.18 (0.01-3.38, 0.254)	0.07 (0.01-1.26, 0.072)	0.13(0.01-2.37, 0.170)	0.42(0.01-12.28, 0.611)
Pf exposure time (person-years)	Early MDA	N/A	183	196	192	198	
	Deferred-MDA	N/A	176	159	151	167	
Incidence of Pf infection (per 1000 person-years)	Early MDA	N/A	0(0-20)	46(21-87)	26(8-61)	30(11-66)	
	Deferred-MDA	N/A	510(410-627)	617(501-752)	563(450-696)	478(379-595)	
Comparison between group, AIRR(95% CI, P-value)**				0.21(0.01-3.08, 0.254)	0.09(0.01-1.24, 0.071)	0.15(0.01-2.24, 0.169)	0.08(0.01-0.88, 0.039)

*Significant declining over time in early MDA group, (AOR=0.82, 95% CI 0.74-0.86, p-value<0.001) but this was not significant in Non-MDA group, (AOR = 0.97, 95%CI 0.94-1.01, p-value=0.101).

**Adjusted for Pf prevalence at baseline (village level) and cluster (village) effect.

Table 4. Multilevel mixed-effects Poisson regression on *P. falciparum* infections in early MDA village and compared with deferred-MDA village during tri-monthly follow-up after MDA.

Characteristics	IRR (95%CI)	P-value	Model 1: MDA intervention Adjusted IRR (95%CI)*	P-value	Model 2: MDA coverage Adjusted IRR (95%CI)*	p-value
Intervention						
MDA	0.08(0.01-0.88)	0.039	0.08(0.01-0.91	0.042		
Non-MDA	Reference		Reference			
Coverage						
DA completed 3 rounds	0.06(0.01-0.64)	0.020			0.06(0.01-0.066)	0.022
DA completed 1 and 2 rounds	0.11(0.01-1.74)	0.118			0.11(0.01-1.70)	0.114
MDA not completed in a single round/No MDA	0.50(0.04-6.27)	0.591			0.52(0.04-6.47)	0.611
Non-MDA	Reference				Reference	
Gender						
Male	1.00(0.72-1.39)	0.990	1.01(0.73-1.39)	0.963	1.03(0.74-1.41)	0.878
Female	Reference		Reference		Reference	
Age in year	0.99(0.98-1.00)	0.088	0.99(0.98-1.00)	0.087	0.099(0.98-1.00)	0.089
Fever	0.91(0.50-1.67)	0.759				
Bed net use						
Regular	Reference					
Irregular	1.07(0.80-1.43)	0.646				
Never use	1.10(0.58-2.07)	0.775				
Season						
Wet	1.04(0.84-1.28)	0.725	1.04(0.84-1.28)	0.724	1.05 (0.85-1.29)	0.667
Dry	Reference		Reference		Reference	

*Adjusted for gender, age, fever and season,
IRR=Incidence Rate Ratio

Table 5. Prevalence and incidence of symptomatic *P. falciparum* in early MDA and deferred-MDA villages during tri-monthly follow-up.

Variables	Village Status	Baseline	Follow-up time			
		M0	M3	M6	M9	M12
Number of villagers*	Early MDA	883	889	877	879	899
	Deferred-MDA	1,036	1,029	1,071	1,022	1,022
Number of Pf Clinical Case**	Early MDA	1	0	8	1	0
	Deferred-MDA	2	2	26	8	0
Prevalence of Pf Clinical case 95% CI	Early MDA		0.0(0.0-0.4)	0.9(0.4-1.7)	0.1(0.0-0.6)	0
	Deferred-MDA		0.2 (0.2-0.7)	2.4(1.6-3.5)	0.8(0.3-1.5)	0
P-value			0.192	0.008	0.035	
PF exposure time (person-year)	Early MDA	N/A	222	219	220	220
	Deferred-MDA	N/A	257	268	256	205
Incidence of clinical Pf infection 95% CI (per 1000 person-years)	Early MDA	N/A	0(0-6.6)	36(16-72)	4.6(0.1-25.3)	0
	Deferred-MDA	N/A	8(0.9-28.1)	97(63-142)	31(13.5-61.6)	0
P-value			0.288	0.011	0.037	

* number of villagers who have RDT result

** Confirmed by RDT

Table 6. Recurrent and persistent of *P. falciparum* infections during tri-monthly follow-up (M3, M6, M9 and M12) in early and deferred MDA villages.

Village Status	Number of *P. falciparum* infections at M0	Number of *P. falciparum* infections during all follow-up time points (M3, M6, M9 and M12)				
		Uninfected	Infection 1 time	Infection 2 times	Infection 3 times	Infection 4 times
Deferred MDA village, n (%)	140	39(27.8)	26(18.5)	32(22.8)	22(15.7)	21(15.0)
Early MDA village, n (%)	41	40(97.5)	0	0	1(2.5)	0
p-value*		p<0.001				
Total	181	79(43.6)	26(32.0)	32(17.6)	23(12.7)	21(11.6)

* using z test or Chi-square test.

Table 7. Type of adverse events following mass administrations of dihydroartemisinin-piperaquine plus single low dose of primaquine

Type of adverse events	Number of people with events n= 282	Number of events n= 295	% of event
Common cold	50	51	17.3
Gastritis	22	22	7.5
Diarrhea	22	22	7.5
Vomiting	20	20	6.8
Dizziness	18	18	6.1
Pruritus	16	18	6.1
Watery stool	12	12	4.1
Nausea	8	8	2.7
Headache	8	8	2.7
Cough	4	4	1.4
Others	102	112	38.0

Discussion

The emergence of artemisinin and partner drug resistance leaves few treatment options for countries in the Greater Mekong Sub-region, including Laos [1, 2, 13, 14]. This is a first study in Laos evaluating the impact, feasibility, safety and effectiveness of a MDA consisting of DHA-PP and single low dose primaquine as a potential tool to eliminate *P. falciparum* parasitaemia.

For a MDA to be successful, a high population coverage is essential and mathematical modellers have suggested that at least 80% population coverage is required for MDA to interrupt the local malaria transmission [15-17]. In early MDA villages alone 84% (872/1,036) of the residents participated in the MDA and of those who participated 90% (781/872) completed all three rounds (9 doses). Achieving such a high coverage required concerted action of all involved including intensive community engagement, provision of ancillary care, monetary and non-monetary incentives and the factors embedded in local social and cultural context such as cohesive nature of the communities and decision making dynamics within the households [7, 10, 11].

Plasmodium infections were actively monitored every 3 months for a year in both intervention villages and control villages. Consistent with observations in Myanmar and Cambodia the incidence and prevalence of *P. falciparum* infections was suppressed in villages after the early MDAs [13, 18]. In Laos the effect of MDAs persisted throughout the follow up period.

Future roll out of MDA in Laos may be able to interrupt the transmission of *Plasmodium falciparum* if high coverage can be assured and basic malaria control measures such as access to LLIN and early diagnosis and treatment is assured. A recent MDA in Zambia employed only 2 rounds of DHA-PP and showed a substantial albeit temporary impact on malaria prevalence, cumulative infection incidence and confirmed case incidence rates over a 5 months follow-up period [19]. A scale-up of targeted malaria elimination which includes the basic malaria control measures combined with MDA in hotspots has recently been successfully implemented in Thai-Myanmar border areas [20]. MDA in the real world of malaria control programmes may become more feasible and acceptable as neither blood sampling nor follow up are required.

Two systematic reviews of MDAs suggest that MDA interrupts the malaria transmission temporarily followed by a rebound in malaria prevalence (but not to the level of pre-intervention). In isolated geographical locations such as islands, where introduction of new

malaria cases are low due to minimal or no migration the impact of MDAs is longer lasting [16, 17]. So far, only one study in Vanuatu Island has shown permanent interruption of malaria transmission following MDAs [21, 22].

In general, MDA was well tolerated and safe. Sub-studies accompanying this study reported that adverse events were conflated in villagers' perception with pre-existing illnesses. Community engagement that accompanied this study provided free health care in these villages which could mean that villagers felt more encouraged to attend the health centres and the services provided owing to the fact that economical barriers were removed [7, 10, 11]. In a scale up programme in Thai-Myanmar border areas which includes MDAs, monitoring the adverse events of anti-malarials was critical in the successful implementation of malaria posts and MDAs [20]. Future studies could benefit from exploring factors related to the perception of adverse events and ways to mitigate the negative effect of adverse events on perception. In the current study, adverse events triggered deployment of additional doctors (who stayed in the villages until the study finished) and health centre staff who were mobilized to provide health care [7, 10, 11].

Strengths and limitations

This study randomised only 4 villages into 2 arms with 2 villages in each arm. Despite the limited statistical power, the current study was able to show meaningful differences between study arms. A large multi-centre study of which the current study was part of, has just been completed in the GMS and will provide more robust evidence for the effectiveness of the intervention. The current study could have benefitted by incorporating detailed entomological observations to test the impact in terms of the human biting rate, sporozoite index, and entomological inoculation rate of MDA on vectors [20]. Future implementation of MDA in malaria control programmes will require detailed cost efficacy analyses to guide policy makers. In the current pilot study, the implementation team and the government authorities invested considerable resources including intensive community engagement to achieve high coverage, not only in the participation in MDAs but also in the intensive monitoring and evaluation of the intervention. In scaling up of MDAs, intense community engagement with allocation of incentives for participation may neither be possible, nor required but could ultimately affect participation. The current project was initiated by researchers and relied on the enthusiasm of the villagers. Different forces, e.g. conformity may play a more important role once the

programme is part of a national malaria elimination programme. Scaling up the programme without frequent blood collections, may also increase the community participation.

Conclusions

In a remote, malaria endemic region of Savannakhet Province, Laos, MDA consisting of three rounds DHA-piperaquine with a single low dose of primaquine at monthly intervals was found safe, well tolerated and feasible when accompanied by intensive community engagement. Following the intervention MDA, sub-microscopic malaria prevalence was significantly reduced in intervention villages compared to control villages. A high population coverage with adherence to all 3 rounds was critical for this success. Achieving high population coverage in remote communities requires accompanying community engagement and therefore, for future roll out, an adequate community engagement strategy entailing community collaboration and sharing of responsibility with community members is critical. This study shows significant reduction in *P. falciparum* prevalence and incidence following MDA. MDA can become an effective intervention tool for malaria elimination in Savannakhet Province and potentially other parts of Laos and the GMS. Interruption of transmission and thus malaria elimination can only be achieved if the re-importation of malaria can be prevented. Future studies should integrate MDAs with multi-pronged approaches such as vector control (e.g. ivermectin) and preventive measures (e.g. antimalarial vaccines) with a focus on high risk groups such as mobile populations and forest goers.

DECLARATIONS

Authors' contribution

Concept and design of the study: NJW, AMD, LvS, MM, SP and TP. Field work: KP, BA, GH and TP. Laboratory work: MI, KP and GH. Data curation and analysis: TP, MMu, PP and LvS. Drafting of the manuscript: TP, BA, MM and LvS. Overall supervision: AMD, NJW, NPJD, PNN, LvS, MM, SP, KC, BH, PS and TP. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The data is available upon request to the Mahidol Oxford Tropical Medicine Research Unit Data Access Committee (http://www.tropmedres.ac/data-sharing) complying with the data access policy (http://www.tropmedres.ac/_asset/file/data-sharing-policy-v1-0.pdf)

Consent for publication

Not applicable

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Chapter 3-Mass drug administrations with Dihydroartemisinin-piperaquine and single low dose primaquine to eliminate *Plasmodium falciparum* have only a transient impact on *Plasmodium vivax*: Findings from randomized controlled trials

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Abstract

Background

Mass administrations of antimalarial drugs (MDA) have reduced the incidence and prevalence of *P. falciparum* infections in a trial in the Greater Mekong Subregion. Here we assess the impact of the MDA on *P. vivax* infections.

Methods

Between May 2013 and July 2017, four villages in each Myanmar, Vietnam, Cambodia and Lao PDR were selected based on high prevalence of *P. falciparum* infection. Eight of the 16 villages were randomly assigned to receive MDA. The MDA consisted of three-monthly rounds of three-day treatment courses of dihydroartemisinin-piperaquine and, except in Cambodia, a single low-dose of primaquine. Cross-sectional surveys were conducted at quarterly intervals to detect Plasmodium infections using ultrasensitive qPCR. The difference in the cumulative incidence between the groups was assessed through a discrete time survival approach, the difference in prevalence through a difference-in-difference analysis, the difference in the number of participants with a recurrence of *P. vivax* infection through a mixed-effect logistic regression.

Results

3,790 (86%) residents in the intervention villages participated in at least one MDA round, of whom 2,520 (57%) participated in three rounds. The prevalence of *P. vivax* infections fell from 9.31% to 0.89% at month 3 but rebounded by six months to 5.81%. There was no evidence that the intervention reduced the cumulative incidence of *P. vivax* infection (95% confidence interval [CI] Odds ratio (OR): 0.29 to 1.36), despite a reduction of 37% (OR: 0.63). Similarly, there was no evidence of MDA related reduction in the number of participants with at least one recurrent infection (OR: 0.34; 95% CI: 0.08 to 1.42).

Conclusion

MDA with schizontocidal drugs had a lasting effect in reducing *P. falciparum* infections but only a transient effect on the prevalence of *P. vivax* infections. Provision of radical cure with an 8-aminoquinoline will be needed for the rapid elimination of vivax malaria.

Keywords: P. vivax, dihydroartemisinin, piperaquine, primaquine, mass drug administration

Introduction

Malaria transmission has fallen in the Greater Mekong Subregion (GMS) since the early 2000s. *P. falciparum* infections have declined at a faster rate than *P. vivax* infections resulting in the current dominance of *P. vivax* in most parts of the GMS [1]. This trend has stalled over the last years coinciding with the emergence and spread of multidrug-resistant *P. falciparum* [1]. The spread of resistance is cause for international concern, as the spread of multidrug-resistant *P. falciparum* from Asia to sub-Saharan Africa, has happened previously with devastating public health consequences. [2]. In areas of low malaria transmission, mass antimalarial drug administrations (MDA) have the potential to accelerate the elimination of malaria. The MDA described here resulted in a substantial reduction of *P. falciparum* infections over a 12-month period [3].

P. vivax, in contrast to *P. falciparum*, has persistent liver stages (hypnozoites) which cause relapse. The relapse pattern varies geographically and in southeast Asia, *P. vivax* relapses frequently with short inter-relapse intervals [4]. Repeated relapses impact adversely on health and quality of life. Multiple vivax relapses are a particular burden in children affecting growth, development and education [5]. Clearing hypnozoites requires a radical cure with a course of 8-aminoquinolines, but this class of drugs is underutilized related to the risk of haemolysis in individuals with glucose-6 phosphate-dehydrogenase (G6PD) deficiency.

Here we report a post hoc analysis of the concomitant effect of an MDA with dihydroartemisinin-piperaquine (DP) plus a single low dose primaquine (SLDPQ) on the prevalence, incidence, and frequency of recurrence of *P. vivax* infections in the Greater Mekong Region. SLDPQ is a potent *P. falciparum* gametocytocide but has no measurable activity against *P. vivax* hypnozoites nor does it cause clinically relevant haemolysis in G6PD deficient people.

Methodology

Trial design

Pre-trial *Plasmodium* prevalence surveys using ultrasensitive PCR (uPCR) in 62 villages in the four countries identified the villages with the highest *P. falciparum* prevalence. In each country, four villages with high *P. falciparum* prevalence and all-year access were included in the study. Village pairs of similar size, access and *P. falciparum* prevalence were randomly

allocated to intervention or control using a computer-generated random sequence, except in Myanmar where the drug allocation was decided by flipping a coin. Participants were enrolled by specially trained field staff with the help of local health workers. Participants and field staff were not blinded to the treatment allocation. However, laboratory personnel, who performed the uPCR off-site, did not know which samples were from the intervention or control villages, thereby reducing potential bias.

Study site

The study was conducted in rural Eastern Kayin (Karen) State of Myanmar; in two provinces of Vietnam (Binh Phuoc and Ninh Thuan provinces: the former is in the Southeast region of the country and shares a border with Cambodia, while the latter embraces the South-Central Coast of Vietnam); in Samlout District of Battambang province, Western Cambodia which shares a border with Thailand), and in Nong Districts of Savannakhet province, southern Lao PDR which shares a border with Vietnam. The location of the study sites is shown in **Fig.** 1. Detailed descriptions of the study sites have been published recently [6-9].

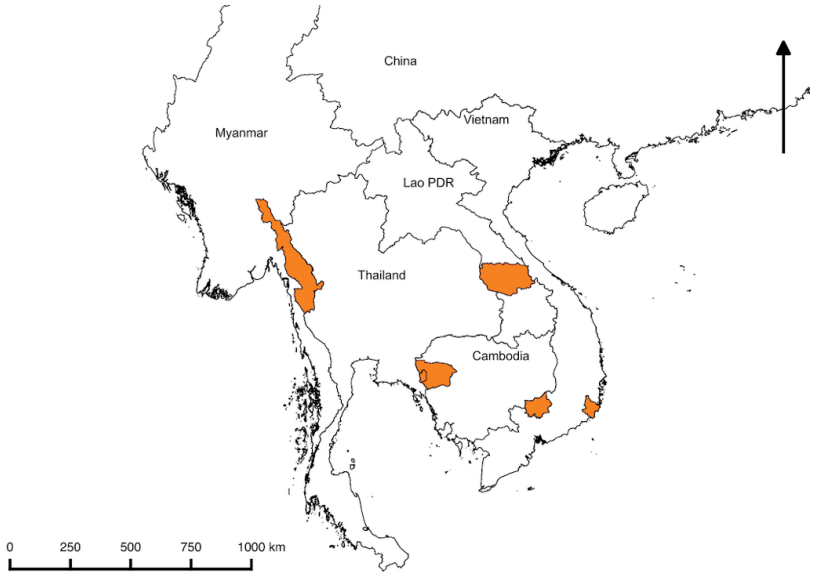

Figure 1. Map of the Greater Mekong Subregion. the areas highlighted in orange are study sites: Kayin (Karen) state, Myanmar; Battambang province, Cambodia; Savannakhet Province, Lao PDR; Binh Phuoc and Ninh Thuan province, Vietnam.

Participants

Every person in the selected villages was eligible to be included in the surveys if they gave informed consent, were older than 6 months, were not ill at the time of the survey, and had no known allergy to the study drugs. All pregnant women were excluded from the MDA in Cambodia and Lao PDR, while in Myanmar and Vietnam only pregnant women in first trimester were excluded. Breastfeeding women were invited to participate but did not receive SLDPQ. Participants who experienced serious adverse events potentially related to study drugs or could not tolerate the study drugs were withdrawn from the next MDA round but remained in the study.

Intervention

The intervention consisted of three rounds of DP on three consecutive days. One SLDPQ which clears *P. falciparum* gametocytes but has no effect on hypnozoites was added to each round of antimalarials. The treatment regimen was given at the start and then one and two months after start of the study. SLDPQ was not administered in Cambodia for regulatory reasons. A weight-based regimen containing a total dose of approximately 7 mg/kg dihydroartemisinin and 55 mg/kg piperaquine phosphate was used (Eurartesim®, Sigma Tau, Italy or D-Artepp®, Guilin Pharmaceutical Co, Guilin, People's Republic of China), while 0.25 mg base/kg PQ was given as a single dose (manufactured by the Government Pharmaceutical Organization, Thailand). The intervention was extended to the initial control villages after 12 months of follow-up, except in Myanmar where the control villages received the intervention after nine months for operational reasons (difficult access during rainy season).

Data collection and laboratory methods

The study started in Myanmar in May 2013, followed by Vietnam in November 2013, Cambodia in July 2015 and Lao PDR in April 2016. Data were collected through community-wide surveys at baseline, and then every three months. Surveys were preceded by a community census to re-enumerate the study population and to provide intensive community engagement in order to improve high coverage [10-14]. Information collected included demographics, bednet use, duration of stay in the forest, and adverse events. Malaria prevalence at each survey was assessed by quantitative uPCR. 3 mL of blood was taken from participants older than 4 years old, and 0.5 mL from younger children. The blood samples were stored in cool boxes in the field for about 5-6 hours before being separated in the local laboratories and then kept in -

20°C freezers. Samples from Myanmar, Cambodia, and Lao PDR were shipped on dried ice to the Mahidol-Oxford Research Unit (MORU) in Bangkok, Thailand for uPCR while samples collected in Vietnam were sent to the Oxford University Clinical Research Unit (OUCRU) in Ho Chi Minh City, Vietnam. DNA was extracted from thawed packed red blood cells using automated DNA extraction, dried in a centrifugal vacuum concentrator, and used as template for PCR detection and quantification of Plasmodium. The methodology and performance characteristics of the quantitative ultrasensitive PCR (uPCR) have been described previously [15].

Sample size

The sample size described here was for assessing the impact of MDA on the *P. falciparum* elimination. Four-village clusters per country, was chosen mainly for operational and practical reasons. It is acknowledged that although the intra-cluster correlation coefficients (ICCs) were generally very low in this region, there remains a problem of generalisability of findings when such few numbers of clusters are used [16] . A formal sample size calculation suggested that 16 villages with a minimum of at least 152 individuals in each village recruited and followed-up would provide 80% power to detect a 95% fall in prevalence from a 10% initial prevalence, independently controlled by country. This calculation assumes the highest ICC of 0.082 for *P. falciparum* baseline prevalence in the region that was observed in Laos as a post-study analysis of the ICCs [16].

Outcomes

The aim of our post hoc analysis was to describe the impact of the MDA on the prevalence of *P. vivax* infections at each quarterly survey, cumulative incidence of *P. vivax* parasitaemias derived from uPCR results of the quarterly surveys, and frequency of recurrent *P. vivax* infections. Recurrent infections after treatment of the blood stage infection can be due to recrudescence of a persistent infection, relapse due to activation of hypnozoites, or reinfection following a new infectious mosquito bite.

Statistical analysis

The changes of prevalence of *P. vivax* infection at the separate surveys were assessed by a difference-in-difference (DiD) approach for which we fitted a logistic model that incorporated effect modification between the intervention groups and the time period of the survey. We

assessed these differences between baseline and each follow-up time point (month 3, 6, 9, and 12) with the exception of month 12 data from Myanmar (where for logistic reasons the cross-over MDA in control villages had to be conducted on M9 instead of M12). None of the parameters in the difference-in-difference analysis was constrained to zero. The cumulative uPCR-derived incidence of *P. vivax* parasitaemias over the 12-month period was assessed using a survival analysis approach in which participants were censored when diagnosed, lost to follow-up, end of the observation period, which ever came first. The effect of the intervention on the incidence of *P. vivax* parasitaemias was assessed through a discrete time survival approach using a pooled mixed effects logistic regression analysis incorporating random effects for country and village. A participant was considered to have a recurrent *P. vivax* infection if there were two or more episodes with a positive uPCR result.

As consecutive positive uPCR tests could reflect continuous blood stage infection or frequent relapses, we defined an episode in two ways. In the first approach, each positive uPCR test was considered as a separate episode (i.e. relapse or reinfection). In the second approach, consecutive positive uPCR results were considered to belong to the same continuous infection (Table S1). In two additional sensitivity analyses we considered a missing uPCR test results either as positive or negative (Tables S2-S7). We assessed the impact of MDA on the recurrence of *P. vivax* parasitaemias through mixed-effects logistic regression with random effects for country and village.

All analyses used an individual weight that corrected for panel attrition during the observation period, and non-response at the separate surveys [17]. The panel-attrition weight is the inverse of the probability of participating in all cross-sectional surveys given personal characteristics and characteristics present during follow-up. The cross-sectional non-response weight is the inverse of the probability of participating in a single survey, given the individual's personal characteristics. Non-response weights were re-scaled to the original study sample to avoid inflating the degrees-of-freedom. The final analysis weight is the product of all cross-sectional non-response weights and the single panel-attrition weight. Personal characteristics used in the weighting procedure included age, sex, occupation, resident status, length of stay in the village, and village. Additional follow-up characteristics used in the panel-attrition weight were a history of previous malaria, number of previous malaria episodes, previous positive rapid diagnostic test for malaria, and experience of adverse event in study. For both types of weights, we used the chi-square automatic interaction detection (*chaid*) approach to derive at groups of similar probability of participation [18]. We allowed the *chaid* procedure to expand the number

of groups if there would be at least 25 observations in the resulting groups. Alpha-values for splitting and combining groups were set at 0.1. Inclusion of the predictors was unordered, while continuous variables were grouped in three similar-sized groups using tertiles. All statistical tests used an alpha level of 5%, below which statistical significance was assumed. All analyses used the original treatment assignment groups and were performed in STATA 14.1 (Stata Corp., College Station, Texas, USA).

Ethics statement

The studies were approved by the Cambodian National Ethics Committee for Health Research (0029 NECHR, dated 04 Mar 2013), the Institute of Malariology, Parasitology and Entomology in Ho Chi Minh City (185/HDDD dated 15 May 2013), the Institute of Malariology, Parasitology and Entomology in Qui Nhon (dated 14 Oct 2013), the Lao National Ethics Committee for Health Research (Ref No 013-2015/NECHR), Government of the Lao PDR and the Oxford Tropical Research Ethics Committee (1015-13, dated 29 Apr 2013). Individual informed consent was obtained from each participant or parent/guardian in the case on minors. A fingerprint was obtained for illiterate participants countersigned by a literate witness. (ClinicalTrials.gov Identifier: NCT01872702)

Results:

Participant characteristics were similarly distributed in intervention and control villages (**Table 1**).

Coverage

At baseline survey (M0), there were 4,135 and 4,310 people living in the intervention and control villages while 3,333/4,135 (80%) and 3,426/4,310 (79%) participated in the study, respectively. During three MDA rounds (M0, M1 and M2), 4,423 people were living in the intervention villages. Of those, 3,790 (85%) completed at least one round (3 doses) of DP-MDA and 2,520 (57%) completed 3 rounds (**Fig 2**).

Table 1. Baseline sociodemographic, history of malaria and bednet use data of the control and intervention villages

Characteristic	Control villages	Intervention villages	Overall
Cumulative number of participants	4,734	4,246	8,980
Number of first-time participation, n (%)			
Baseline	3,430 (72)	3,529 (83)	6,959 (77)
M03	558 (12)	316 (7)	874 (10)
M06	299 (6)	179 (4)	478 (5)
M09	238 (5)	130 (3)	368 (4)
M12	209 (4)	92 (2)	301 (3)
Age year, median (n, IQR)	20 (4,728, 8-35)	21 (4,241, 9-36)	20 (8,969, 9-36)
Sex, n (%)			
Male	2,412 (51)	2,196 (52)	4,608 (51)
Female	2,322 (49)	2,050 (48)	4,372 (49)
Occupation, n (%)			
Farmer	1,780 (38)	1,860 (44)	3,640 (41)
Child	413 (9)	266 (6)	679 (8)
Student	500 (11)	398 (9)	898 (10)
Others	194 (4)	225 (5)	419 (5)
Missing	1,847 (39)	1,497 (35)	3,344 (37)
History of malaria, n(%)			
Yes	754 (16)	758 (19)	1,512 (17)
Missing	1,910 (40)	1,492 (35)	3,402 (38)
Bednet use, n(%)			
Regular	3,117 (66)	2,643 (62)	5,760 (64)
Irregular	470 (10)	555 (13)	1,025 (11)
Never use	90 (2)	122 (3)	212 (2)
Missing	1,057 (22)	926 (22)	1,983 (22)
Malaria infection by uPCR, n (%)			
P.vivax	432 (9)	281 (7)	713 (8)
P. falciparum	180 (4)	154 (4)	334 (4)
P. vivax+P. falciparum	94 (2)	57 (1)	151 (2)

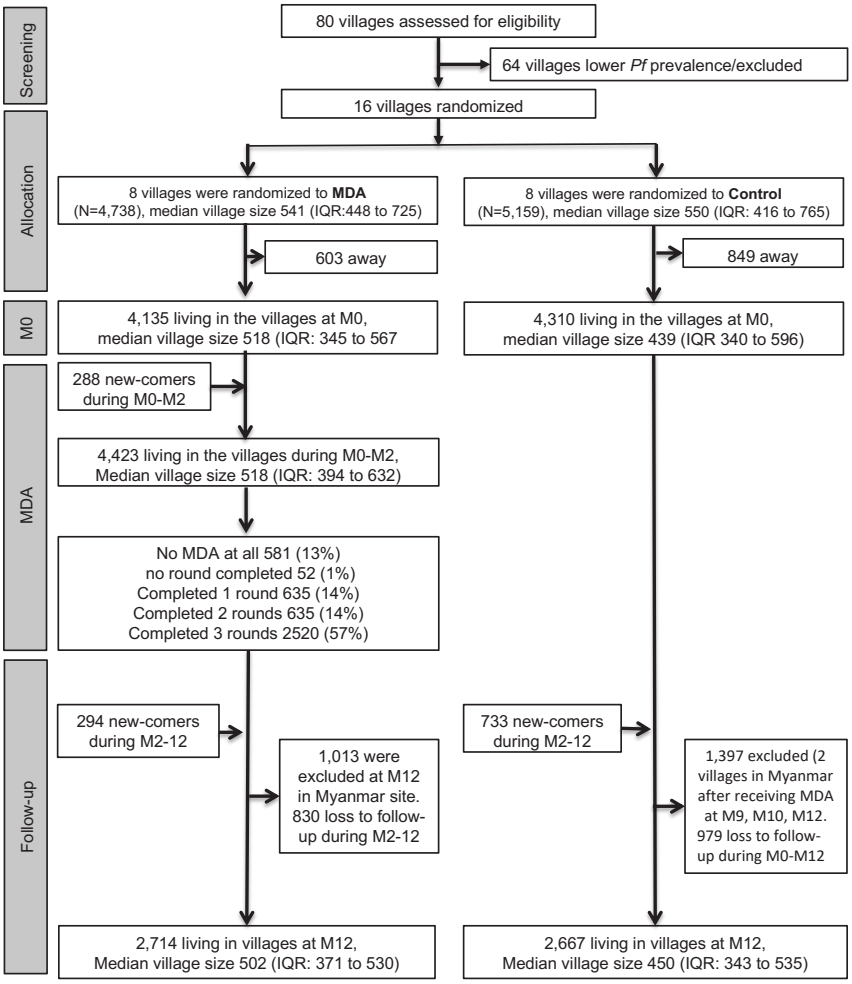

Figure 2. CONSORT flow diagram of MDA allocation and follow-up

Effect on prevalence

The prevalence of *P. vivax* parasitaemias decreased in both the intervention and control villages during the 12-month follow-up. The prevalence dropped sharply from 9.31% at baseline to 0.89% after three rounds of MDA at M3 follow-up (one month after the last MDA round) but rebounded to 5.81% by M6 follow-up after which the difference in prevalence between the intervention and the control villages was no longer statistically significant (coefficient (95%CI):-0.02 (-0.05, 0.003) (**Fig. 3**). An analysis by country showed that the prevalence of *P. vivax* parasitaemias rebounded in each study site except for Lao PDR where the *P. vivax*

prevalence dropped following the MDA and thereafter stayed low throughout the study period (**Fig. S1**).

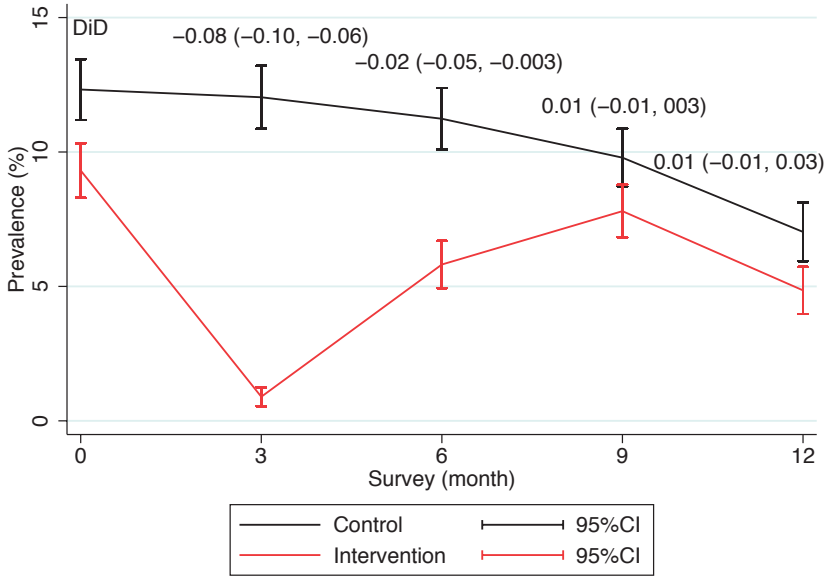

Figure 3. Changes in the prevalence of P. vivax infection during 12-month follow-up in the control and intervention villages. The month 12 data from Myanmar are not included because of logistic reasons, the cross-over MDA in the control villages had to be conducted on M9 instead of M12. DiD, difference in difference, coefficient (95% confident interval).

Effect on the incidence of *P. vivax* parasitaemias

The Kaplan-Meier survival curves (**Fig 4**) show a slower and lower cumulative uPCR-derived incidence of *P. vivax* detected parasitaemias in the intervention group compared to the control group. The uPCR-derived incidence of *P. vivax* detected parasitaemias in the control villages by month 12 was highest in Vietnam, Lao PDR, and was lowest in Cambodia (**Figure S2**). In Lao PDR the uPCR-derived *P. vivax* incidence of detected parasitaemias remained very low in the intervention villages over the follow-up period. In the other three sites, the incidence of detected parasitaemias in intervention villages rapidly approached the incidence seen in the control villages. There was no evidence that the intervention reduced the cumulative incidence of *P. vivax* infection (95% confidence interval [CI] Odds ratio (OR): 0.29 to 1.36) within a year follow-up, despite an average reduction in the population of 37% (OR: 0.63), This conclusion

holds for each country separately (**Fig 5**). The intra-cluster correlation coefficient (ICC) for the incidence of detectable *P. vivax* parasitaemias or village (as a cluster), accounting for the random effect of country, was in the range from 0.17 to 0.63, estimated at baseline and every 3 months up to month 12. The weighted-average ICC was 0.55 over the one-year follow-up.

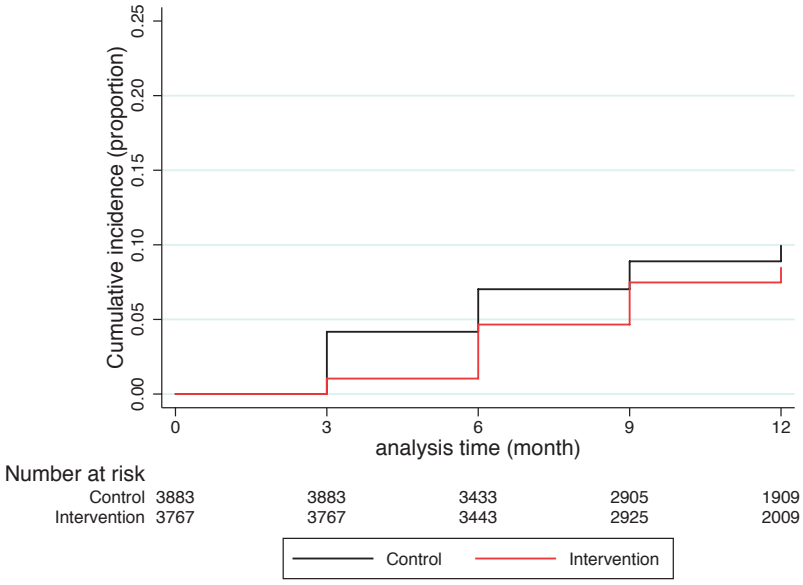

Figure 4. Cumulative uPCR-derived incidences of P. vivax infection between intervention and control villages. The month 12 data from Myanmar are not included because of logistic reasons, the cross-over MDA in the control villages had to be conducted on M9 instead of M12.

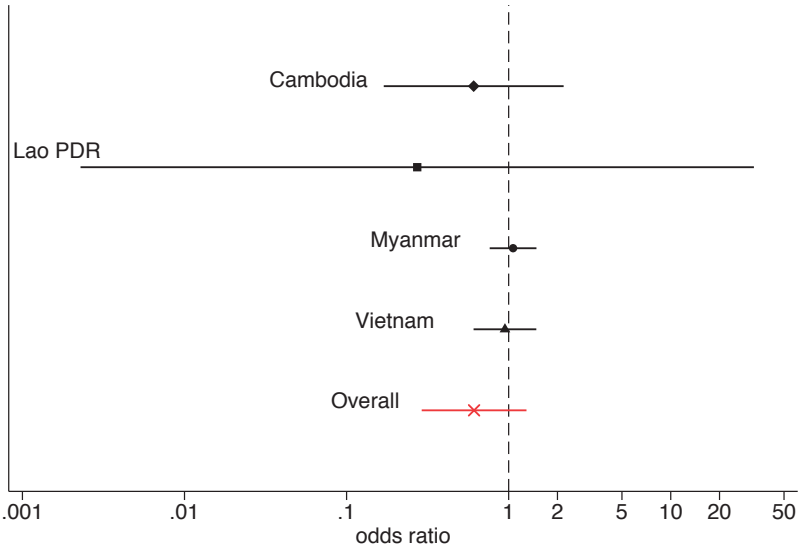

Figure 5. Forest plot of country odds ratios in the uPCR-derived incidence of P. vivax infections.

Effect on recurrence of *P. vivax* parasitaemias and number of episodes detected

The number of participants with recurrent parasitaemias was lower in the intervention villages than in the control villages whether each positive uPCR tests was coded as a separate episode (OR: 0.34) or consecutive positive uPCR tests were coded together as one continuous episode (OR: 0.62). The estimates of the effect of MDA on recurrent parasitaemias varied-widely and did not reach statistical significance overall (95%CI: 0.08-1.42, and 0.18-2.12, respectively) (**Table 2**). Overall 809 (17.1%) of the participants in the control villages had at least one recurrent *P. vivax* episode compared to 562 (13.2%) in the intervention villages (**Table 3**). The sensitivity analyses defining missing value to negative or to positive showed similar outcomes (**Table S2 & S3**).

Table 2. Multilevel logistic regression with random effect for country and village on P. vivax episode and analysis weight (panel attrition and non-response)

Variable	Control (deferred MDA) villages n=4,734	Intervention (early MDA) villages n=4,246	p-Value
Each positive test = one episode, n			
Participants with recurrence, n (%)	408 (8.6)	183 (4.3)	
Odds Ratio (95%CI)	Ref.	0.34 (0.08-1.42)	0.138
Consecutive positive as one episode			
Participants with recurrence, n (%)	170 (3.6)	113 (2.7)	
Odds Ratio (95%CI)	Ref.	0.62 (0.18-2.12)	0.443

MDA, mass drug administration; CI, confidence interval; Ref., reference

Table 3. Comparison of the number of P. vivax episodes in the control and intervention villages.

No. episodes	Each positive test = one episode		Consecutive positive test = one episode	
	Control n=4,734	Intervention n=4,246	Control n=4,734	Intervention n=4,246
	n (%)	n (%)	n (%)	n (%)
0	3925 (82.9)	3684 (86.8)	3925 (82.9)	3684 (86.8)
1	401 (8.5)	379 (8.9)	639 (13.5)	449 (10.6)
2	194 (4.1)	124 (2.9)	160 (3.4)	108 (2.5)
3	136 (2.9)	48 (1.1)	10 (0.2)	5 (0.1)
4	62 (1.3)	10 (0.2)		
5	16 (0.3)	1 (0)		

Discussion

This study found no evidence of a sustained effect of MDA with dihydroartemisinin-piperaquine on the prevalence of *P. vivax* infections over a one-year follow-up. The MDA reduced the prevalence of *P. vivax* infections for three to six months after the last round after which a rebound in the prevalence of *P. vivax* parasitaemias was seen in all sites except for the sites in Lao PDR. The most likely reason for the absence of a rebound in Lao PDR is the lower

P. vivax incidence compared to the other sites resulting in a lower risk for hypnozoite carriage, relapse and reinfection. Comparing *P. vivax* and *P. falciparum* prevalence changes between M3 and M6 suggests that the prevalence of *P. vivax* increased faster than *P. falciparum* prevalence. One explanation for this observation could be that vivax hypnozoites fuel the speedy rebound of *P. vivax* prevalence. An alternative perhaps complementary explanation could be that the *P. vivax* transmission is higher at least in some of the study sites. MDA without a full course of 8-aminoquinolines clears only the blood stages of *P. vivax* and not the hypnozoites. As dihydroartemisinin-piperaquine clears *P. vivax* infections, recrudescent infections are unlikely the cause for recurrent *P. vivax* infections. Relapses, and to a lesser degree reinfection, are the most likely source for the observed *P. vivax* recurrences, and are a source of ongoing transmission [19]. In SE Asia, *P. vivax* infections tend to relapse after short intervals (typically three weeks), although the residual concentrations of slowly eliminated antimalarial drugs like piperaquine have a prophylactic effect which can lead to prolonged intervals [20]. Following the treatment of symptomatic infections with dihydroartemisinin-piperaquine, relapses emerge typically around six weeks after treatment [21, 22]. The radical cure of *P. vivax* infections requires a 7- or 14-day course of primaquine or single dose of tafenoquine. The SLDPQ aimed at blocking transmission by clearing *P. falciparum* gametocytes which in contrast to *P. vivax* gametocytes are resistant to schizontocidal drugs [23].

MDA including radical cure with 8-aminoquinolines have been conducted in the former Soviet republics, Afghanistan and North Korea, and they contributed to the elimination of malaria from the USSR (24). Mass primaquine administrations were used in Azerbaijan in 1971 to eliminate *P. vivax*. Despite the high prevalence of G6PD deficiency, primaquine administration was safe with few reported severe adverse events [24]. In China, primaquine mass administration was implemented at a large scale in “spring treatment” involving over 200 million people at high risk for vivax malaria, and were effective in reducing the incidence of *P. vivax* malaria [25]. MDA with a three day-course of chloroquine and primaquine in Nicaragua showed a reduction of both *P. falciparum* and *P. vivax*, but *P. vivax* returned, as in our study, to endemic levels within months after the MDA [26].

Our study has limitations: 1) The intervention, MDA with DP and SLDPQ, was designed to eliminate *P. falciparum* and not *P. vivax* infections. Our post-hoc analyses describe the concurrent effect of the intervention targeting *P. falciparum* on *P. vivax*. 2) Treating villages in the uninterrupted presence of infective reservoirs in the surrounding areas will inevitably

result in reimportation of infections, as we observed in the return of *P. falciparum* infections after 12 months [3]. A more regional approach to targeted malaria elimination including MDA would be required to reduce the risk of re-importation of Plasmodium infections. 3) The prevalence estimate at month 12 could be an underestimated due to the absence of data from Myanmar (where for logistic reasons the evaluation was conducted at month 9). In every site, except Laos, the prevalence of *P. vivax* increased between month 6 and 12 in the intervention villages [6]. 4) Entomological aspects were only evaluated in one study site [27]. Entomological data from more study sites may have helped to gain further insights into malaria transmission dynamics. 5) We sampled study participants in quarterly intervals. More frequent sampling would have provided a better understanding of the persistence of infections. The uPCR derived incidence of *P. vivax* parasitaemia reported in our study is much higher than the incidence of clinical vivax malaria but allows us to estimate the impact of the MDA on asymptomatic reservoir of *P. vivax* infections.

Our findings highlight the challenges but also suggest approaches to the elimination of all malarias including vivax malaria. The mass administration of dihydroartemisinin-piperaquine made a lasting and significant impact on *P. falciparum* infections [3], and on the *P. falciparum* entomological inoculation rate [27]. To provide a sustained reduction of *P. vivax* transmission, first and foremost radical cure with an 8-aminoquinoline is needed. But all 8-aminoquinolines cause haemolysis in G6PD deficient individuals. There are several approaches for the treatment of G6PD deficient individuals a) exclusion from the radical cure MDA b) use the one weekly primaquine 0.75mg/kg regimen for 8 weeks c) an alternative primaquine dosing regimen with incremental, yet safe increase in dosing. A regimen allowing primaquine to be stopped in case of symptoms or signs of haemolysis proved safe and effective without G6PD testing in the former USSR [24]. Pregnant women and young children should not receive 8-aminoquinoline. Robust and accurate point-of-care tests for the diagnosis of G6PD deficiency could play a pivotal role in the elimination of vivax malaria. A range of new diagnostic tests is currently in development [28, 29]. Second, high participation in mass treatment programmes is critical in clearing asymptomatic reservoirs. More than 80% of residents are thought having to participate in MDA to interrupt transmission but this estimate is not based on empirical data [30]. Although the coverage of at least one MDA round reached that target, the coverage of three MDA rounds was low. Finally, understanding the reservoirs of the nearby places and movement of people between places is essential in scaling up MDAs.

Conclusion

An MDA with schizontocidal and gametocytocidal medication for clearing only blood-stage infection made a short-lasting impact on *P. vivax* prevalence and a reduction in recurrent infections but there no evidence of an effect on the long-term reduction of incidence, prevalence, and number of recurrent *P. vivax* infections over 12-month follow-up. This is in contrast to the impact of MDA on *P. falciparum* infections, where we showed a significant longer lasting impact [3]. The combination of effective blood schizontocidal and hypnozoiticidal drugs will be essential for the elimination of all malarias.

Declaration of interests

We declare no competing of interests in any form related to employment, consultancy, patents, products in development, or marketed product, etc. None of our authors is affiliated with commercial entity

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Supporting information

Table S1. The coding of number of recurrent episodes in 11 scenarios, Green=uPCR negative and Red=uPCR positive

Scenario	PCR test result per survey round 1 to 5: green = negative, red = positive					Interpretation: Counts of positive episodes	
	1	2	3	4	5	If each test is considered as a single episode	If consecutive positives are considered single episodes
1						0	0
2						1	1
3						2	2
4						2	1
5						3	3
6						3	2
7						3	1
8						4	2
9						4	2
10						4	1
11						5	1

Table S2. Multilevel logistic regression with random effect country and village on *P. vivax* recurrent episodes

Variable	Available data		Missing test = positive		Missing test = negative	
	Control	Intervention	Control	Intervention	Control	Intervention
Each positive test = one episode	N = 4,734	N = 4,246	N = 4,734	N = 4,246	N = 4,734	N = 4,246
Participants with recurrence, n(%)	408 (8.62)	183 (4.31)	575 (12.15)	366 (8.62)	408 (8.62)	183 (4.31)
OR (95%CI)	1	0.34 (0.08-1.42)	1	0.46 (0.13-1.62)	1	0.34 (0.08-1.42)
p-Value		0.138		0.227		0.138
Consecutive positive as one episode						
Participants with recurrence, n(%)	170 (3.59)	113 (2.66)	263 (5.56)	209 (4.92)	170 (3.59)	113 (2.66)
OR (95%CI)	1	0.62 (0.18-2.12)	1	0.67 (0.22-2.03)	1	0.62 (0.18-2.12)
p-Value		0.443		0.475		0.443

Control, deferred-MDA villages; Intervention, early-MDA villages; CI, confidence interval

* multilevel logistic regression with random effect country and site, and analysis weight (panel attrition and non-response)

Table S3. Comparison of the number of *P. vivax* episodes in control and intervention villages

	Each positive test = one episode											
	Available data				**Missing test = positive**				**Missing test = negative**			
	Control N=4734		Intervention N=4246		Control N=4734		Intervention N=4246		Control N=4734		Intervention N=4246	
Number episodes	n	%	n	%	n	%	n	%	n	%	n	%
0	3925	82.9	3684	86.8	3240	68.4	3126	73.6	3925	82.9	3684	86.8
1	401	8.5	379	8.9	919	19.4	754	17.8	401	8.5	379	8.9
2	194	4.1	124	2.9	311	6.6	243	5.7	194	4.1	124	2.9
3	136	2.9	48	1.1	174	3.7	103	2.4	136	2.9	48	1.1
4	62	1.3	10	0.2	72	1.5	18	0.4	62	1.3	10	0.2
5	16	0.3	1	0	18	0.4	2	0	16	0.3	1	0
	Consecutive positive test = one episode											
	Available data				**Missing test = positive**				**Missing test = negative**			
	Control N=4734		Intervention N=4246		Control N=4734		Intervention N=4246		Control N=4734		Intervention N=4246	
Number episodes	n	%	n	%	n	%	n	%	n	%	n	%
0	3925	82.9	3684	86.8	3240	68.4	3126	73.6	3925	82.9	3684	86.8
1	639	13.5	449	10.6	1231	26	911	21.5	639	13.5	449	10.6
2	160	3.4	108	2.5	250	5.3	201	4.7	160	3.4	108	2.5
3	10	0.2	5	0.1	13	0.3	8	0.2	10	0.2	5	0.1

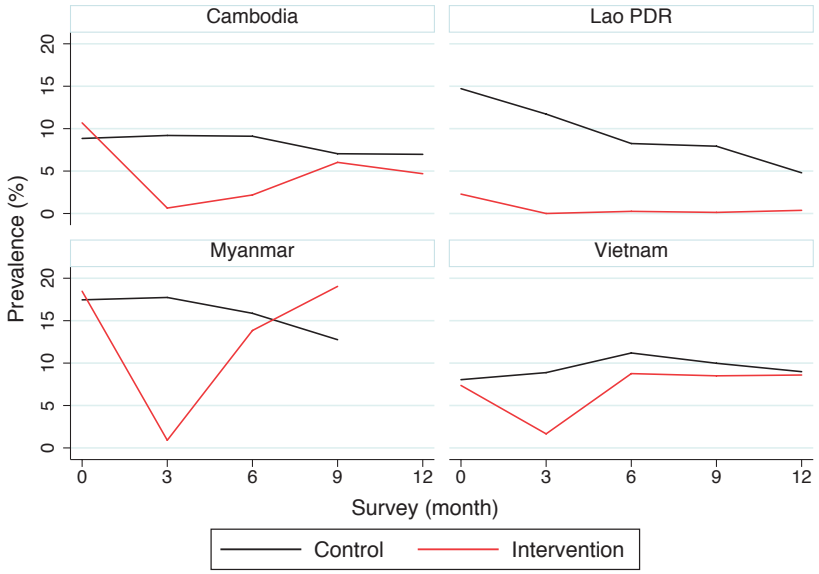

Fig. S1: *P. vivax* prevalence

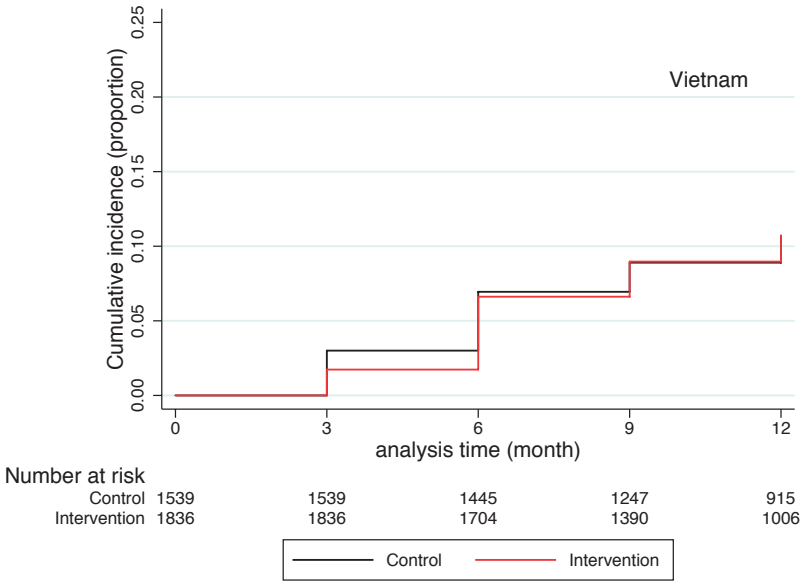

Fig. S2. *P. vivax* incidence, Vietnam

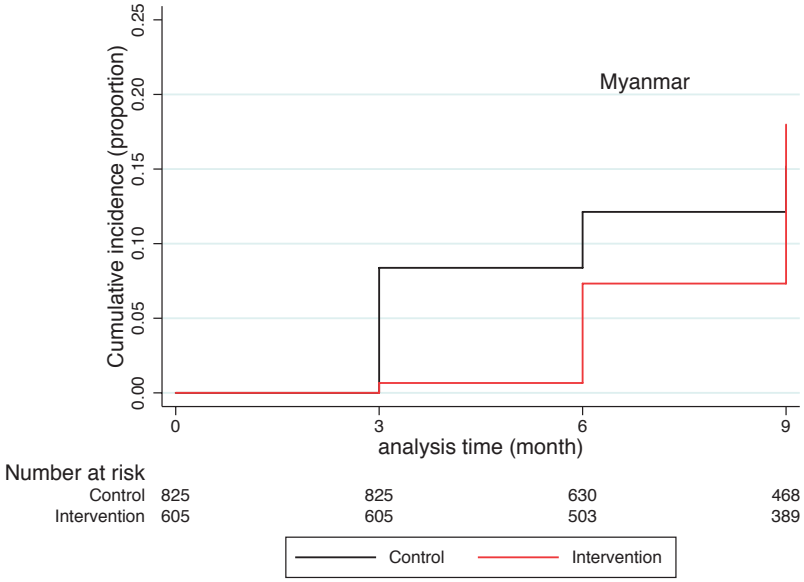

Fig. S2. *P. vivax* incidence, Myanmar

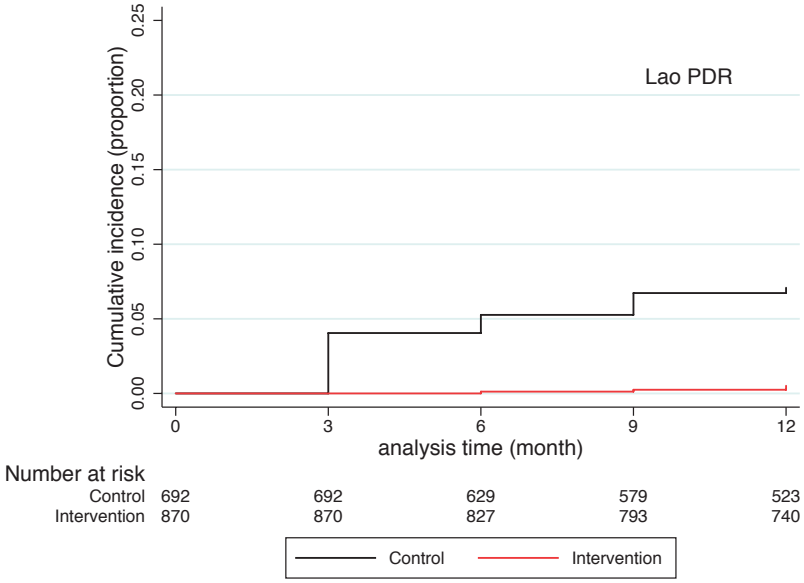

Fig. S2. *P. vivax* incidence, Laos

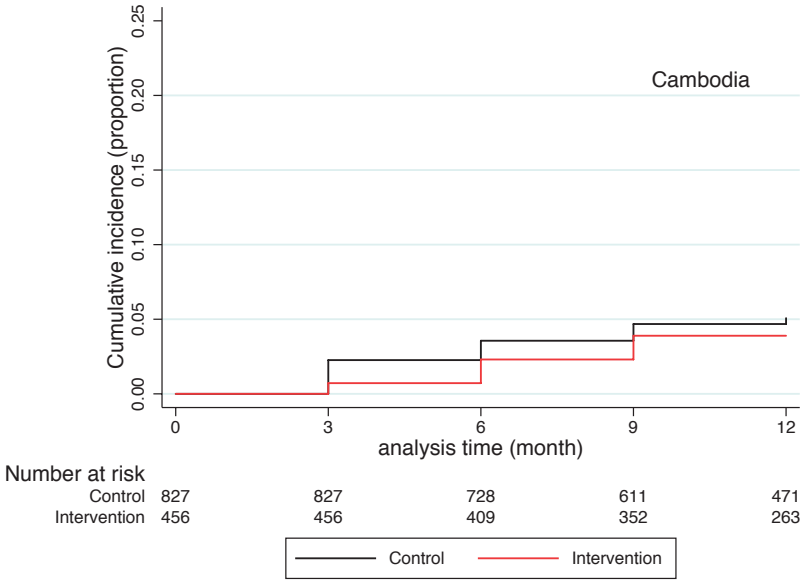

Fig. S2. *P. vivax* incidence, Cambodia

Chapter 4-The use of ultrasensitive quantitative-PCR to assess the impact of primaquine on asymptomatic relapse of *Plasmodium vivax* infections: a randomized, controlled trial in Lao PDR

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Malaria journal 2020, **19**(1):4.

Abstract

Background

Trials to assess the efficacy of the radical cure of *P. vivax* malaria with 8-aminoquinolines require that the majority of post-treatment relapses are identified, but there is no consensus on the optimal duration of follow-up in either symptomatic or asymptomatic vivax malaria. The efficacy of a 14-day course of primaquine on the cumulative incidence of recurrent asymptomatic *P. vivax* infections detected by ultrasensitive quantitative PCR (uPCR) as a primary endpoint was assessed.

Methods

A randomized, placebo-controlled, single-blind trial was conducted in four villages of the Lao PDR during 2016-2018 nested in a larger project evaluating mass drug administrations (MDA) with dihydroartemisinin-piperaquine (DP) and a single low-dose primaquine to clear *P. falciparum* infections. Eligible participants aged older than 9 years old with mono- or mixed *P. vivax* infection detected by uPCR were randomized to receive either 14 days of primaquine (0.5 mg/kg/day) or placebo during the last round of MDA (round 3) through directly observed therapy. Participants were checked monthly for 12 months for parasitaemia using uPCR. The primary outcome was cumulative incidence of participants with at least one recurrent episode of *P. vivax* infection.

Results

20 G6PD-normal participants were randomized in each arm. 5 (29%) of 20 participants in the placebo arm experienced asymptomatic, recurrent *P. vivax* infections, resulting in a cumulative incidence at month 12 of 29% in the placebo. None of the 20 participants in the intervention arm had recurrent infections (p=0.014 Fisher's exact test). Participants with recurrent *P. vivax* infections were found to be parasitaemic for between one and five sequential monthly tests. The median time to recurrence of *P. vivax* parasitaemia was 178 days (range: 62-243 days).

Conclusion

A 14-day course of primaquine in addition to DP-MDA was safe, well-tolerated, and prevented recurrent asymptomatic *P. vivax* infections. Long follow up for up to 12 months is required to capture all recurrences following the treatment of asymptomatic vivax infection. To eliminate all malarias in settings where *P. vivax* is endemic, a full-course of an 8-aminoquinolines should be added to MDA to eliminate all malarias.

Trial registration: This study was registered with ClinicalTrials.gov under NCT02802813 on 16th June 2016. https://clinicaltrials.gov/ct2/show/NCT02802813

Key words: malaria, P. vivax, PCR, primaquine, relapse

Background

Plasmodium vivax remains one of the major public health problems in malaria endemic countries where 2.5 billion people are at risk of infections [1]. The control of *P. vivax* has been slower than the control of *P. falciparum* due to its ability to lie dormant in liver cell (hypnozoites), causing relapse weeks to months after the initial attack. *P. vivax* gametocytes appear quite early after successful blood feeding and before the presence of clinical symptoms, resulting in mosquito infection and transmission. Moreover, *P. vivax* infections are usually of low density which might be missed by conventional diagnostic tests [2]. Primaquine and tafenoquine, both 8-aminoquinolines, are the only licensed drugs with activity against hypnozoites for radical treatment of *P. vivax* [3-5] but are underutilised due to their potential to cause haemolysis in glucose-6-phosphate-dehydrogenase (G6PD) deficient people.

Trials to assess the efficacy of radical cure of *P. vivax* malaria with 8-aminoquinolines require prolonged follow up of a large sample of participants to detect clinically relevant reductions in the number of recurrent clinical *P. vivax* malaria episodes. However, there is no consensus on the optimum duration of follow-up in therapeutic assessments of symptomatic vivax malaria, and no guidance at all for therapeutic assessments in asymptomatic infections. These asymptomatic recurrences are epidemiologically important as they are the likely reservoir of the infection [6]. Indeed, since the application of PCR to malaria, asymptomatic plasmodium carriers have been increasingly recognised as they are substantially more prevalent than clinical cases and probably serve as infective reservoirs [7, 8]. The recent development of a highly sensitive quantitative PCR (uPCR) to identify and quantify low-density malaria parasites by using a relative large blood volume, allows reliable detection of parasite densities as low as 22 parasites/ml of blood [9]. In order to eliminate malaria, treatment of asymptomatic *P. vivax* carriers is critical to prevent the persistent *P. vivax* infections. Detection of asymptomatic parasitaemia by uPCR could be a critical trial endpoint in the assessment of the anti-relapse potential of anti-malarial drug regimens in asymptomatic infections. The objective of this study was to assess the efficacy of a 14-day radical cure with primaquine using the incidence of asymptomatic *P. vivax* infections detected by uPCR as endpoint.

Methodology

Trial design

This sub-study was nested within a large multicentre targeted malaria elimination project, a mass drug administration (MDA) trial in the Greater Mekong Subregion which included four villages of Nong District, Savannakhet Province, Lao PDR (**Fig. 1**)[10]. Two of the four villages were randomized to receive three rounds of MDA, each consisting of a three-day course of dihydroartemisinin-piperaquine (DP) and a single low dose (0.25 mg/kg) of primaquine (SLDPQ). The other two villages served as controls and received MDA after 12 months of surveillance [10]. Participants in the MDA villages found to be infected with *P. vivax* by uPCR at the baseline survey or month 0 were invited to participate in the primaquine trial described here during MDA round 3. Participants in the control villages, who were found to be infected during cross-sectional surveys, were invited to participated during crossover MDA round 3 (month 14 of MDA trial). This sub-study was a nested, randomized, single-blind, treatment trial of asymptomatic vivax infections in participants without G6PD deficiency with asymptomatic *P. vivax* mono or mixed-infection detected during the MDA trial [11].

Study site

The Lao PDR is a land-linked country in Southeast Asia bordered by China and Myanmar in the north, Vietnam in the east, Thailand in the west and Cambodia in the south. The country is composed of 18 provinces, which are further subdivided into 147 districts. Malaria epidemiology is highly heterogeneous; the five-southern provinces are the most malaria prevalent and accounted for 97% of cases reported in Lao PDR. *P. falciparum* is still the predominant parasite species, but *P. vivax* accounted for nearly 47% of reported cases in 2014. Lao PDR together with its neighbouring countries plans to eliminate malaria in the Greater Mekong Subregion by 2030 [12]. The national first line treatment for *P. vivax* is a 3-day course of artemether-lumefantrine plus 14-day primaquine and the second line treatment is 3-day chloroquine plus 14-day primaquine. A radical cure with primaquine policy was adopted by the national malaria control programme (CMPE) in 2011 but has not yet been widely implemented due to the lack of G6PD testing.

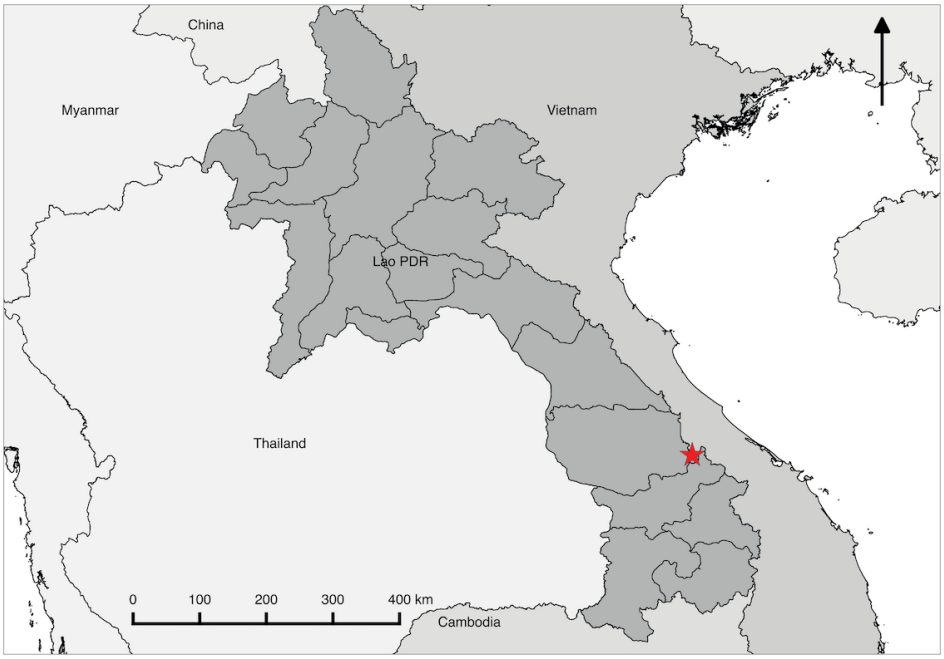

Fig. 1. Map of study site (red star indicates study site)

Participants

Male, and non-pregnant and non-breastfeeding females older than 9 years were eligible to participate if asymptomatic *P. vivax* mono- or mixed infections were detected by ultrasensitive qPCR during the cross-sectional surveys of MDA trial. People with following conditions were excluded: unable to take oral treatment, previous episode of haemolysis or severe haemoglobinuria following primaquine, known hypersensitivity or allergic to study drugs, blood transfusion in the last 90 days, acute malaria episode requiring treatment or febrile condition at the time of recruitment, anaemia with haemoglobin less than 9 g/dL. Participants who took medication that might interfere with pharmacokinetics of primaquine were also excluded. Participants were recruited at two different time points. First recruitment was in the two intervention villages at the beginning of the MDA trial, and second, 12 months later in the two control villages of the initial cRCT when participants received the MDA at the end of the surveillance period. All study participants had received 3 rounds consisting each of three doses DP+SLDPQ except for two participants who received two rounds of DP+SLDPQ. A single

round DP+SLDPQ is sufficient to clear *P. vivax* blood stages (schizontocides) and a SLDPQ given has no effect on the hypnozoites of *P. vivax*.

Intervention

Participants who met the inclusion criteria including informed consent were randomly assigned to 14 days of primaquine (0.5mg/kg for 14 days) or placebo in addition to the 3 day-course of dihydroartemisinin-piperaquine (7mg/kg/day DHA and 55mg/kg/day piperaquine) they had received during MDA. Day 0 for the current sub-study corresponded to month 2 of the MDA trial (third MDA round) in the intervention villages or month 14 in the control villages which was also the third round of the MDA. DP used in our study was manufactured by Guilin Pharmaceutical Company, China. Primaquine and placebo were manufactured by the Government Pharmaceutical Organization, Thailand, and both had similar appearances.

Outcomes

The primary outcome was the cumulative incidence of asymptomatic *P. vivax* recurrences detected by uPCR over 12-month follow-up. Secondary outcomes were parasite densities, time to first recurrence, frequency of recurrent asymptomatic and clinical malaria episodes, and the changes in haemoglobin (Hb) concentration, and the number of adverse events over the first 28 days (until 14 days after the last dose of primaquine or placebo). Time to parasite clearance could not be assessed in this trial.

Sample size

The sample size was chosen for mainly pragmatic reasons with the aim to enrol up to sixty participants. Prior to study start neither the asymptomatic *P. vivax* prevalence in Savannakhet nor the impact of primaquine on asymptomatic *P. vivax* infections was known. Assuming that relapse would be detectable by uPCR in 30% of the participants in the control arm a sample size of 60 participants, 30 per arm, would be sufficient to detect that difference between groups in clearing parasitaemias based on alpha value of 0.05, a power of 80%, and 20% loss to follow-up.

Randomization

The computer-generated randomization list was prepared centrally at the Mahidol-Oxford Tropical Medicine Research Unit with a group ratio of 1:1. Regimen allocation was kept in a

series of sealed, opaque envelopes that were sequenced numerically. Participants were sequentially assigned to the envelopes, which contained the random treatment allocation.

Blinding

The treatment allocation was concealed to participants and Laboratory technicians who performed uPCR throughout the study.

Procedures

On day 0, a physical examination was conducted, socio-demographic data, a history of illness and medication in the last 28 days was collected and 3 ml of blood was taken for haemoglobin measurement and uPCR before taking study drugs. Directly observed therapy (DOT) was used to ensure adherence. Drugs were administered with biscuits and soya milk to reduce gastro-intestinal side effects. After drug administration, participants were observed for an hour. If a participant vomited within 30 minutes, the full dose was repeated. If the participant vomited after 30 minutes but less than 1 hour, half of the dose was given. Temperature and adverse events were collected on daily basis over the first 14 days and then on day 28. All adverse events either related or unrelated to study drugs during this period were recorded. If hospitalization, death, or a drop in haemoglobin by 25% compared to baseline occurred, it was be recorded as serious adverse event. Follow-up blood samples were taken on Day 2, 6, 13, 28 and then monthly over one year for uPCR and haemoglobin measurement. During the follow-up visits temperature and history of illness during the preceding month were recorded. Data from each participant were recorded in a standardized case record form. Participants found to have recurrent infections detected by uPCR during follow-up period without clinical symptoms were not treated. Participants with clinical signs and symptoms of malaria and positive for plasmodium infection by rapid diagnostic test were treated according to Lao national malaria treatment guideline.

Laboratory procedures:

Sample collection:

A 3 ml blood sample was collected into an EDTA anticoagulated tube at baseline and each schedule time, which was kept in an ice packed cool box and transported within 6 hours from the villages to the local laboratory. On arrival at the laboratory, 200 microlitre samples were aliquoted for haemoglobin measurement, and the remaining blood was processed and separated

into red blood cell pellets, buffy coat and plasma. Each aliquot was stored at -20ºC in a freezer along with an additional negative control in the sample pool. The samples were then transported on dry ice to the molecular laboratory of the Mahidol Oxford Tropical Medicine Research Unit (MORU) in Bangkok, Thailand for uPCR analysis.

DNA extraction and PCR amplification

A highly sensitive and specific high-volume quantitative PCR method was used, which has a lower limit of detection of 22 parasites/ml [9]. In short, an automated DNA extraction method (QIAsymphony and DSP DNA midi kit; Quiagen, Germany) was used to purify DNA from thawed red blood cells. The purified DNA was concentrated, dried and then used as a template for PCR detection and quantification of Plasmodium. DNA of Plasmodium was detected and quantified using 18S rRNA-targeting primers and hydrolysis probes. For Plasmodium-positive samples, an attempt was made to identify the species using *P. falciparum* and *P. vivax*-specific PCR primers [9].

Other field laboratory work:

Haemoglobin levels were measured in the field by using the HemoCue® Hb 301 system (Hemocue AB, Angelholm, Sweden) by trained laboratory technician following the manufacturer's recommendation. G6PD deficiency was tested by using fluorescent spot test (FST) (the Trinity Biotech Plc, IDA Business Park, Bray, Co Wicklow, Ireland), which showed a perfect match with spectrophotometry at 30% cut-off activity [13].

Statistical analysis

All data collection was transferred into databases for data management and cleaning using Macro electronic data capture. An Intention-to-treat (ITT) analysis was performed to determine primary and secondary outcomes, with the ITT defined as all randomized participants who took at least one dose of primaquine. The cumulative incidence of *P. vivax* infections over 12-month follow-up was assessed by survival analysis. Follow-up data were censored for participants without events throughout the follow-up period, and right censored at the day of their first recurrence or the day when they were last seen which ever came first. The difference between the two survival curves was assessed through Kaplan Meier estimates at month 12 using the log-rank test. Time to first recurrence was calculated as time from the start of the intervention (D0 of administering the 14-day primaquine regimen) to time when a follow-up sample turned positive and displayed in number of days and range. Given the small sample size and the small

number of outcome events, we did not perform any other analyses (e.g. Cox regression), as adequate inference of statistical models in this situation is not possible. To count the total number of recurrent *P. vivax* episodes per person through the available follow-up, there was no censoring to include multiple episodes. We assessed the effect of primaquine on haemoglobin levels by using a multilevel mixed effect linear model with an unstructured covariance to accommodate their repeated measurements. Adverse events were reported by frequency. Statistical significance was assumed at the 5% level. The analysis was performed using Stata version 14.1 (StataCorp, Texas, USA).

Results

The first 18 participants were enrolled in Jun 2016 and another 22 participants in Jun 2017. The last follow-up visit was on 15th Jun 2018. In total, 40 participants were randomized (20 in each arm). The baseline characteristics of trial participants were balanced between the treatment arms (**Table 1**). 16 (80%) participants in each arm completed the 12 month-follow-up period. Four participants in each arm did not complete 12-month follow-up; in the intervention arm on day 4, day 14, month 8 and month 10, and in the control arm on day 5, day 6, day 14 and month 8 (**Figure 2**). The reason for leaving the study by the first 14 days, was "enough of frequent blood draws", while the participants who left by Month 8 or month 10 were lost to follow-up.

The primaquine treatment was given at the start of DP during the MDA round 3 with the median dosing of 0.52 mg/kg per day (range:0.35 to 0.77 mg/kg).

Over a period of 12-month follow-up, none of participants developed clinical *P. vivax* infection, but one participant in placebo arm developed clinical *P. falciparum* at month 5 and was treated with three-day course of artemether-lumefantrine according to Lao national malaria treatment guideline and he recovered well.

Plasmodium vivax recurrent infections

Five participants had at least one recurrent *P. vivax* infection in the placebo arm, resulting in a cumulative incidence at month 12 of 29% (95% Confidence Interval [CI]: 13.4-56.9), and none in the primaquine arm (p=0.014) (**Figure 3**). The median time to first recurrence in the placebo arm was 178 days (range: 62-243 days). The pattern of recurrent infections was variable (**Figure 4**). Participants with recurrent *P. vivax* infections were found to be parasitaemic between one and five sequential monthly tests. The participant with the highest parasite density

at M0 (Recurrent 4; 284,873 genomes/ml) had no apparent lag between the first and five subsequent tests. The participant with the lowest parasite density (Recurrence 1; 5,190 genomes/ml) tested only once positive at M06 (six months after the start of the trial). No clinical *P. vivax* cases were detected during the follow-up period.

Table 1. Participant characteristics at baseline

Variable	Drug allocation		Total, N=40
	Placebo, n=20	Primaquine, n=20	
Age in year, median (Range)	32.5 (10-76)	19 (10-54)	25.5 (10-76)
Male (%)	14 (70)	12 (60)	26 (65)
Weight kg, mean (95%CI)	44.5 (39.7-49.3)	44.6 (38.2-50.9)	44.5 (40.6-48.5)
Hb[#] g/dl, mean (95%CI)	13.7 (13.1-14.4)	13.5 (12.9-14.1)	13.6 (13.2-14.1)
Recruitment year, n			
Jun-16	9	9	18
Jun-17	11	11	22
uPCR			
During surveys of MDA trial			
mono-PV infection	16	17	33
Mixed PV infection	4	3	7
At Day 0			
PV infection	0	0	0

[#] Haemoglobin

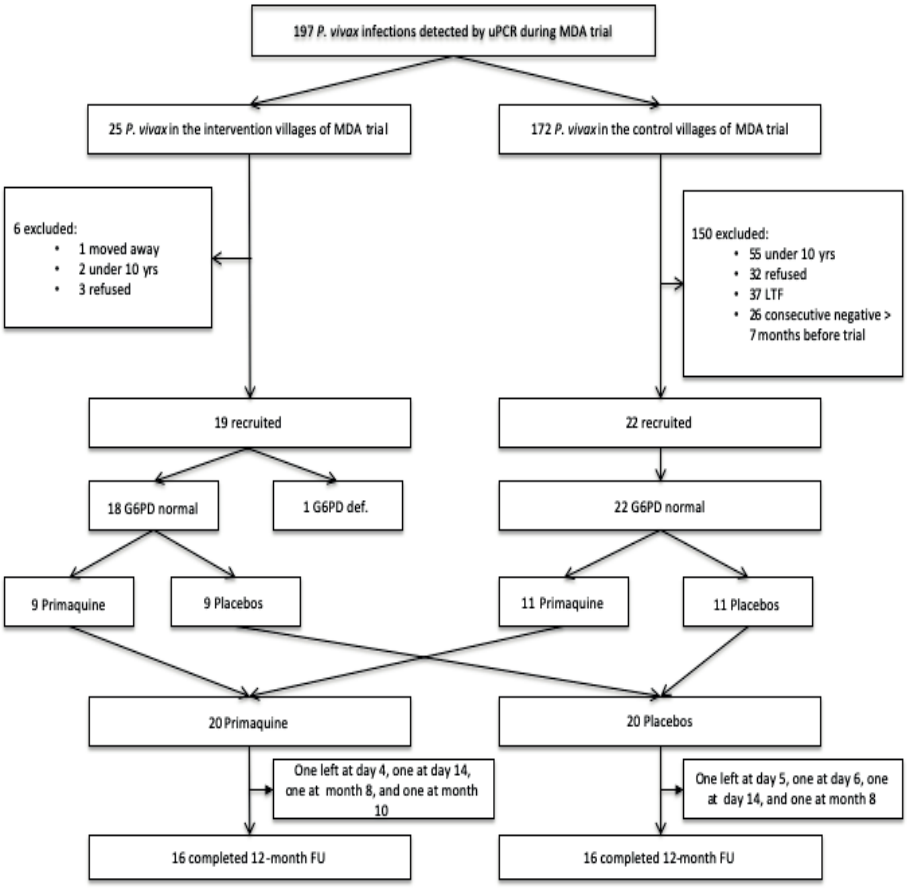

Fig. 2. Consort flow chart of recruitment *uPCR* ultrasensitive polymerase chain reaction, *G6PD* glucose 6 phosphate dehydrogenase deficiency, *FU* follow-up

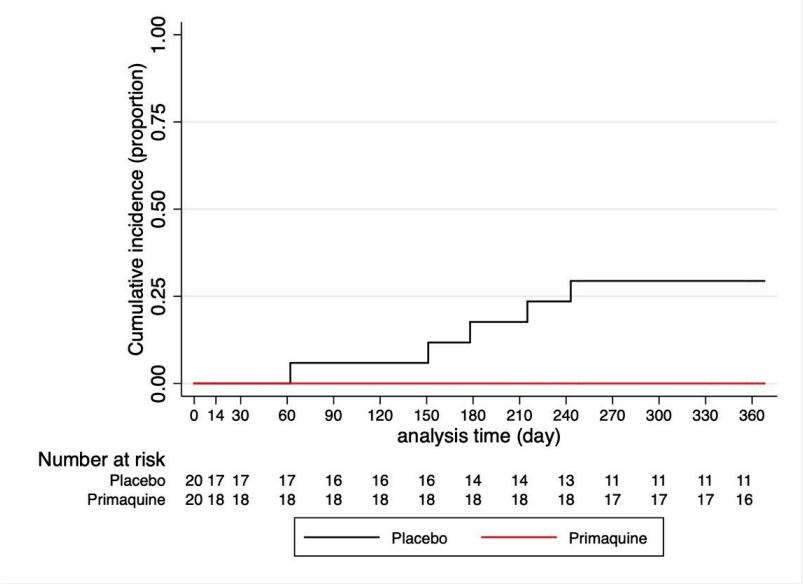

Fig. 3. Cumulative recurrent incidence of P. vivax infections by intervention

Effect of primaquine on haemoglobin level over the first 28 days

There was a small decrease in haemoglobin level of 0.225 g/dL and 0.080 g/dL on day 2 and day 13, respectively in the primaquine group but this decrease was not clinically significant (**Figure 5**). A multilevel mixed effect model to assess the effect of drug and time on haemoglobin level showed that the average haemoglobin level of participants who took primaquine was 0.228 g/dL (95%CI: -1.058 to 0.602) lower than those who took the placebo (p-value: 0.59).

Adverse events

Two participants reported adverse events in the primaquine arm which were considered as related to study drugs; one participant felt dizzy, while the other felt dizzy and nauseated 30 minutes after taking study drugs. Both adverse events were mild and self-limited. Two adverse events were detected over the first 28 day-follow-up in the placebo arm, one participant reported watery stool, which was considered as a possibly relation to study drug, and the other had a foot injury which was considered unrelated. No patient complained of red or black urine and no serious adverse events were reported.

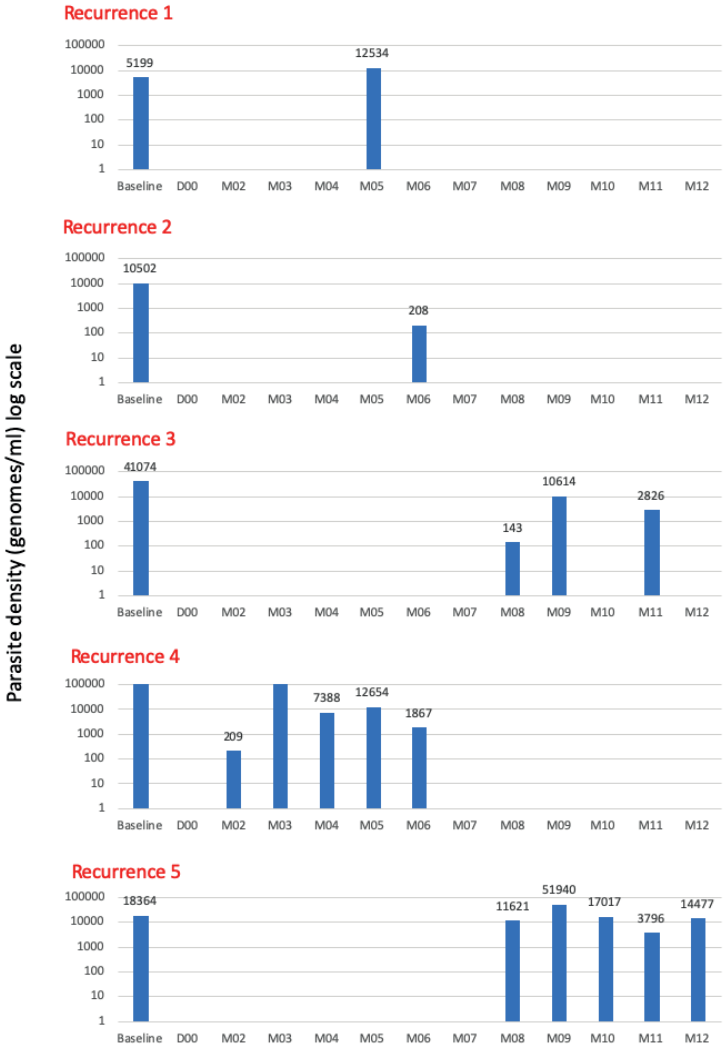

Fig. 4. the pattern of recurrent *P.vivax* infections in 5 study participants all in the placebo group. The x-axis shows the time of the survey in relation to the drug administration (D=Day, M=Month; Baseline of Recurrent 1, 2, and 3=M0 of malaria elimination project; Baseline of Recurrent 4 and 5=During cross sectional surveys of malaria elimination project either M6, M9 or M12). The y-axis shows the density (genomes/ml) on a log scale. The numbers above the columns indicate the parasite density at that point in time.

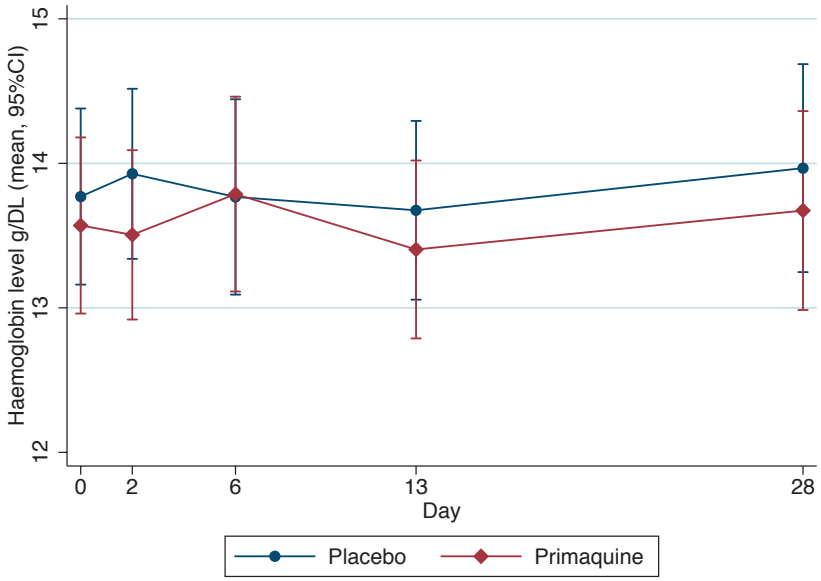

Fig. 5. Changes in haemoglobin level of participants in primaquine and placebo arms over the first 28 days after drug administration

Discussion

In this placebo-controlled evaluation, nested within the study of dihydroartemisinin-piperaquine mass antimalarial drug treatment conducted in Lao PDR, a 14-day primaquine regimen of 0.5 mg/kg/day following a three-day course of DP was well tolerated and effective in the prevention of recurrences over a period of 12-month follow-up in participants with asymptomatic *P. vivax* infection. None of the participants in primaquine arm had recurrent *P. vivax* infections. The primaquine dose used in our study was double the standard dose recommended by the Lao national malaria treatment guideline. However, the World Health Organization, US Centre for Disease Control and many European countries have recommended this higher dose for *P. vivax* infections in East Asia and Oceania [14]. Provided patients with G6PD deficiency are excluded, this dose has been shown to be safe [15, 16]. Although safety in G6PD heterozygotes not identified by the fluorescent spot test remains an open question [17]. In this small study, the higher primaquine dose was safe and well tolerated by our participants without a clinically relevant drop in haemoglobin levels. The administration of at least one full course of schizontocidal drugs, DP with a SLDPQ without a full course of an 8-aminoquinoline, had no apparent impact on recurrent vivax infections. The study highlights once more the critical need for the radical cure with an 8-aminoquinoline to eliminate all malarias in vivax endemic regions [18]. The radical treatment of *P. vivax* can consist of 14-day primaquine or a single dose of tafenoquine. To adhere to 14 days of primaquine for successful treatment is important but can be challenging. As a consequence, many trials tried to shorten the regimen varying the cumulative dose of primaquine and the duration of treatment. 7-day high dose primaquine (total dose of 7 mg/kg) is as efficacious as standard 14-day high-dose primaquine in radical treatment of vivax malaria at one year follow-up, but quantitative G6PD testing is required as there is a higher risk of haemolysis in treatments with a higher daily primaquine dose [15, 16]. However, the duration less than 7 days proved to be less effective [19]. Takeuchi *et al.* compared DOT for 14 days of primaquine versus non- DOT primaquine, and found Non-DOT group experienced more recurrences [20]. Novel, robust, quantitative G6PD test are already available and more products are under development [21, 22]. The combination of reliable G6PD testing combined with safe and effective 8-aminoquinoline regimes holds promise for the elimination of all malarias.

Recurrent asymptomatic *P. vivax* infections were seen throughout the follow-up period but only in the control group which had not received primaquine. Recurrent infections can have three possible causes. They can be caused by recrudescent or persistent infections which is

unlikely considering the delay between schizonticidal treatment and observed infection. They can also be due to re-infections caused by a new mosquito bite. This explanation is also not very likely in this study as no new infections were observed in the participants who had received a full course primaquine which clears all hypnozoites. Within one month of primaquine treatment the participants in the primaquine group had the same risk of becoming re-infected as in the placebo group but had no infections. This observation suggests that the *P. vivax* transmission in the study site is low and the recurrent infections in the control group are most likely due to relapses due to the activation of hypnozoites.

This study shows the potential of using uPCR as a tool to assess the primary endpoint of recurrent infections without obvious clinical outcomes. Participants with recurrent infections showed no clinical signs related to their *P. vivax* infections which are likely to include gametocytes at some point in time and hence continue to contribute to the transmission of *P. vivax* [2, 23]. It is of note that the participant with the highest parasite load at enrolment had the shortest lag time to the first recurrence and was found to be infected with *P. vivax* during the following 5 surveys. By contrast, the participant with the lowest parasite density at enrolment had the first documented recurrent infection six months after enrolment. This observation would support the notion of a parasite density related recurrence rate. However, this is speculative as the number of participants with a recurrent infection is very low in this study. The South East Asian region is known to have a short lag time to the first recurrent infection which is on average 41 days [1, 24]. Short interval relapses are usually caused by tropical *P. vivax* strain, while temperate and sub-tropical strains have long incubation periods for relapse [25]. Time from initial infection to relapse and relapse frequency are not only determined by geographic origin of *P. vivax* strains but also number of inoculated sporozoites received from infected mosquitoes. The more sporozoites the liver harbours, the greater the chance to get illness early and the greater the frequency of relapses [26].

For comparison with historical studies, the outcome of our study can be rephrased as an incidence rate in the placebo group of 35.6 recurrent episodes/100 person-years (95% CI: 14.8 to 85.5). This rate of recurrent asymptomatic infections detected by uPCR compares well with the recurrence rate observed following clinical vivax malaria episodes in historical studies. In a recent large vivax malaria treatment trial [17], the recurrence rate over a year was 48.7 recurrent episodes /100 person-years (95% CI: 43.4-54.4). As relapses following treatment of asymptomatic infections appear to have less periodicity (and thus early clustering) than those which follow symptomatic infections, long follow up is required. It is, therefore, unlikely that

treatment trials in people with subclinical infections can curtail the study period required for trials. Trialists may still be interested in recruiting people with subclinical infections to study radical curative treatments, as the prevalence of subclinical infections is much higher than of clinical episodes. None of the *P. vivax* infections detected in our study had any clinical signs or symptoms of malaria.

The study has several limitations. The sample size was very small; the number of people with *P. vivax* infections was lower than expected, limiting our enrolment to 40 participants. Secondly, the study was conducted following 3 rounds of MDA. While the first 18 participants (in the intervention villages of MDA trial) were recruited and randomized for trial within 2 months of the first positive uPCR, the remaining 22 participants were recruited in control villages 12 months later. Despite this, the distribution of participants with recurrent infections was similar in these two periods of time. There were no differences in climate between the two years, something that could have influenced the risk for reinfection. thirdly, uPCR is a sophisticated tool that cannot be used in the field leading to a delay between blood collection and uPCR result. lastly, more frequent follow-up of blood draws with uPCR might give insight into persistence of the infection.

Conclusion

In the context of mass drug administration and correct G6PD testing, a 0.5 mg/kg/day dose of primaquine for 14 days following three rounds of dihydroartemisinin-piperaquine was safe, well-tolerated and effective in the prevention of recurrence of asymptomatic *P. vivax* infections. The elimination of all malarias could be much accelerated by the roll out of the radical cure with high dose primaquine or tafenoquine.

Declarations

Ethics

Participants provided written informed consent by signature or thumbprint for participants older than 15 years. Children aged between 12 and 15 years co-signed or thumbprinted with their parents or guardians. This study received ethical approval by the National Ethics Committee for Health Research (074NIOPH/NECHR, 11th January 2016), Ministry of Health, Lao PDR and Oxford Tropical Research Ethics Committee (OxTREC Reference 19-16, 18th May, 2016), University of Oxford.

Trial registration: This study was registered with ClinicalTrials.gov under NCT02802813 on 16th June 2016. https://clinicaltrials.gov/ct2/show/NCT02802813

Consent for publication: not applicable as no personal data are included

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

Competing interests: The authors have declared that no competing interests exist.

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Abbreviations:

G6PD, Glucose 6 Phosphate Dehydrogenase; uPCR, ultrasensitive quantitative Polymerase Chain Reaction; Lao PDR, Lao People's Democratic Republic; MORU, Mahidol-Oxford Research Unit MDA, Mass Drug Administration; DP, dihydroartemisinin-piperaquine; SLDPQ, single low dose primaquine; Hb, haemoglobin

Author Contributions:

KP wrote the first draft of this article. KP, LvS, NPJD, AMD, PNN, NJW, and MMa developed the study concept. KP, PS, and PP curated the data. KP, FvL, MMu analysed the data. NPJD, AMD, NJW acquired the funding for the study. LvS, KP, PNN, MMa administered the project. KP, FvL, MI, GH, TP, BA, TJP, CP, MD, NPJD, FC, AMD, PNN, NJW, LvS, and MMa supervised the project. All authors read and approved the final manuscript.

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Part II-Asymptomatic malaria

Chapter 5-Asymptomatic Plasmodium infections in 18 villages of southern Savannakhet Province, Lao PDR (Laos)

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Malaria journal 2016, **15**(1):296.

Abstract

Background

A large fraction of *Plasmodium* infections do not cause clinical signs and symptoms of disease and persist at densities in blood that are not detectable by microscopy or rapid diagnostic tests. These infections may be critical as a transmission reservoir in areas of low malaria endemicity. Understanding the epidemiology of these infections would be helpful for malaria elimination.

Methods

A cross-sectional survey was conducted in Thapangthong and Nong Districts of Savannakhet Province, Lao PDR, to determine the prevalence of parasitaemia. A total of 888 blood samples were collected from afebrile volunteers aged ≥15 years in 18 villages during March and July 2015. *Plasmodium* infections were diagnosed by rapid diagnostic tests (RDT) and high volume, ultra-sensitive quantitative polymerase chain reaction (uPCR).

Results

uPCR detected *Plasmodium* infections in 175 of 888 samples (20%). The species distribution was *Plasmodium falciparum* 3.6% (32/888), *Plasmodium vivax* 11.1% (99/888), mixed infections with *P. falciparum* and *P. vivax* 1.6% (14/888) and *Plasmodium* of undetermined species 3.4% (30/888;). RDT identified only 2% (18/888) positive cases. Using uPCR as reference, the sensitivity and specificity of RDTs were 28 and 100%, respectively, in detecting *P. falciparum* infections, and 3 and 99% in detecting asymptomatic *P. vivax* infections. The K13 kelch propeller domain C580Y mutation, associated with reduced susceptibility to artemisinin derivatives, was found in 75% (12/18) of *P. falciparum* isolates from Thapangthong and in 7% (2/28) from Nong ($p < 0.001$). In a multivariate analysis, males were more likely to have *P. vivax* infections (adjusted odds ratio (aOR)4.76 (95% CI 2.84-8.00)) while older villagers were at lower risk for parasitaemia (aOR for increasing age 0.98 (95% CI 0.96-0.99)).

Conclusion

There is a high prevalence of asymptomatic malaria in southern Savannakhet. Artemisinin-resistant *P. falciparum* strains are replacing susceptible strains in Thapangthong District and are already present in the more remote Nong District. This worrying trend has wider implications for Laos and could reverse the gains achieved by the successful control of malaria in Laos and the Greater Mekong Sub-region (GMS). Rapid elimination of *P. falciparum* has to be a top priority in Laos as well as in the wider GMS.

Keywords: Asymptomatic malaria, RDT, uPCR, Prevalence, Lao PDR.

Background

Substantial progress has been made in the control of malaria in the Lao PDR (Laos), in particular in the north of the country. The five southernmost provinces, Savannakhet, Salavan, Sekong, Champasack, and Attapeu accounted for 90% of all malaria patients reported in the country in 2008 [1]. A nationwide malaria survey conducted between 2006 and 2008, including 495 health centres in Savannakhet Province, reported an overall incidence of 11.5 *Plasmodium falciparum* cases per 1,000 people. The survey ranked Savannakhet as the province with the third highest *P. falciparum* cases recorded in 2008 [2].

In Laos, many remote health centres rely on rapid diagnostic tests (RDT) for malaria diagnosis. Few regional and district-level health centres have access to microscopy [3]. The nationwide prevalence survey carried out during 2006-2008 was based on passive case reporting by provincial and district hospitals, provincial malaria stations, health centres, and village health workers (VHWs). Case detection was based on either RDTs or microscopy [1,2]. The majority of malaria infections remained undetected since only symptomatic cases were captured [4-7]. People with asymptomatic *Plasmodium* infections can carry very low parasite densities, for extended periods, which are undetectable by microscopy or RDTs [5,6]. Mosquitoes feeding on blood samples from individuals with sub-microscopic *Plasmodium* infections can become infected [8,9]. Thus, sub-microscopic carriers contribute to malaria transmission [10,11].

The elimination of malaria in the greater Mekong Sub-region (GMS) has become particularly urgent with the emergence and spread of artemisinin resistance, the failure of artemisinin combination therapy (ACT) partner drugs, and the threat of untreatable malaria [12]. Current recommendations to prevent further spread of drug-resistant malaria from Southeast Asia advocate regional malaria elimination [13,14]. As a part of National Strategic Plan for Malaria Control and Elimination 2011-2015, Laos has adopted the goal of eliminating malaria by 2030 [1,15].

To gain a better understanding of which villages need to be targeted for malaria elimination, a survey was conducted in 18 villages of southern Savannakhet Province, which has a historically high malaria prevalence based on village malaria worker records.

Methods

Study site and design

The study was conducted in southern Savannakhet Province, Laos. The province is ~600 km south from Vientiane, the capital city of Laos. It has a total area of 21,774 sq km and includes 15 districts [16]. Savannakhet is the most populous province of Laos with a total population of ~843,245 people, representing about 14% of the population of the country. The province has one provincial hospital, 15 district hospitals and 115 health centres. This health system covers approximately 89% of the province's geographical area [17]. Cross-sectional surveys were conducted in Thapangthong and Nong Districts (see **Figure 1**). The districts and villages were chosen based on the previous high malaria incidence in provincial epidemiological records. Villagers were informed by local health centre staff of the reasons for the survey and requested to arrive at a suitable location within each village. A mobile study team with blood collection equipment, tools for anthropometry and essential medicines conducted the study in each village.

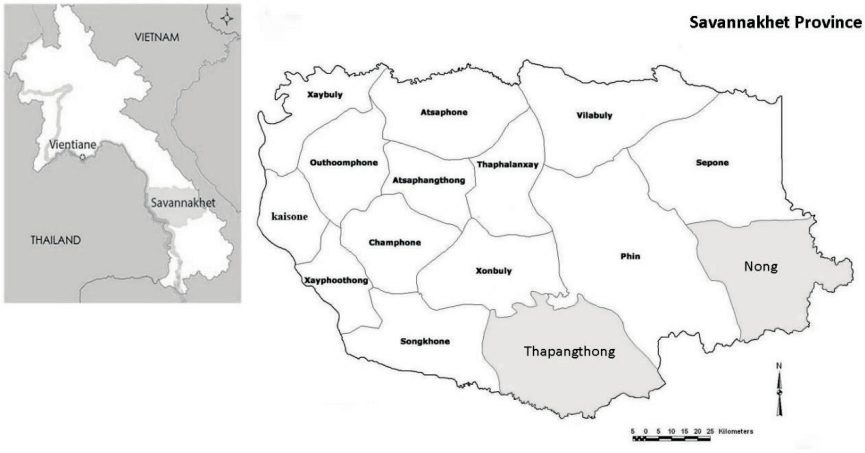

Figure 1. Study sites within Savannakhet province

Study participants and procedures

A short description of the study was announced at village meetings. Additional explanations about the study were provided to each participant during the consent process before blood sample collection. Volunteers of age ≥15 years were enrolled into the study. Written consent

was obtained from each volunteer before participation. Travel costs were reimbursed and vitamin B complex and/or haematinics were given to the study participants based on the judgment of study clinicians. Information on demographics (age, sex, weight, height), tympanic temperature, history of fever and history of illness during the previous 48 hours, a history of malaria, anti-malarial drug treatment, recent travel, and bed net use was collected using the Open Data Kit (ODK®) application on a smartphone.

Sample collection

Villagers who met the inclusion criteria were asked to give 3 mL venous blood samples which were collected into EDTA tubes and stored in ice pack cooling boxes until their transportation to a centralized field laboratory in the two districts (within six hours of blood collection). Upon return to the centralized laboratory, the whole blood was separated and the red blood cell pellets were frozen promptly and stored at -20^0C for up to seven days. Each sample was labelled with a barcode to ensure blinding and negative controls were added to the sample pool. The samples were transported on dry ice to the molecular parasitology laboratory in Bangkok, Thailand for analysis.

All participants were tested on site for malaria using the SD Bioline Ag Pf/Pan (Standard Diagnostics Inc) RDT. Those with positive RDTs were treated with artemether/lumefantrine, as per Laos national treatment guidelines. The RDT test was performed and interpreted by an experienced laboratory technician according to manufacturer's recommendation.

DNA extraction and PCR amplification

DNA was extracted from thawed packed red blood cells using an automated DNA extraction machine (QIAsymphony and DPS DNA midi kit; Qiagen, Germany). DNA was dried, concentrated and then used as a template for PCR detection and quantification of *Plasmodium*. Quantitative ultrasensitive PCR (uPCR) analysis was performed as described elsewhere [18]. Briefly, DNA of *Plasmodium* was detected and quantified using 18S rRNA-targeting primers. The limit of detection was ~22 parasites/mL. For *Plasmodium*-positive samples, an attempt was made to identify the species using *P. falciparum*- and *Plasmodium vivax*-specific PCR protocols as described previously [18].

To detect polymorphisms associated with reduced susceptibility to artemisinin derivatives, the open-reading frame of the PF3D7_1343700 kelch propeller domain was amplified using a nested PCR protocol [12,19]. Purified PCR products were sequenced at Macrogen, Republic

of Korea, and analysed using BioEdit version 7.1.3.0., using the 3D7 kelch13 sequence as reference (Accession: XM_001350122.1). The definition of single nucleotide polymorphisms (SNPs) was based on analytical approaches described previously [12,20].

Statistical analysis

Asymptomatic malaria was defined as a *Plasmodium* infection detected in study participants who were afebrile (tympanic temperature of <37.5°C) at the time of the survey and had no history of fever in the preceding 48 hours. Sub-microscopic infections were here defined as a *Plasmodium* infection with densities too low to be detectable by microscopy and malaria RDT but detectable by uPCR.

Descriptive statistics were used to analyse the baseline characteristics of the study population. The Chi-squared test and Kruskal-Wallis test were used to test associations between variables and *Plasmodium* infection in univariate analyses. Multiple logistic regression models were constructed and used to explore associations between *P. falciparum* mono-infections and independent co-variables as well as for *P. vivax* mono-infections and independent co-variables. Variables which were significant at the $p < 0.05$ level in the univariate analysis were evaluated in a multivariable regression model. The models were run for any *Plasmodium* infections and then for *P. falciparum* and *P. vivax* as dependent variables. P-values less than 0.05 were considered statistically significant. Statistical analysis was performed using STATA 14.0 (StataCorp, College Station, TX, USA).

Ethics statement

Ethical approval for the study was received from the Lao National Ethics Committee for Health Research (Ref No 013-2015/NECHR), Government of the Laos and the Oxford Tropical Research Ethics Committee (1015-13). Individual informed consent was obtained from each participant.

Results

A total of 888 volunteers, 433 from eight villages in Thapangthong District and 455 from ten villages in Nong District participated in the study. The surveys were conducted between 21 and 24 March, 2015 in Thapangthong District and between 22 and 26 July, 2015 in Nong District.

Table 1: Baseline characteristics of the study population (n=888)

Characteristics	Results					P-Value**
	Negative	Positive				
		P. falciparum	*P. vivax*	Mixed (*Pf*+*Pv*)*	*Plasmodium* spp.	
Sex						
Male (n=479)	354 (74%)	16 (3%)	76 (16%)	10 (2%)	23 (5%)	**<0.001**
Female (n=409)	359 (88%)	16 (4%)	23 (6%)	4 (1%)	7 (2%)	
Age group						
15 – 35 years (n=505)	383 (76%)	22 (4%)	73 (15%)	8 (2%)	19 (4%)	**0.009**
36 – 55 years (n=234)	196 (84%)	7 (3%)	20 (9%)	5 (2%)	6 (3%)	
56 – 80 years (n=149)	134 (90%)	3 (2%)	6 (4%)	1 (0.7%)	5 (3%)	
Mean age in years (n=888; SD)	37.0 (16.2)	32.0 (13.9)	30.9 (13.6)	32.9 (13.9)	34.7 (15.7)	**0.005**
History of Fever in the last 48 hours						
Yes (n=277)	223 (80%)	17 (6%)	26 (9%)	4 (1%)	7 (3%)	0.056
No (n=611)	490 (80%)	15 (3%)	73 (12%)	10 (2%)	23 (4%)	
Feeling ill in the last 48 hours						
Yes (n=451)	370 (82%)	20 (4%)	43 (10%)	5 (1%)	13 (3%)	0.186
No (n=437)	343 (79%)	12 (3%)	56 (13%)	9 (2%)	17 (4%)	
History of malaria						
Yes (n=493)	367 (74%)	21 (4%)	76 (15%)	10 (2%)	19 (4%)	**<0.001**
No (n=385)	336 (87%)	11 (3%)	23 (6%)	4 (1%)	11(3%)	
Episodes of malaria						
0 Episodes (n=395)	346 (88%)	11 (3%)	23 (6%)	4 (1%)	11 (3%)	**<0.001**
1 to 3 episodes (n=409)	320 (78%)	20 (5%)	47 (12%)	7 (2%)	15 (4%)	
>3 episodes (n=84)	47 (56%)	1 (1%)	29 (35%)	3 (4%)	4 (5%)	
Stay overnight in the forest in the last 3 months*						
<2 weeks ago (n=183)	136 (74%)	7 (4%)	30 (16%)	3 (2%)	7 (4%)	**<0.001**
2 to 4 weeks ago (n=27)	16 (59%)	1 (4%)	8 (30%)	1 (4%)	1 (4%)	
>4 weeks ago (n=66)	43 (65%)	1 (2%)	15 (23%)	4 (6%)	3 (5%)	
No forest visits (n=612)	518 (85%)	23 (4%)	46 (8%)	6 (1%)	19 (3%)	
Travelled outside the village in the last 3 months*						

Yes (n=232)	188 (81%)	10 (4%)	23 (10%)	4 (2%)	7 (3%)	
No (n=656)	525 (80%)	22 (3%)	76 (12%)	10 (2%)	23 (4%)	0.902
Bed net use						
Every day (n=682)	559 (82%)	26 (4%)	67 (10%)	8 (1%)	22 (3%)	0.132
Sometimes (n=143)	108 (76%)	3 (2%)	24 (17%)	4 (3%)	4 (3%)	
Never (n=63)	46 (73%)	3 (5%)	8 (13%)	2 (3%)	4 (6%)	

*Mixed infection with *P. falciparum* and *P. vivax* ** chi-squared test

Demographic characteristics of participants

The overall median age was 33 years (IQR 22-47 years). More than half (57%) of the participants were in the age range between 15 and 35 years and 479/888 (54%) were male. Only two among 888 participants (0.2%) had a fever (≥ 37.5°C) at the time of the survey (**Table 1**). One febrile participant (39.3°C) was blood smear- and RDT-negative but was later found to be infected by *P. vivax* using uPCR. The other participant (37.6°C) was not parasitaemic. The mean weight and height of participants were 48 kg (SD=7.6) and 153 cm (SD=7.3), respectively.

Prevalence of *Plasmodium* infections

Overall, *Plasmodium* parasites were detected in 175/888 individuals (20%) by uPCR (**Table 2**). 99/888 (11%) had *P. vivax* mono-infections, 32/888 (4%) had *P. falciparum* mono-infections and 14/888 (2%) had mixed infections (*P. vivax* and *P. falciparum*). The *Plasmodium* species could not be determined in specimens from 30/888 (3.4%) patients. The prevalence of *P. vivax* mono-infections was significantly lower in Nong (5% (22/455)) than in Thapangthong (18% (77/433); p <0.0001). In contrast, the prevalence of *P. falciparum* mono-infections was higher in Nong (5% (24/455)) compared to Thapangthong (2% (8/433); p=0.1; **Table 2**). There was also high variability in *Plasmodium* prevalence within districts. In the eight villages in Thapangthong District the *Plasmodium* infection prevalence ranged between 12 and 39% and in the ten villages in Nong District between 4 and 56%.

Resistance markers

The mutation C580Y in the PF3D7_1343700 kelch propeller domain, which is associated with reduced susceptibility to artemisinin derivatives, was found in 32% (of 14/44) *P. falciparum* strains (**Table 2**). No other kelch polymorphisms were detected. The C580Y mutation was

found in 75% (12/18) of *P. falciparum* strains detected in Thapangthong and in 7% (2/28) strains detected in Nong ($p < 0.001$).

Plasmodium prevalence by RDT

RDTs detected eight participants (8/888; 0.9%) with *P. falciparum* mono-infections, six participants (6/888; 0.7%) with non-*P. falciparum* infections and four participants (4/888; 0.5%) with mixed infections (*P. falciparum* and non-*P. falciparum;* **Table 3**). Using uPCR as reference, the sensitivity and specificity of RDTs were 28 and 100%, respectively, in detecting *P. falciparum* infections and 3 and 99% in detecting *P. vivax* infections (**Table 4**).

Table 2: Parasite prevalence detected by uPCR by village and district

Villages	Negative	Positive				PfK-13 resistance Marker C580Y
		PF	*PF+PV*	*PV*	*P species*	
Thapangthong district						
Maiphosy (n=50)	38 (76%)	0 (0%)	1 (2%)	11 (22%)	0 (0%)	1/1 (100%)
Bouttaphan (n=59)	42 (71%)	1 (2%)	2 (3%)	14 (24%)	0 (0%)	3/3 (100%)
Maixe (n=58)	47 (81%)	2 (3%)	0 (0%)	7 (12%)	2 (3%)	1/2 (50%)
Phouphanang (n=56)	37 (66%)	2 (4%)	1 (2%)	15 (27%)	1 (2%)	3/3 (100%)
Phoumaly (n=52)	42 (81%)	1 (2%)	1 (2%)	6 (12%)	2 (4%)	2/2 (100%)
Nalao (n=52)	40 (77%)	1 (2%)	2 (4%)	7 (13%)	2 (4%)	0/3 (0%)
Khampia (n=49)	30 (61%)	1 (2%)	1 (2%)	14 (29%)	3 (6%)	2/2 (100%)
Kengkhai (n=57)	50 (88%)	0 (0%)	0 (0%)	3 (5%)	4 (7%)	0/0
Total (n=433)	**326 (75%)**	**8 (2%)**	**8 (2%)**	**77 (18%)**	**14 (3%)**	**12/16 (75%)**
Nong district						
Oi Tan Tip (n=50)	22 (44%)	4 (8%)	5 (10%)	15 (30%)	4 (8%)	0/8 (0%)
Denvilay (n=50)	46 (92%)	1 (2%)	0 (0%)	2 (4%)	1 (2%)	0/1 (0%)
Asing na (n=19)	15 (79%)	1 (5%)	0 (0%)	2 (11%)	1 (5%)	0/1 (0%)
Asing saly (n=29)	27 (93%)	0 (0%)	0 (0%)	0 (0%)	2 (7%)	0/0
Phounmakmy (n=57)	46 (81%)	9 (16%)	0 (0%)	0 (0%)	2 (4%)	0/8 (0%)
Kaysone (n=50)	46 (92%)	2 (4%)	0 (0%)	1 (2%)	1 (2%)	0/1 (0%)
Thathe (n=49)	45 (92%)	1 (2%)	1 (2%)	1 (2%)	1 (2%)	1/2 (50%)
Xuangtai (n=50)	44 (88%)	3 (6%)	0 (0%)	0 (0%)	3 (6%)	0/1 (0%)
Paloy (n=47)	45 (96%)	1 (2%)	0 (0%)	1 (2%)	0 (0%)	1/1 (100%)
Salang (n=54)	51 (94%)	2 (4%)	0 (0%)	0 (0%)	1 (2%)	0/2 (0%)
Total (n=455)	**387 (85%)**	**24 (5%)**	**6 (1%)**	**22 (5%)**	**16 (4%)**	**2/28 (7%)**
Total (n=888)	**713 (80%)**	**32(4%)**	**14(2%)**	**99(11%)**	**30(3%)**	**14/44 (32%)**

Table 3: *Plasmodium* species identification by RDT and qPCR

	qPCR					
RDT	**Negative**	**P. falciparum**	**P. vivax**	**Mixed***	***Plasmodium* spp.**	**Total**
Negative	708	23	96	13	30	870
P. falciparum	0	7	0	1	0	8
Non-falciparum	4	0	2	0	0	6
Mixed infection*	1	2	1	0	0	4
Total	713	32	99	14	30	888

* *P. falciparum* and *P. vivax*

Table 4: RDT performance characteristics (sensitivity, specificity, positive predictive value, negative predictive value)

		RDT			
		Sensitivity % (95% CI)	Specificity % (95% CI)	PPV (95% CI)	NPV (95% CI)
uPCR	*P. falciparum* (n=36)	27.8 (14.2 – 45.2)	99.9 (99.1 – 100)	90.9 (57.1 – 99.5)	96.5 (94.8 – 97.6)
	P. vivax (n=109)	2.7 (0.7 – 8.2)	99.3 (98.3 – 99.7)	37.5 (10.2 – 74.1)	86.7 (84.1 – 88.9)

PPV – Positive predictive value; NPV – Negative predictive value.

Factors associated with *Plasmodium* infections

Five covariates were independently and significantly associated with *P. vivax* mono-infections (**Table 5**): male sex (Odds Ratio adjusted for age and district 4.76 (95% CI 2.84-8.00)), increasing age (aOR 0.98 (95% CI 0.96-0.99)), residing in Nong District (aOR 0.16 (95% CI 0.10-0.27)), a history of three or more malaria episodes (aOR 1.56 (95% CI 1.08-2.25)), and an overnight stay in a forest in the last three months (aOR 2.45 (95% CI 1.17-5.11)). There was a statistically significant association between a history of fever in the previous 48 hours and *P. falciparum* mono-infections (aOR 2.47 (95% CI 1.20-5.08; **Table 6**)). Increasing age was found to be protective against *P. falciparum* infection in the univariate analysis (OR 0.97 (95% CI 0.96-1.00)) but not in the multivariate analysis (aOR 0.98 (0.95-1.00)).

Table 5: Multiple logistic regression analysis to identify independently significant variables associated with *P. vivax* mono-infections (n=99)

Covariates	Univariate Analysis	p-value	Multivariate analysis	p-value
	Crude OR (95% CI)		**Adj. OR (95% CI)**	
Sex Male (n=812)	3.35 (2.06 5.46)	**<0.001**	4.76 (2.84 to 8.00)[a]	**<0.001**
Age (n=812)	0.97 (0.96 to 0.99)	**0.001**	0.98 (0.96 to 0.99)[b]	**0.002**
District Nong (n=812)	0.21 (0.13 to 0.34)	**<0.001**	0.16 (0.10 to 0.27)[c]	**<0.001**
History of Fever in the previous 48 hours (n=812)	0.78 (0.49 to 1.26)	0.31	1.02 (0.61 to 1.72)[d]	0.92
Feeling ill in the previous 48 hours (n=812)	0.71 (0.47 to 1.09)	0.12	0.98 (0.62 to 1.55) [d]	0.92
History of 3 or more malaria episodes (n=802)	9.28 (4.96 to 17.36)	**<0.001**	1.56 (1.08 to 2.25) [d]	**0.02**
Stay overnight in the forest in the last 3 months (n=812)	3.06 (2.00 to 4.70)	**<0.001**	2.45 (1.17 to 5.11) [d]	**0.02**
Travelled outside the village in the last 3 months (n=812)	0.85 (0.51 to 1.39)	0.50	0.92 (0.54 to 1.57) [d]	0.76
Bed net never used (n=812)	1.28 (0.58 to 2.79)	0.31	1.55 (0.66 to 3.66) [d]	0.45

[a] Adjusted for age and district, [b] Adjusted for sex and district, [c] Adjusted for age and sex, [d] Adjusted for age, sex and district

Table 6: Multiple logistic regression analysis to identify independently significant variables associated with *P. falciparum* mono-infections (n=32)

Covariates	Univariate Analysis	p-value	Multivariate analysis	p-value
	Crude OR (95% CI)		**OR adj. (95% CI)**	
Sex Male (n=745)	1.01 (0.50 to 2.06)	0.97	0.84 (0.87 to 4.65) [a]	0.65
Age (n=745)	0.97 (0.96 to 1.00)	**0.01**	0.98 (0.95 to 1.00) [b]	**0.08**
District Nong (n=745)	1.89 (0.84 to 4.3)	0.12	2.02 (0.87 to 4.65) [c]	0.10
History fever in the last 48 hours (n=745)	2.49 (1.22 to 5.08)	**0.01**	2.47 (1.20 to 5.08) [d]	**0.01**
Feeling ill in the last 48 hours (n=745)	1.55 (0.74 to 3.21)	0.24	1.70 (0.81 to 3.59) [d]	0.16
History of any malaria (n=735)	1.75 (0.83 to 3.68)	0.14	1.68 (0.79 to 3.61) [d]	0.18
History of 3 or more malaria episodes (n=735)	0.67 (0.09 to 5.30)	0.70	0.87 (0.10 to 7.40) [d]	0.90
Stay overnight in the forest in the previous 3 months (n=745)	1.04 (0.47 to 2.28)	0.92	1.04 (0.44 to 2.41) [d]	0.94
Travelled outside the village in the previous 3 months (n=745)	1.27 (0.59 to 2.73)	0.54	1.91 (0.55 to 2.58) [d]	0.66
Bed net never used (n=745)	1.50 (0.44 to 5.11)	0.52	1.18 (0.34 to 4.10) [d]	0.80

[a] Adjusted for age and district, [b] Adjusted for sex and district, [c] Adjusted for age and sex, [d] Adjusted for age, sex and district

Discussion

This is the first survey conducted in Laos of sub-clinical and sub-microscopic malaria prevalence using uPCR. The study found that one-fifth of the afebrile population in southern Savannakhet was carrying *Plasmodium* infections, a third of which may be resistant to artemisinin derivatives. The prevalence of asymptomatic *Plasmodium* infection was very heterogeneously distributed not only between districts but also between villages separated by short distances.

There has been a shift from the traditional dominance of *P. falciparum,* which used to be the most prevalent (≥90%) species in Laos, towards *P. vivax* [21-25]. An increase in the proportion of *P. vivax* infections has been reported from Thailand [26], Cambodia [27], Solomon Islands [28], Amazon [29,30], and central America [31] as falciparum malaria incidence has declined. A recent, large, multi-centre, cross-sectional survey conducted in Cambodia, Vietnam and Thai-Myanmar border found almost twice as many *P. vivax* infections compared to *P. falciparum* infections among a total of 988 *Plasmodium* infections [32]. Current malaria control practices in Laos are more effective in the control of *P. falciparum* than *P. vivax*. The complete, radical cure of vivax infections requires a 14-day course with 8-aminoquinolines, such as primaquine, and this is not implemented currently. There remains considerable reluctance to use primaquine in radical cure while G6PD testing is unavailable and there is therefore a significant haemolytic risk.

A high geographical heterogeneity was also found in the distribution of the K13 mutation C580Y which is associated with resistance against artemisinin derivatives [12,19]. In Thapangthong District 75% of the tested *P. falciparum* strains had the K13 C580Y mutation. In contrast, in Nong District the K13 mutation was only detected in 7% of *P. falciparum* strains. This finding supports the hypothesis that with decreasing falciparum malaria transmission resistant strains replace wild type strains [14].

The heterogeneity in the prevalence of *Plasmodium* infections may at least in part be explained by environmental factors, such as the location of homes in relation to mosquito breeding sites, the design and construction materials of the home, and protective measures taken by the residents. The heterogeneity in the prevalence of *Plasmodium* infections in this study is consistent with an earlier study conducted in Laos [2]. The reason for heterogeneity suggested by the authors included the proximity of deep forest and the characteristics of the principal

vectors in Laos which are *Anopheles dirus* and *Anopheles minimus* [33]. The dispersal range of *An. dirus* and *An. minimus,* combined with the anthropophilic behaviour of *An. minimus* was thought to explain at least some of the variability in malaria prevalence between villages [33,34]. In a study conducted in Cambodia, villages proximal to forested areas had a higher prevalence of *Plasmodium* infections compared to villages more distant from forested areas [35]. Detailed observations on the micro-epidemiology of *P. falciparum* infections found similar large differences between villages in sub-Saharan Africa and in Europe in the last century [36]. Different environmental (mosquito densities, abundance of larval habitats) and human-mosquito behaviour (exposure to mosquitoes due to occupation, such as working in the forest) can explain in large part the geographical variation in species epidemiology [28]. Genetically determined host factors such as red cell abnormalities, and possibly immune response genes, may also contribute to differences observed between villages [37].

The sensitivity of RDTs in this study was 28% for *P. falciparum* infections and 3% for *P. vivax* infections using uPCR as reference. The wide discrepancy in prevalence measured by RDT compared to uPCR in this current study is consistent with a multi-national survey, which used the same uPCR detection technique [32]. This earlier study, conducted in Cambodia, Thai-Myanmar border and Vietnam, found a *Plasmodium* prevalence of 4% by RDT compared to 20% by uPCR. A survey using RDT conducted in Laos in 2006-2008 [1,2] found a 1.2% prevalence of *P. falciparum* infections - similar to the 0.9% *P. falciparum* prevalence detected by RDT in the current study.

Several factors were associated with *P. vivax* mono-infections. Being a male was associated with higher prevalence of *P. vivax* infection than being female. The increased risk of contracting malaria for males may be explained by work-related exposure in the field and forested areas [3]. A similar gender imbalance was also observed on the Thailand-Myanmar border, in Cambodia [27] and Ethiopia [5,38]. A history of sleeping in the forest was found to be associated with increased *P. vivax* prevalence. Forest workers have been traditionally a high-risk group for malaria in the Greater Mekong Sub-region [3,39-43]. Control of forest malaria has proven to be highly challenging and innovative approaches will be needed to interrupt malaria transmission among forest workers.

The peak risk for *Plasmodium* infections in this study was in the age group 15-35 years and decreased with older age. Younger participants may have been less exposed to vectors while

older participants may have developed sufficient immunity to clear infections. A similar peak prevalence in adults has been observed in past studies in Laos [44] and Cambodia [35].

Conclusion

The study found a high prevalence of asymptomatic malaria as detected by uPCR and three times more *P. vivax* than *P. falciparum* infections in the southern Savannakhet Province. The distribution of infections was very heterogeneous, with some villages nearly free of infections while in other villages more than half of the village population was infected. Artemisinin-resistant *P. falciparum* strains appear to have replaced susceptible strains in Thapangthong District and are already present in the more remote Nong District. This worrying trend has wider implications for Laos and could reverse the gains achieved by the successful control of malaria in Laos and the GMS. The rapid elimination of *P. falciparum* should be a top priority in Laos and the GMS before malaria there becomes untreatable. Such elimination will require targeted interventions in high prevalence villages.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KP, BA, GH, TP, LvS, NJW, AD, PNN, and MM: study design; MI: molecular biology; GH: data management; LvS: statistics. All authors contributed to final, submitted draft

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Chapter 6-Perceptions of asymptomatic malaria infection and their implications for malaria control and elimination in Laos

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Abstract

Background

In the Greater Mekong Sub-region (GMS), malaria elimination efforts are targeting the asymptomatic parasite reservoirs. Understanding community perceptions about asymptomatic malaria infections and interventions that target this reservoir is critical to the design of community engagement. This article examines knowledge, attitudes, perceptions and practices related to asymptomatic malaria infections and mass drug administration (MDA) in malaria-endemic villages in southern Savannakhet Province, Laos.

Methods

A questionnaire consisting of questions on socio-demographic characteristics, knowledge, attitudes, perceptions and practices on malaria and MDA was administered to each household head or representative (n=281) in four villages. These topics were also further discussed in 12 single-gender focus group discussions (FGDs). The FGDs were conducted in all four villages and consisted of eight to 10 participants.

Results

A minority (14.2%; 40/281) of respondents agreed that a seemingly healthy person could have malaria parasite in his or her blood. Half (52%; 146/281) disagreed and one third (33.8%, 95/281) were unsure. Respondents who responded that "MDA aims to cure everyone" [AOR=4.6; CI: 1.6-13.1], "MDA is to make our community malaria free" [AOR=3.3; CI: 1.3-8.1] and "I will take part in future MDA" [AOR=9.9; CI: 1.2-78.8] were more likely to accept the idea of asymptomatic malaria. During FGDs, respondents recalled signs and symptoms of malaria (fever, chills and headache), and described malaria as a major health problem. Symptomatic and asymptomatic malaria infections were associated with their work in the forest and living conditions. Measures described to eliminate malaria included using mosquito nets, wearing long-sleeved clothes and taking medicine when symptomatic. Most respondents were unaware of MDA as a tool to eliminate malaria.

Conclusions

Awareness of asymptomatic malaria infections, and MDA as a tool to eliminate malaria, was low. With the need to target asymptomatic malaria carriers for elimination efforts in the GMS, as well as informing target groups about asymptomatic infection, accompanying community engagement must build trust in interventions through the active collaboration of government stakeholders, key local persons and community members. This entails training and devolving responsibilities to the community members to implement and sustain the control and elimination efforts.

Keywords: knowledge, attitude, perceptions, practices, MDA, malaria control, malaria, formative research, community engagement, Laos.

Introduction

The emergence of antimalarial resistance in the Greater Mekong sub-Region (GMS) has triggered greater efforts to contain its spread within the region and beyond [1, 2]. This, together with general declines in malaria incidence, has prompted malaria control programs in the region to set targets for the elimination of *falciparum* malaria by 2025 and all malaria species by 2030 [3]. The Lao People's Democratic Republic (Laos) has adopted the goal of eliminating malaria by 2025 [4].

Achieving such goals is challenging for several reasons. First, the emerging resistance to all anti-malarials poses a serious challenge for containment [5]. Second, malaria tends to cluster in populations that live along international borders, within forested areas and in forest fringes [3]. These communities are often geographically isolated and highly mobile, have low levels of literacy, experience language barriers, include ethnic minorities, endure a lack of health care services and experience high levels of human-mosquito interaction [6, 7].

With recent declines in clinical malaria, there is increasing evidence that asymptomatic parasite carriers play a critical role in sustaining transmission [2, 8-11]. Seemingly healthy individuals can be chronic carriers of asexual and sexual (gametocytes) forms of the parasite that are critical for transmission [10, 12]. This is particularly the case in low- and seasonal-transmission areas where people with chronic, asymptomatic infections may serve as the infectious reservoir for transmission in subsequent seasons [12]. Data from Cameroon, The Gambia, Mali and Senegal indicate that over 25% of individuals with sub-microscopic gametocytes were capable of infecting mosquitoes [13]. A recent study in Laos found 20% (175/888) of the seemingly healthy individuals were infected with *Plasmodium* species [4].

Elimination programs must therefore target asymptomatic malaria reservoirs to eliminate rapidly the disease. Mass drug administration (MDA), whereby a complete dose of antimalarial treatment is provided to the entire targeted population, regardless of infection status, is one such approach [2, 14, 15]. In the absence of more promising interventions, the threat of a public health emergency if multidrug resistant malaria reaches Africa has prompted scientists to consider MDA as an approach to accelerate elimination in addition to other malaria prevention and control [2, 16]. Together with the ethical justification for such an approach [15, 16], recently scaled up MDA was found effective in Thai-Myanmar border [17]. Including MDA, strategies such as mass screening and treatment (MSAT), focal screening and treatment (FSAT) and voluntary screening and testing (VSAT) for malaria require testing all people in a

geographical area and treating only positive cases [18, 19]. All of these approaches entail people taking anti-malarials when asymptomatic.

Recent MDAs for targeted falciparum malaria elimination have highlighted the challenges related to convincing healthy people to take anti-malarials [6, 20-23]. The rationale for this approach involves complex biological concepts and communicating them to people with low levels of formal education and frequently across language barriers [6, 20, 24]. Furthermore, local declines in clinical *falciparum* cases might mean that malaria is no longer a priority in target communities and reduce the willingness of some people to take anti-malarials [21].

The situation is further complicated by the presence of *P. vivax* and *P. falciparum* in South East Asia [25] and *P. vivax* has been found to dominate the overall prevalence of plasmodium infections [4, 9, 10, 26]. This complex *P. vivax* parasite has a latent liver stage, hypnozoites, which can cause relapsing infections for months and years [4, 26-28]. *P. vivax* persistence can sustain a parasite reservoir for ongoing transmission and radical therapy targeting this parasite requires an addition of a 14 days course of primaquine [28], which carries the risk of hemolysis in G6PD-deficient individuals [10, 29, 30]. In remote communities of the GMS, knowledge about malaria species and the mechanisms of infection is however low [6, 21, 24]. Yet after the elimination of *P. falciparum* infections *P. vivax* infections are likely to persist.

To date, three articles have reported malaria-related knowledge, attitudes and practices in Laos [31-33]. However, none have explored perceptions of asymptomatic malaria infections in rural malaria endemic areas.

This article draws on mixed-methods formative research conducted prior to a pilot study of MDA for targeted malaria elimination (TME) in Nong District, southern Savannakhet Province, Laos. The article explores understandings of asymptomatic malaria infections in a geographically remote area, with a view to designing community engagement strategies to accompany interventions that target the parasite reservoir (such as MDA, MSAT and VSAT).

Materials and Methods

Study context

Four villages in Nong District were chosen for the TME study on the malaria prevalence survey conducted during June and July 2015 in Thappangthong and Nong District, Savannakhet Province [4]. All four villages; Oi Tan Tip (OTP), Phoun Mak Mee (PMM), Tha The (TT) and

Xuang Tai (XT) are home to members of ethnic minority groups (*Mangkong*=200; 71.2%, *Tree*=64; 22.8%, and *Phu Thai*=6). Oi Tan Tip is farthest (≥50km) from the Nong District town, lying close to the Vietnam border, and Xuang Tai was closest (≥10 km). The other villages, PMM and TT are about 25 km and 15 km away from the district town respectively.

Residents of all four villages primarily rely on subsistence farming, including migratory, swidden cultivation of staple foods, such as sticky rice, maize, and cash crops such as cassava. Besides staple food, community members visit local forests to collect foods, such as bamboo shoots, wild rodents, beetles and other insects. Most community members reared domestic animals, such as cows, pigs, chicken, buffaloes and goats, which are also their source of cash. For instance, when in urgent need of money, community members sell these animals to Vietnamese mobile traders for cash.

There are limited health facilities in the study villages. In PMM, one health centre is located within the settlement, but the residents of the other villages had to travel from 5km (for TT) to over (10km for OTP) to reach the nearest health centre.

Study participants and questionnaire

Based on the census conducted for TME study, a total of 281 households (OTP = 65, PMM = 74, TT = 82 and XT = 60) were included in this survey. A questionnaire was administered to one household member above the age of 18 years, preferably household head from all households in the four study villages. Two Laotian social scientists interviewed respondents at their household following written informed consent. If representatives of the household were not present during the survey, they were followed up during the consecutive days. None of the household representative refused to participate. Respondents representing a total of two households were away and interviewed once they returned. Each interview lasted for 30 to 45 minutes.

Each questionnaire was divided into five parts: 1) general information about the interview, interviewer and interviewee; 2) socio-demographic characteristics, such as age, sex, religion and marital status; 3) health seeking behaviour; 4) malaria-related knowledge, attitudes and practices; 5) knowledge and attitudes toward MDA. Based on the research question of this study, all sections except section 3 submitted as manuscript elsewhere are included. Variables in the questionnaire were designed after carefully reviewing the past studies from Laos [34-36]

and elsewhere [37-41]. The questionnaire was designed in English, then translated into Laotian and back translated into English to ensure validity.

The questionnaires were pre-tested with members of the study team and 15 volunteer participants around Mahosot Hospital in Vientiane. Further pre-testing was carried out in Nong District in six households. Any ambiguity found was corrected and simplified. After the questionnaire was finalized, it was entered into the Open Data Kit (ODK; Available online: https://opendatakit.org/) application on a smartphone.

Data management and analysis

All data entered into ODK were extracted into an Excel sheet (Microsoft Excel 2013) and analyzed in IBM SPSS Statistics for Windows, Version 24.0, Armonk, NY: IBM Corp.

Each respondent was asked whether a healthy looking person could have malaria parasite in his/her blood. The response to this question was treated as an outcome variable and were further dichotomized into "Yes" and "No" to explore the associations with respondents' awareness of the possibility of asymptomatic malaria. Following a descriptive analysis (frequency and percentage), the data were analysed using Chi squared and Fisher's exact test as appropriate to determine associations. All variables (socio-demographic, knowledge, attitude, practice and perceptions on malaria and MDA) were tested for association with the outcome variable and an association was considered significant at p value ≤ 0.05.

Further analysis using logistic regression was carried out in which variables were selected based on the significant association with the perception on asymptomatic malaria infections and the relevance to the research question. For the logistic regression analysis, thus selected variables were dichotomized into two categories; with presence of certain characters/conditions = 1 and absence = 0. First, a univariate analysis was conducted with the outcome variable (perception on asymptomatic malaria infection categorized as "Yes"=1 and "No"=0) to calculate crude odds ratios. In the multi-variate analysis, selected variables were included into the model to calculate the adjusted odds ratio. Statistical significance was considered if p value ≤ 0.05.

Focus group discussions

To explore further the issues addressed in the questionnaire, a series of focus group discussions were also undertaken in all four study villages. In total, 12 FGDs were conducted with 100

participants from the four villages and their sub-villages. Each FGD involved eight to 10 participants, selected by simple randomization (lottery method) during village meetings. Aware of the tendency for conformism, and patriarchy in the study communities [20, 24], to encourage the active participation of female respondents, discussions were conducted in single-sex groups by a male or female social scientist. Based on a joint decision among participants, the discussions took place at one of their houses.

An open-ended questionnaire guide was used to lead the discussion and each FGD lasted for about 30 to 45 minutes. The FGDs were held in *Pasha Lao* and the local language with translation provided by a TME staff member or bilingual volunteer from the village. People gave informed oral consent at the community meeting and each selected participant provided individual consent.

All FGDs were audio-recorded and transcribed and translated into English by the FGD facilitators. Translated and transcribed FGD transcripts were analyzed using qualitative data analysis software (QSR NVivo 11). All transcripts were coded line by line using pre-set themes (deductive approach) as nodes including addition of nodes for emerging themes (inductive approach). Based on the research question, all the nodes were analyzed for the pattern and the content and are presented in the manuscript below.

Ethics approval and consent to participate

Ethical approval for the study was received from the Lao National Ethics Committee for Health Research (Ref. No. 013-2015/NECHR), Government of the Lao PDR and the Oxford Tropical Research Ethics Committee (1015-13). Written informed consent were sought from each participant before each interview and FGD.

Results

Questionnaire-based survey

Socio-demographic characteristics and awareness of asymptomatic malaria infections

Most respondents were aged below 50 years (mean age = 38.9 years; 229/281; 81.5%), male (201/281; 71.5%), household head (188/281; 66.9%), from the ethnic minorities (269/281; 95.7%), identified as animist (272/281; 96.8%), illiterate (214/281; 76.2%), rice farmers (254/281; 90.4%) with a monthly income (192/281; 68.3%) of less than 500,000 Kip (60 USD).

Of the 281 participants, only 40 (14.2%) reported that a seemingly healthy person could have malaria parasite in his/her blood. Over half (146/281; 52%) rejected this idea and a third (95/281; 33.8%) responded that they did not know (**Table 1** and **S1 Tables**).

Respondents' literacy (illiterate: 22/214; 10.3% versus literate: 18/67; 26.9%; p=0.001), education status (not attended school: 22/204; 10.8% versus attended school: 18/77; 23.4%; p=0.008), occupation (farmer: 29/254; 11.41% versus non-farmer: 11/27;40.7 ;<0.001), monthly income (lowest income group: ≤500,001 kip: 14/192; 7.3% versus middle income group: 500,001 to 2,000,000 kip: 11/41; 26.8%; p<0.001), influenced their response about the possibility of asymptomatic malaria infection.

Table 1: Socio-demographic characteristics of participants in relation to perception on asymptomatic malaria infections (n=281)

Characteristics	Total	Possibility of asymptomatic malaria infections		p-value
	Number (%)	Yes (n=40)	No (n=241)	
Village				
OTP	65 (23.1)	16 (40)	49 (20.3)	<0.001
PMM	74 (26.3)	2 (5)	72 (29.9)	
TT	82 (29.2)	5 (12.5)	77 (32)	
XT	60 (21.4)	17 (42.5)	43 (17.8)	
Respondent status				
Family head	188 (66.9)	29 (72.5)	159 (66)	0.35
Wife of family head	70 (24.9)	10 (25)	60 (24.9)	
Other	23 (8.2)	1 (2.5)	22 (9.1)	
Age Group				
≤30 years	91 (32.4)	13 (32.5)	78 (32.4)	0.28
31-50 years	138 (49.1)	23 (57.5)	115 (47.7)	
≥51 years	52 (18.5)	4 (10)	48 (19.9)	
Mean=38.9±14.5, min=18 and max=100				
Sex				
Male	201 (71.5)	30 (75)	171 (71)	0.37
Female	80 (28.5)	10 (25)	70 (29)	
Ethnicity*				
Lao Theung	269 (95.7)	32 (80)	237 (98.3)	<0.001
Other	12 (4.3)	8 (20)	4 (1.7)	
Religion				
Buddhist	9 (3.2)	8 (20)	1 (0.4)	<0.001
Animist	272 (96.8)	32 (80)	240 (99.6)	
Marital Status				
In relationship	262 (93.2)	39 (97.5)	223 (92.5)	0.21

Characteristics	Total	Possibility of asymptomatic malaria infections		p-value
	Number (%)	Yes (n=40)	No (n=241)	
Not in relationship	19 (6.8)	1 (2.5)	18 (7.5)	
Literacy				
Literate	67 (23.8)	18 (45)	49 (20.3)	0.001
Illiterate	214 (76.2)	22 (55)	192 (79.7)	
Education in years				
Not attended School	204 (72.6)	22 (55)	182 (75.5)	0.008
Attended School	77 (27.4)	18 (45)	59 (24.5)	
Occupation				
Farmer	254 (90.4)	29 (72.5)	225 (93.4)	<0.001
Other	27 (9.6)	11 (27.5)	16 (6.6)	
Monthly Income				
≤500,000 kip	192 (68.3)	14 (35)	178 (73.9)	<0.001
500,001 to 2,000,000 kip	41 (14.6)	11 (27.5)	30 (12.4)	
≥ 2000,001	27 (9.6)	11 (27.5)	16 (6.6)	
Don't know	21 (7.5)	4 (10)	17 (7.1)	
Do you have toilet facility at home?				
Yes	22 (7.8)	4 (10)	18 (7.5)	0.38
No	259 (92.2)	36 (90)	223 (92.5)	
Did you migrate from any other village?				
Yes	61 (21.7)	13 (32.5)	48 (19.9)	0.06
No	220 (78.3)	27 (67.5)	193 (80.1)	
How far is the forest from your house in km?				
≤1 km	97 (34.5)	10 (25)	87 (36.1)	0.24
1.1 to 2 km	76 (27)	13 (32.5)	63 (26.1)	
≥2.1 km	49 (17.4)	5 (12.5)	44 (18.3)	
NA	59 (21)	12 (30)	47 (19.5)	
How often do you go to forest?				
Everyday	171 (60.9)	24 (60)	147 (61)	0.26
Every alternate day	68 (24.2)	7 (17.5)	61 (25.3)	
≥Weekly	42 (14.9)	9 (22.5)	33 (13.7)	

*Ka Tarng=1 (0.4%), Lao Loum=3 (1.1%), Mangkong=200 (71.2), Phu Thai=6 (2.1%), Ta Oi=3 (1.1%), Tree=64 (22.8%), Vietnamese=4 (1.4%)

Malaria-related knowledge, practices and perceptions

Almost all respondents, 260/281 (92.5%) had heard about malaria before (**Table 2, 3** and **S1 Tables**). Among these 260 respondents, most could recall the symptoms of malaria such as fever: 231/260; 88.8%, headache: 190/260; 73.1% and chills: 227/281; 87.3%. In the past, the majority received information on malaria from health workers (193/281; 74.2%) and knew that malaria was transmitted by mosquitoes (230/281; 81.9%). Respondents who recalled that

mosquitoes transmit malaria (37/230; 16.1%; compared to those who did not: 3/51; 5.9%; p=0.004) were likely to report the possibility of asymptomatic malaria infection. The majority of respondents recalled that using a mosquito net at home (274/281; 97.5%) and wearing long sleeves in the forest (262/281; 93.2%) can prevent mosquito bites. Also, the majority (256/281; 91.1%) recalled sleeping under the mosquito net the previous night at home. On further prompting, most respondents described malaria as a deadly disease (265/281; 94.3%) and were confident that malaria was cured by medicine (275/281; 97.9%).

Table 2: Knowledge, practices, perceptions and attitudes towards malaria of participants in relation to perception on asymptomatic malaria infections (n=281)

Characteristics	Total	Possibility of asymptomatic malaria infections		p-value
	Number (%)	Yes (n=40)	No (n=241)	
Have you heard of malaria before?				
Yes	260 (92.5)	38 (95)	222 (92.1)	0.71
No	18 (6.4)	2 (5)	16 (6.6)	
Don't know	3 (1.1)	0	3 (1.2)	
What are the symptoms of malaria? (n=260)*				
Fever	231 (88.8)	33 (86.8)	198 (89.2)	0.42
Headache	190 (73.1)	32 (84.2)	158 (71.2)	0.06
Muscle pain	46 (17.7)	12 (31.6)	34 (15.3)	0.01
Vomiting	45 (17.3)	7 (18.4)	38 (17.1)	0.5
Chills	227 (87.3)	32 (84.2)	195 (87.8)	0.34
Where did you get the information on malaria from? (n=260)*				
Village Meetings	3 (1.2)	1 (2.6)	2 (0.9)	0.39
Health workers	193 (74.2)	24 (63.2)	169 (76.1)	0.07
Banners	2 (0.8)	2 (5.3)	0	0.02
Other	49 (18.8)	8 (21.1)	41 (18.5)	0.42
Don’t know	41 (15.8)	13 (34.2)	28 (12.6)	0.002
Where would you like to get health information from?*				
Village Meetings	59 (21)	9 (22.5)	50 (20.7)	0.47
Health workers	137 (48.8)	23 (57.5)	114 (47.3)	0.15
Banners	56 (19.9)	6 (15)	50 (20.7)	0.27
Entertainment	52 (18.5)	9 (22.5)	43 (17.8)	0.3
Other	13 (4.6)	2 (5)	11 (4.6)	0.57
Don’t know	31 (11)	5 (12.5)	26 (10.8)	0.46
Malaria is transmitted from*				
Water	14 (5)	2 (5)	12 (5)	0.61
Soil	1 (0.4)	1 (2.5)	0	0.14
Forest	55 (19.6)	9 (22.5)	46 (19.1)	0.37
Mosquito	230 (81.9)	37 (92.5)	193 (80.1)	0.04
God	1 (0.4)	0	1 (0.4)	0.85

Characteristics	Total	Possibility of asymptomatic malaria infections		p-value
	Number (%)	Yes (n=40)	No (n=241)	
Uncleaned Surrounding	30 (10.7)	4 (10)	26 (10.8)	0.57
Don't know	29 (10.3)	0	29 (12)	0.009
How do you prevent mosquito bites at home?*				
Using mosquito net	274 (97.5)	40 (100)	234 (97.1)	0.33
Using repellants	5 (1.8)	1 (2.5)	4 (1.7)	0.53
By smoking	2 (0.7)	0	2 (0.8)	0.73
Wearing Sleeves	16 (5.7)	3 (7.5)	13 (5.4)	0.4
How do you prevent mosquito bites at forest?*				
Using mosquito net	1 (0.4)	0	1 (0.4)	0.85
Burning fire	11 (3.9)	2 (5)	9 (3.7)	0.48
Using repellants	7 (2.5)	5 (12.5)	2 (0.8)	0.001
By smoking	1 (0.4)	1 (2.5)	0	0.14
Wearing Sleeves	262 (93.2)	39 (97.5)	223 (92.5)	0.21

*Multiple answers were possible and percentage does not add to 100, analyses were made against "yes" and "no".

Table 3: Knowledge, practices, perceptions and attitudes towards malaria of participants in relation to perception on asymptomatic malaria infections (n=281)

Characteristics	Total	Possibility of asymptomatic malaria infections		p-value
	Number (%)	Yes (n=40)	No (n=241)	
Did you sleep under the mosquito net last night?				
Yes	256 (91.1)	38 (95)	218 (90.5)	0.27
No	25 (8.9)	2 (5)	23 (9.5)	
If Yes, how often do you sleep under the mosquito net?				
Everyday	247 (96.5)	35 (92.1)	212 (97.2)	0.13
Sometimes (>2-3 days in a week)	9 (3.5)	3 (7.9)	6 (2.8)	
How many mosquito nets do you have at your home?				
≤2	156 (55.5)	23 (57.5)	133 (55.2)	0.46
≥3	125 (44.5)	17 (42.5)	108 (44.8)	
Do you think you can get malaria if anybody in your family/neighbor has malaria?				
Yes	164 (58.4)	27 (67.5)	137 (56.8)	0.26
No	83 (29.5)	11 (27.5)	72 (29.9)	
Don't know	34 (12.1)	2 (5)	32 (13.3)	
If Yes, how? (n=164)*				
Touch	75 (45.7)	8 (29.6)	67 (48.9)	0.05
Air	11 (6.7)	0	11 (8)	0.12
Mosquito	108 (65.9)	21 (77.8)	87 (63.5)	0.11
Water	33 (20.1)	4 (14.8)	29 (21.2)	0.32

Characteristics	Total	Possibility of asymptomatic malaria infections		p-value
	Number (%)	Yes (n=40)	No (n=241)	
Food	34 (20.7)	4 (14.8)	30 (21.9)	0.29
How do we know if a person has malaria?*				
Blood	178 (63.3)	30 (75)	148 (61.4)	0.06
Fever	73 (26)	14 (35)	59 (24.5)	0.11
Health worker	90 (32)	11 (27.5)	79 (32.8)	0.32
Other	33 (11.7)	2 (5)	31 (12.9)	0.11
Is malaria a deadly disease?				
Yes	265 (94.3)	40 (100)	225 (93.4)	0.24
No	12 (4.3)	0	12 (5)	
Don't know	4 (1.4)	0	4 (1.7)	
Are you scared of malaria?				
Yes	277 (98.6)	39 (97.5)	238 (98.8)	0.58
No	3 (1.1)	1 (2.5)	2 (0.4)	
Don't know	1 (0.4)	0	1 (0.4)	
Can malaria be cured by medicine?				
Yes	275 (97.9)	38 (95)	237 (98.3)	0.4
No	3 (1.1)	1 (2.5)	2 (0.8)	
Don't know	3 (1.1)	1 (2.5)	2 (0.8)	

*Multiple answers were possible and percentage does not add to 100, analyses were made against "yes" and "no".

Knowledge, perceptions and attitudes towards MDA

Most respondents described malaria as an important problem in their communities (178/281; 63.3%) (**Table 4** and **S1 Tables**). Respondents who agreed with the idea of asymptomatic malaria infections also agreed that a healthy person with malaria parasites in his or her blood can transmit the infection (23/103; 22.3%; $p<0.001$). When asked if medicine should be provided to community members with asymptomatic malaria infections, three-quarters (78/103) answered “yes”, and this was to “to cure all villagers” (11/29; 37.9%). The majority (241/281; 86%) had not heard about the malaria elimination programme in their village. More than half (162/281; 57.7%) stated that malaria can be eliminated from their village and with regard to ways (options) to eliminate the disease, they two thirds selected giving medicine to all the villagers (105/162; 64.8%), half chose using mosquito nets (81/162; 50%) and a sixth opted for cleaning the surroundings (23/162; 14.2%). The majority of respondents who thought that malaria elimination was possible were more likely to recognize the possibility (Yes: 30/162; 18.5% versus No and Don’t know: 10/109; 9.1%; $p=0.08$) of asymptomatic malaria infections. Most respondents showed enthusiasm (208/281; 74%) for participation in future

malaria elimination campaign as a volunteer and were likely to recognize (Yes: 35/208; 16.8% versus No: 5/60; 8.3%; p=0.08) the possibility of asymptomatic malaria infections.

Table 4: Knowledge, perceptions and attitudes towards MDA of participants in relation to perception on asymptomatic malaria infections (n=281)

Characteristics	Total	Possibility of asymptomatic malaria infections		p-value
	Number (%)	Yes (n=40)	No (n=241)	
Is malaria a big problem in your community?				
Yes	178 (63.3)	29 (72.5)	149 (61.8)	0.06
No	43 (15.3)	8 (20)	35 (14.5)	
Don't know	60 (21.4)	3 (7.5)	57 (23.7)	
Do you think a person in your village can have malaria parasite without being sick?				
Yes	34 (12.1)	26 (65)	8 (3.3)	<0.00
No	144 (51.2)	9 (22.5)	135 (56)	
Don't know	103 (36.7)	5 (12.5)	98 (40.7)	
Can a healthy person with malaria parasite in his body transmit to others?				
Yes	103 (36.7)	23 (57.5)	80 (33.2)	<0.00
No	94 (33.5)	13 (32.5)	81 (33.6)	
Don't know	84 (29.9)	4 (10)	80 (33.2)	
If Yes, should we provide medicine to all the villagers?				
Yes	78 (75.7)	17 (73.9)	61 (76.3)	0.26
No	19 (18.4)	6 (26.1)	13 (16.3)	
Don't know	6 (5.8)	0	6 (7.5)	
If Yes, why? (n=78)*				
To cure all villagers	29 (37.2)	11 (64.7)	18 (29.5)	0.01
To eliminate malaria from the village	15 (19.2)	4 (23.5)	11 (18)	0.42
To prevent malaria transmission in the	41 (52.6)	9 (52.9)	32 (52.5)	0.59
To prevent us from malaria	34 (43.6)	7 (41.2)	27 (44.3)	0.52
Have you heard of malaria elimination in your village?				
Yes	40 (14.2)	8 (20)	32 (13.3)	0.14
No	224 (79.7)	32 (80)	192 (79.7)	
Don't know	17 (6)	0	17 (7.1)	
Do you think malaria can be eliminated from your village?				
Yes	162 (57.7)	30 (75)	132 (54.8)	0.04
No	66 (23.5)	7 (17.5)	59 (24.5)	
Don't know	53 (18.9)	3 (7.5)	50 (20.7)	
If Yes, how? (n=162)*				
By giving medicine to all villagers	105 (64.8)	24 (80)	81 (61.4)	0.04
By using mosquito net	81 (50)	17 (56.7)	64 (48.5)	0.27
By taking regular medicine	9 (5.6)	1 (3.3)	8 (6.1)	0.47
By cleaning surrounding	23 (14.2)	6 (20)	17 (12.9)	0.23
Don't know	11 (6.8)	1 (3.3)	10 (7.6)	0.35
Other	30 (18.5)	6 (20)	24 (18.2)	0.49

Characteristics	Total	Possibility of asymptomatic malaria infections		p-value
	Number (%)	Yes (n=40)	No (n=241)	
Would you participate in malaria elimination as a volunteer?				
Yes	208 (74)	35 (87.5)	173 (71.8)	0.08
No	60 (21.4)	5 (12.5)	55 (22.8)	
Don't know	13 (4.6)	0	13 (5.4)	
If Yes, why? (n=208)*				
I want to make my community free from	127 (61.1)	29 (82.9)	98 (56.6)	0.002
I want to help my community	85 (40.9)	14 (40)	71 (41)	0.53
Malaria is a big problem in my community	6 (2.9)	2 (5.7)	4 (2.3)	0.26
Other	14 (6.7)	0	14 (8.1)	0.06
Don't know	21 (10.1)	1 (2.9)	20 (11.6)	0.09
Have your ever heard about MDA before?				
Yes	257 (91.5)	36 (90)	221 (91.7)	0.66
No	16 (5.7)	2 (5)	14 (5.8)	
Don't know	8 (2.8)	2 (5)	6 (2.5)	
Would you take part in MDA in future?				
Yes	198 (70.5)	39 (97.5)	159 (66)	<0.00
No	82 (29.2)	1 (2.5)	81 (33.6)	
Don't know	1 (0.4)	0	1 (0.4)	

*Multiple answers were possible and percentage does not add to 100, analyses were made against "yes" and "no".

Multivariate logistic regression

Using the logistic regression model, the following factors were associated with recognizing the possibility of asymptomatic malaria infections (**Table 5**). 1. Interest in taking part in future MDAs (AOR=9.9; CI=1.2 to 78.8; p=0.02). Respondents were asked if MDA for malaria was going to happen in their village in future, would they take part. Those who responded yes were likely to recognize the possibility of asymptomatic infection. 2. Understanding that the rationale of MDA is to cure all people, including those with asymptomatic infections (AOR=4.6; CI=1.6 to 13.1; p=0.004). Respondents who responded that providing antimalarials to all community members (was to cure all people with malaria and asymptomatic infections) were likely to recognize the possibility of asymptomatic malaria infection 3. Respondents who indicated a desire to participate in MDA as a volunteer (assistant) (AOR=3.3; CI: 1.3 to 8.1; p=0.008). Respondents motivated to contribute as a volunteer in MDA were more likely to accept the possibility of asymptomatic malaria infection.

Table 5: Logistic regression on knowledge, attitude and perceptions predicting the perception on asymptomatic malaria infections

Covariates and analyzed sample	Total	Univariate Analysis	Multivariate analysis
	Number (%)	Crude OR (95% CI)	AOR* (95% CI)
Animist religion (n=281)	272 (96.8)	0.01 (0.002 - 0.13)	0.09 (0.001 - 0.14)
Living for less than 10 years in the village (n=61)	28 (45.9)	6.0 (2.58 - 14.03)	2.85 (0.79 - 10.19)
Forest less than 1 km away (n=281)	97 (34.5)	0.59 (0.27 - 1.26)	0.19 (0.04 - 0.9)
MDA is to cure all people (n=78)	29 (37.2)	4.69 (2.0 - 10.92)	4.66 (1.65 - 13.15)
I want to make my community free from malaria	127 (61.1)	3.84 (1.83 - 8.0)	3.35 (1.36 - 8.15)
I will take part in MDA in future (n=281)	198 (70.5)	20.11 (2.71 -	9.97 (1.26 - 78.85)

*AOR=Adjusted Odds Ratio; adjusted with age and sex

Focus group discussions

Characteristics of the participants

One hundred community members were chosen at random from the four study villages and their sub-villages to participate in six gender-specific FGDs (**Table 6**). The mean age of respondents was 37 years, ranging from 18 to 80 years. Fifty two of the 100 respondents were male. All participants were farmers and the majority (82/100) had not attended school.

Knowledge of malaria

Almost all FGD participants showed knowledge of malaria. Locally termed *"Aai moi kap"* (disease mosquito bite) or *"Aai singyet"* (disease chills), respondents often recalled headache, fever, chills, shivering, vomiting, body pain as symptoms. Some also mentioned vomiting, thirst, cough and cold, and diarrhea.

Table 6: Socio-demographic characteristics of FGD Participants (n=100)

		Village: Number (%)					
	Total	Oi	Tantip	PMM	Keng and	TT main	XT
Age Group							
≤35 years	51 (51)	7 (13.7)	12	6 (11.8)	13 (25.5)	7 (13.7)	6 (11.8)
>36 years	49 (49)	9 (18.4)	4 (8.2)	12	5 (10.2)	9 (18.4)	10
Mean=37.58±12.71; Range=18-80 years							
Sex							
Male	52 (52)	8 (15.4)	8 (15.4)	10	10 (19.2)	8 (15.4)	8 (15.4)
Female	48 (48)	8 (16.7)	8 (16.7)	8 (16.7)	8 (16.7)	8 (16.7)	8 (16.7)
Occupation							
Farmer	100	16 (16)	16 (16)	18 (18)	18 (18)	18 (18)	16 (16)
Education Groups							
Not attended	82 (82)	12	10	13	17 (20.7)	14	16
1-5 years	16 (16)	4 (25)	5 (31.3)	5 (31.3)	1 (6.3)	1 (6.3)	0
>6 years	2 (2)	0	1 (50)	0	0	1 (50)	0

Asymptomatic malaria infection as a concept

The concept of asymptomatic infection was new to participants and only few agreed that a healthy person could have malaria parasites in his or her blood. However, respondents also suspected that they could perhaps have a type of *malaria*, which they associated with the nature of their work and the cleanliness of conditions in their village. When probed about whether these healthy-looking but infected people could be infectious, almost all participants agreed.

I: How do you know if somebody has malaria?

FGD7S1: When we go to forest and mosquito bite us.

FGD7S2: If a person goes to forest and mosquito bites but we cannot know from the look.

FGD7S3: If you see the mosquito bite and if that person has chills that means he/she has got malaria

[After a brief pause]

I: Let's continue. How do you know if someone has malaria?

FGD7S5: By checking temperature

FGD7S6: By mosquito bite.

FGD7S7: if somebody takes medicine and got cured, we can know that person has malaria.

FGD7S8: We know it if somebody goes to hospital and finds out that he/she has malaria.

FGD with eight female participants at a sub-village of TT

I: Do you think that a healthy person (who is walking around and working normally) can be infected?

FGD6S1 (after discussing amongst themselves first): All of us have malaria parasites inside our body because we are thin, we do not live clean, we always go to the forest and mosquito bites us. But for your project [TME] members, you do not have malaria parasite in your body because your lifestyle and living is the opposite to ours.

FGD with 10 male participants at PMM

Malaria as a health problem in the villages

Many respondents described malaria as a major problem in their village. This was linked to their recognition of malaria as potentially fatal (if left untreated), past experiences when many community members died of the disease, or the out of pocket expenses for treatment, which can be a major burden for households. Respondents described sleeping under a mosquito net as essential to protect from the malaria and that inadequate number of mosquito nets prompted concern about this disease. Respondents also explained the presence of malaria in their villages in terms of their living conditions, particularly a reported lack of cleanliness, and the nature of their work in the forests, where they often receive mosquito bites.

I: Is malaria a big problem in your village and if so why?

FGD6S1: Yes. It is big because a lot of people had malaria, many died and they had to spend a lot of money to treat it. (All other agreed).

FGD with 10 male participants at PMM

I: Malaria is a big problem in your village?

FGD5S2: Yes!

I: Why?

FGD5S2: We eat and stay unclean.

FGD5S8: Our clothes are unclean too, because we don't have money to buy soap for washing clothes.

FGD5S7: Mosquito net is very old and broken.

FGD with 8 female participants at PMM

Ways to eliminate malaria

The respondents gave multiple suggestions regarding how malaria could be eliminated: providing mosquito nets, wearing long sleeves, taking medicine when sick, cleaning the environment surrounding their homes, improving water and sanitation, and building health centers in their villages.

I: Do you think malaria can be eliminated from the village? Why and How?

FGD12S2: Yes, we all the villagers have to sleep under the mosquito net. During the day, we have to wear long-sleeves.

FGD12S4: By using mosquito net.

FGD12S5: Medicine, mosquito net and toilet facility.

FGD12S6: Toilet facility: agreed with S5.

FGD with 8 male participants at XT

I: Do you think malaria can be eliminated from the village? Why and How?

FGD3S2: Yes [malaria can be eliminated], if the project helps.

FGD3S3: Yes, it is possible to eliminate if the project can build a health center here and check our health. And it should have enough doctors.

FGD3S8: By giving out mosquito nets.

FGD with 8 female participants at sub-village of OTP

Taking an anti-malarial when healthy was a new idea. However, most respondents said that they would take it to eliminate malaria from the village and protect themselves, particularly because malaria was recognized as a deadly disease.

Discussion

Asymptomatic malaria infection as a concept

This is the first study to explore awareness of asymptomatic malaria infections in remote malaria-endemic communities in Laos. Asymptomatic malaria infection was a new concept and most respondents did not agree with the idea that a seemingly healthy person could have malaria parasites in his or her body. For those who were open to the concept of asymptomatic malaria, infection was often perceived to be associated with living conditions (lack of cleanliness and mosquito nets) and outdoor work (swidden farming and forest visits). Associating malaria with forest work and hygiene is not unique to Laos: research in sub-Saharan Africa and South East Asia has shown that community members often associate the risk of getting malaria from work in the forest and with poor hygiene [21, 42-44]. This is reflected in epidemiological research that has associated working in the forest and residing near the forest fringes with *Plasmodium* infections [45, 46].

Difficulties with grasping the idea of asymptomatic malaria infection have been reported elsewhere. In Cambodia even after intense community engagement accompanying MDA, community members and some study staff members struggled with the concept of asymptomatic *Plasmodium* infections [21]. Partial understanding, however, did not prevent community members from participating in MDA in Cambodia, Laos, or other sites [6, 20-24]. A multitude of factors embedded in local social and cultural context, community engagement, provision of health care and both monetary and non-monetary incentives have been found to affect the participation in Laos and elsewhere [6, 20, 21, 23, 24].

Malaria as a health problem in the villages

In southern Savannakhet, as in western Cambodia, past experiences of malaria-related morbidity, mortality and treatment costs meant that malaria was described as a major health problem [21]. Malaria as a health concern was one of the factors that motivate community members to take part in subsequent pilot studies of MDA in these villages [24]. In addition, members of these remote communities often have little access to healthcare because of a lack

of local facilities, poverty and poor transport infrastructure [6, 20, 24]. Thus constrained by the limited health care infra-structure and the economic burden that malaria entailed, respondents were further motivated by the perceived/real health benefits directly linked to MDA and the ancillary care TME provided [20, 24].

Ways to eliminate/prevent malaria

Conventional malaria prevention measures – using mosquito nets, wearing long sleeves, treatment with anti-malarials when sick – were well known to respondents and were consistent with the findings of other studies [31-33]. Unsurprisingly, respondents were unaware of MDA as a potential malaria prevention strategy but showed enthusiasm towards participating in future MDAs. Intention to participate and enthusiasm to volunteer in MDA was also found amongst respondents in Cambodia [41]. In Laos, this was also reflected by a relatively high participation rate in subsequent MDA (>87% participated in three rounds of MDA) [6, 20, 24]. Participation in MDA was associated with attending study meetings, awareness of the study aim and the perception that MDA was worthwhile. Decisions about participation were also influenced by other household and community members [20]. Often community members were motivated to participate in the MDAs because of the perceived potential health benefits rather than understanding the scientific concept and rationale of MDA [20, 24].

Mass drug administrations were accompanied by intensive community engagement. This entailed informing and involving stakeholders and authority figures from central to the local level; local volunteers who were recruited and trained, and were integral to the design and implementation of activities; formative research to rapidly gain insight into the local social and cultural context, responsiveness whereby the approach was adapted according to the needs of the community and sharing control/leadership with the community in designing and executing program activities [6]. These elements were key in executing activities such as meetings, design of health education tools and house to house visits and aimed primarily to promote uptake of MDA [6, 20, 24].

Implications for malaria control programs

Malaria control programs in the region are dependent on the peripheral health system and may not be able to undertake such intense community engagement and sensitization as in the pilot study that followed this research [6]. Programs however should incorporate the active collaboration of stakeholders, including policy makers, local health care workers, traditional

healers and community members. This entails building a collaborative partnership with communities wherein members of the target populations can be trained to form a community malaria task force. Sharing responsibilities with community members can garner the "sense of ownership" and sustain malaria control and elimination efforts. A collaborative approach is likely to require more preparatory work, however, it was found economical compared to the vertical disease control programs in Africa [47, 48]. Such methods have been increasingly advocated and were found to be economical and sustainable in disease control programs [47-49].

Strengths and limitations

Data were collected as part of formative research to inform subsequent community engagement for a TME pilot study in Laos. As a part of formative research, multiple questions on related theme were asked to explore how their responses varied, this enabled us to understand in-depth perceptions, knowledge, practice and attitudes. Three researchers collected data to reduce the possibility of personal bias caused by the influence of a single data collector. Cultural modesty and conformism including social desirability may have biased some of the data. Although using quantitative data analysis including logistic regression helped to consolidate the findings, the magnitude and accuracy of such data are limited by the lack of ability to explain the variable in detail. Nevertheless, incorporating a mixed method approach (quantitative and qualitative methods) enabled to elicit information from all households including the exploration of reasons behind particular responses (variables). This study derived the data from on-site translation from bilingual translators who were fluent in *Lao* and local language. The language spoken locally has no written script and some nuances and meaning could have been lost during translation. Care was therefore taken to involve several bi-lingual community members to whom study staff could seek clarification about local terminology.

It is possible that village heads who had previously been involved in sensitization meetings regarding the pilot study conducted by higher authorities beforehand had disseminated information on MDA and its rationale to fellow community members, which could have affected some of our results.

Conclusion

In remote areas of Laos, prior to sensitization, awareness of asymptomatic malaria infections and MDA as a tool to eliminate malaria was low. This reflects attitudes to asymptomatic, *P.*

falciparum infection elsewhere in the GMS. Nonetheless, malaria remains a major health concern and community members can generally describe its signs, symptoms and transmission. In the context of declining clinical *falciparum* and the higher proportion of *vivax* malaria, maintaining enthusiasm to clear asymptomatic *falciparum* parasite reservoir presents a challenge. Malaria control programs should invest in community engagement that, as well as seeking to inform people about asymptomatic infection, builds trust through the active collaboration of government stakeholders, key community members (community health workers, traditional healers and local leaders) and the wider community. This entails training and devolving responsibilities to the community members to sustain the control and elimination efforts.

DECLARATIONS

Authors' contribution

Concept and design of the study: BA, CP, MM, LvS and PYC. Quantitative questionnaire: design, data management, curation and analysis: BA, MM, LvS, KP, GH, XS, PK, TJP. FGD guide and analysis: CP, BA, MM. Drafting of the manuscript: BA and CP. Overall supervision: AMD, NJW, NPJD, PNN, LvS, MM and TP. All authors read and approved the final proof.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The data is available upon request to the Mahidol Oxford Tropical Medicine Research Unit Data Access Committee (http://www.tropmedres.ac/data-sharing) complying with the data access policy (http://www.tropmedres.ac/_asset/file/data-sharing-policy-v1-0.pdf)

Consent for publication

Not applicable

Supporting Files

S1 Questionnaire Asymptomatic Malaria Infections

S1 Tables: Additional analysis

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S1 Appendix: Additional analysis (Tables A1 to A4)

Table A1: Socio-demographic characteristics of participants in relation to perception on asymptomatic malaria infections (n=281)					
	Total	**Possibility of asymptomatic malaria infections**			**p-value**
Characteristics	**Number (%)**	**Yes (n=40)**	**No (n=146)**	**Don't know (n=95)**	
Village					
OTP	65 (23.1)	16 (40)	22 (15.1)	27 (28.4)	**<0.001**
PMM	74 (26.3)	2 (5)	48 (32.9)	24 (25.3)	
TT	82 (29.2)	5 (12.5)	43 (29.5)	34 (35.8)	
XT	60 (21.4)	17 (42.5)	33 (22.6)	10 (10.5)	
Respondent status					
Family head	188 (66.9)	29 (72.5)	103 (70.5)	56 (58.9)	**0.009**
Wife of family head	70 (24.9)	10 (25)	26 (17.8)	34 (35.8)	
Other	23 (8.2)	1 (2.5)	17 (11.6)	5 (5.3)	
Age Group					
≤30 years	91 (32.4)	13 (32.5)	51 (34.9)	27 (28.4)	0.31
31-50 years	138 (49.1)	23 (57.5)	64 (43.8)	51 (53.7)	
≥51 years	52 (18.5)	4 (10)	31 (21.2)	17 (17.9)	
Mean=38.9±14.5, min=18 and max=100					
Sex					
Male	201 (71.5)	30 (75)	113 (77.4)	58 (61.1)	**0.02**
Female	80 (28.5)	10 (25)	33 (22.6)	37 (38.9)	
Ethnicity*					
Lao Theung	269 (95.7)	32 (80)	144 (98.6)	93 (97.9)	**<0.001**
Other	12 (4.3)	8 (20)	2 (1.4)	2 (2.1)	
Religion					
Buddhist	9 (3.2)	8 (20)	1 (0.7)	0	**<0.001**
Animist	272 (96.8)	32 (80)	145 (99.3)	95 (100)	
Marital Status					
In relationship	262 (93.2)	39 (97.5)	132 (90.4)	91 (95.8)	0.13
Not in relationship	19 (6.8)	1 (2.5)	14 (9.6)	4 (4.2)	
Literacy					
Literate	67 (23.8)	18 (45)	37 (25.3)	12 (12.6)	**<0.001**
Illiterate	214 (76.2)	22 (55)	109 (74.7)	83 (87.4)	
Education in years					
Not attended School	204 (72.6)	22 (55)	101 (69.2)	81 (85.3)	**0.001**
Attended School	77 (27.4)	18 (45)	45 (30.8)	14 (14.7)	
Occupation					
Farmer	254 (90.4)	29 (72.5)	137 (93.8)	88 (92.6)	**<0.001**
Other	27 (9.6)	11 (27.5)	9 (6.2)	7 (7.4)	

Table A1: Socio-demographic characteristics of participants in relation to perception on asymptomatic malaria infections (n=281)					
	Total	**Possibility of asymptomatic malaria infections**			**p-value**
Characteristics	**Number (%)**	**Yes (n=40)**	**No (n=146)**	**Don't know (n=95)**	
Monthly Income					
≤500,000 kip	192 (68.3)	14 (35)	117 (80.1)	61 (64.2)	**<0.001**
500,001 to 2,000,000 kip	41 (14.6)	11 (27.5)	15 (10.3)	15 (15.8)	
≥ 2000,001	27 (9.6)	11 (27.5)	6 (4.1)	10 (10.5)	
Don't know	21 (7.5)	4 (10)	8 (5.5)	9 (9.5)	
Do you have toilet facility at home?					
Yes	22 (7.8)	4 (10)	13 (8.9)	5 (5.3)	0.5
No	259 (92.2)	36 (90)	133 (91.1)	90 (94.7)	
Did you migrate from any other village?					
Yes	61 (21.7)	13 (32.5)	30 (20.5)	18 (18.9)	0.19
No	220 (78.3)	27 (67.5)	116 (79.5)	77 (81.1)	
How far is the forest from your house in km?					
≤1 km	97 (34.5)	10 (25)	53 (36.3)	34 (35.8)	**0.006**
1.1 to 2 km	76 (27)	13 (32.5)	42 (28.8)	21 (22.1)	
≥2.1 km	49 (17.4)	5 (12.5)	33 (22.6)	11 (11.6)	
NA	59 (21)	12 (30)	18 (12.3)	29 (30.5)	
How often do you go to forest?					
Everyday	171 (60.9)	24 (60)	93 (63.7)	54 (56.8)	0.19
Every alternate day	68 (24.2)	7 (17.5)	38 (26)	23 (24.2)	
≥Weekly	42 (14.9)	9 (22.5)	15 (10.3)	18 (18.9)	
***Ka Tarng=1 (0.4%), Lao Loum=3 (1.1%), Mangkong=200 (71.2), Phu Thai=6 (2.1%), Ta Oi=3 (1.1%), Tree=64 (22.8%), Vietnamese=4 (1.4%)**					

Table A2: Knowledge, practices, perceptions and attitudes towards malaria of participants in relation to perception on asymptomatic malaria infections (n=281)					
	Total	**Possibility of asymptomatic malaria infections**			**p-value**
Characteristics	**Number (%)**	**Yes (n=40)**	**No (n=146)**	**Don't know (n=95)**	
Have you heard of malaria before?					
Yes	260 (92.5)	38 (95)	135 (92.5)	87 (91.6)	0.77
No	18 (6.4)	2 (5)	10 (6.8)	6 (6.3)	
Don't know	3 (1.1)	0	1 (0.7)	2 (2.1)	
What are the symptoms of malaria? (n=260)*					
Fever	231 (88.8)	33 (86.8)	124 (91.9)	74 (85.1)	0.26
Headache	190 (73.1)	32 (84.2)	107 (79.3)	51 (58.6)	**0.001**
Muscle pain	46 (17.7)	12 (31.6)	25 (18.5)	9 (10.3)	**0.016**
Vomiting	45 (17.3)	7 (18.4)	29 (21.5)	9 (10.3)	0.09
Chills	227 (87.3)	32 (84.2)	119 (88.1)	76 (87.4)	0.81
Where did you get the information on malaria from? (n=260)*					
Village Meetings	3 (1.2)	1 (2.6)	2 (1.5)	0	0.39
Health workers	193 (74.2)	24 (63.2)	105 (77.8)	64 (73.6)	0.18
Banners	2 (0.8)	2 (5.3)	0	0	**0.003**
Other	49 (18.8)	8 (21.1)	20 (14.8)	21 (24.1)	0.2
Don't know	41 (15.8)	13 (34.2)	19 (14.1)	9 (10.3)	**0.003**
Where would you like to get health information from?*					
Village Meetings	59 (21)	9 (22.5)	40 (27.4)	10 (10.5)	**0.007**
Health workers	137 (48.8)	23 (57.5)	59 (40.4)	55 (57.9)	**0.014**
Banners	56 (19.9)	6 (15)	39 (26.7)	11 (11.6)	**0.011**
Entertainment	52 (18.5)	9 (22.5)	18 (12.3)	25 (26.3)	**0.019**
Other	13 (4.6)	2 (5)	8 (5.5)	3 (3.2)	0.69
Don't know	31 (11)	5 (12.5)	11 (7.5)	15 (15.8)	0.12
Malaria is transmitted from*					
Water	14 (5)	2 (5)	10 (6.8)	2 (2.1)	0.25
Soil	1 (0.4)	1 (2.5)	0	0	**0.04**
Forest	55 (19.6)	9 (22.5)	34 (23.3)	12 (12.6)	0.11
Mosquito	230 (81.9)	37 (92.5)	125 (85.6)	68 (71.6)	**0.004**
God	1 (0.4)	0	1 (0.7)	0	0.62
Uncleaned Surrounding	30 (10.7)	4 (10)	19 (13)	7 (7.4)	0.37
Don't know	29 (10.3)	0	9 (6.2)	20 (21.1)	**<0.001**
How do you prevent mosquito bites at home?*					
Using mosquito net	274 (97.5)	40 (100)	144 (98.6)	90 (94.7)	0.09
Using repellants	5 (1.8)	1 (2.5)	2 (1.4)	2 (2.1)	0.85
By smoking	2 (0.7)	0	2 (1.4)	0	0.39
Wearing Sleeves	16 (5.7)	3 (7.5)	6 (4.1)	7 (7.4)	0.49
How do you prevent mosquito bites at forest?*					
Using mosquito net	1 (0.4)	0	0	1 (1.1)	0.37

Table A2: Knowledge, practices, perceptions and attitudes towards malaria of participants in relation to perception on asymptomatic malaria infections (n=281)					
	Total	**Possibility of asymptomatic malaria infections**			**p-value**
Characteristics	**Number (%)**	**Yes (n=40)**	**No (n=146)**	**Don't know (n=95)**	
Burning fire	11 (3.9)	2 (5)	7 (4.8)	2 (2.1)	0.53
Using repellants	7 (2.5)	5 (12.5)	1 (0.7)	1 (1.1)	**<0.001**
By smoking	1 (0.4)	1 (2.5)	0	0	**0.049**
Wearing Sleeves	262 (93.2)	39 (97.5)	135 (92.5)	88 (92.6)	0.5
*Multiple answers were possible and percentage does not add to 100, analyses were made against "yes" and "no".					

Table A3: Knowledge, practices, perceptions and attitudes towards malaria of participants in relation to perception on asymptomatic malaria infections (n=281)					
	Total	**Possibility of asymptomatic malaria infections**			**p-value**
Characteristics	**Number (%)**	**Yes (n=40)**	**No (n=146)**	**Don't know (n=95)**	
Did you sleep under the mosquito net last night?					
Yes	256 (91.1)	38 (95)	135 (92.5)	83 (87.4)	0.25
No	25 (8.9)	2 (5)	11 (7.5)	12 (12.6)	
If Yes, how often do you sleep under the mosquito net?					
Everyday	247 (96.5)	35 (92.1)	132 (97.8)	80 (96.4)	0.24
Sometimes (>2-3 days in a week)	9 (3.5)	3 (7.9)	3 (2.2)	3 (3.6)	
How many mosquito nets do you have at your home?					
≤2	156 (55.5)	23 (57.5)	84 (57.5)	49 (51.6)	0.63
≥3	125 (44.5)	17 (42.5)	62 (42.5)	46 (48.4)	
Do you think you can get malaria if anybody in your family/neighbor has malaria?					
Yes	164 (58.4)	27 (67.5)	92 (63)	45 (47.4)	**<0.001**
No	83 (29.5)	11 (27.5)	47 (32.2)	25 (26.3)	
Don't know	34 (12.1)	2 (5)	7 (4.8)	25 (26.3)	
If Yes, how? (n=164)*					
Touch	75 (45.7)	8 (29.6)	52 (56.5)	15 (33.3)	**0.007**
Air	11 (6.7)	0	10 (10.9)	1 (2.2)	0.051
Mosquito	108 (65.9)	21 (77.8)	60 (65.2)	27 (60)	0.3
Water	33 (20.1)	4 (14.8)	26 (28.3)	3 (6.7)	**0.009**
Food	34 (20.7)	4 (14.8)	25 (27.2)	5 (11.1)	0.06
How do we know if a person has malaria?*					
Blood	178 (63.3)	30 (75)	89 (61)	59 (62.1)	0.25
Fever	73 (26)	14 (35)	39 (26.7)	20 (21.1)	0.23
Health worker	90 (32)	11 (27.5)	64 (43.8)	15 (15.8)	**<0.001**
Other	33 (11.7)	2 (5)	18 (12.3)	13 (13.7)	0.34
Is malaria a deadly disease?					
Yes	265 (94.3)	40 (100)	141 (96.6)	84 (88.4)	**0.014**
No	12 (4.3)	0	5 (3.4)	7 (7.4)	
Don't know	4 (1.4)	0	0	4 (4.2)	
Are you scared of malaria?					
Yes	277 (98.6)	39 (97.5)	145 (99.3)	93 (97.9)	0.56
No	3 (1.1)	1 (2.5)	1 (0.7)	1 (1.1)	
Don't know	1 (0.4)	0	0	1 (1.1)	
Can malaria be cured by medicine?					
Yes	275 (97.9)	38 (95)	144 (98.6)	93 (97.9)	0.26
No	3 (1.1)	1 (2.5)	2 (1.4)	0	
Don't know	3 (1.1)	1 (2.5)	0	2 (2.1)	
*Multiple answers were possible and percentage does not add to 100, analyses were made against "yes" and "no".					

Table A4: Knowledge, perceptions and attitudes towards MDA of participants in relation to perception on asymptomatic malaria infections (n=281)					
	Total	**Possibility of asymptomatic malaria infections**			**p-value**
Characteristics	**Number (%)**	**Yes (n=40)**	**No (n=146)**	**Don't know (n=95)**	
Is malaria a big problem in your community?					
Yes	178 (63.3)	29 (72.5)	98 (67.1)	51 (53.7)	**0.005**
No	43 (15.3)	8 (20)	23 (15.8)	12 (12.6)	
Don't know	60 (21.4)	3 (7.5)	25 (17.1)	32 (33.7)	
Do you think a person in your village can have malaria parasite without being sick?					
Yes	34 (12.1)	26 (65)	6 (4.1)	2 (2.1)	**<0.001**
No	144 (51.2)	9 (22.5)	119 (81.5)	16 (16.8)	
Don't know	103 (36.7)	5 (12.5)	21 (14.4)	77 (81.1)	
Can a healthy person with malaria parasite in his body transmit to others?					
Yes	103 (36.7)	23 (57.5)	53 (36.3)	27 (28.4)	**<0.001**
No	94 (33.5)	13 (32.5)	68 (46.6)	13 (13.7)	
Don't know	84 (29.9)	4 (10)	25 (17.1)	55 (57.9)	
If Yes, should we provide medicine to all the villagers?					
Yes	78 (75.7)	17 (73.9)	41 (77.4)	20 (74.1)	0.45
No	19 (18.4)	6 (26.1)	9 (17)	4 (14.8)	
Don't know	6 (5.8)	0	3 (5.7)	3 (11.1)	
If Yes, why? (n=78)*					
To cure all villagers	29 (37.2)	11 (64.7)	15 (36.6)	3 (15)	**0.008**
To eliminate malaria from the village	15 (19.2)	4 (23.5)	8 (19.5)	3 (15)	0.8
To prevent malaria transmission in the village	41 (52.6)	9 (52.9)	18 (43.9)	14 (70)	0.15
To prevent us from malaria	34 (43.6)	7 (41.2)	13 (31.7)	14 (70)	**0.018**
Have you heard of malaria elimination in your village?					
Yes	40 (14.2)	8 (20)	19 (13)	13 (13.7)	**0.046**
No	224 (79.7)	32 (80)	121 (82.9)	71 (74.7)	
Don't know	17 (6)	0	6 (4.1)	11 (11.6)	
Do you think malaria can be eliminated from your village?					
Yes	162 (57.7)	30 (75)	82 (56.2)	50 (52.6)	**0.009**
No	66 (23.5)	7 (17.5)	41 (28.1)	18 (18.9)	
Don't know	53 (18.9)	3 (7.5)	23 (15.8)	27 (28.4)	
If Yes, how? (n=162)*					
By giving medicine to all villagers	105 (64.8)	24 (80)	56 (68.3)	25 (50)	**0.016**
By using mosquito net	81 (50)	17 (56.7)	45 (54.9)	19 (38)	0.12
By taking regular medicine	9 (5.6)	1 (3.3)	7 (8.5)	1 (2)	0.23
By cleaning surrounding	23 (14.2)	6 (20)	13 (15.9)	4 (8)	0.27
Don't know	11 (6.8)	1 (3.3)	3 (3.7)	7 (14)	**0.051**
Other	30 (18.5)	6 (20)	8 (9.8)	16 (32)	**0.006**
Would you participate in malaria elimination as a volunteer?					

Table A4: Knowledge, perceptions and attitudes towards MDA of participants in relation to perception on asymptomatic malaria infections (n=281)					
	Total	**Possibility of asymptomatic malaria infections**			**p-value**
Characteristics	**Number (%)**	**Yes (n=40)**	**No (n=146)**	**Don't know (n=95)**	
Yes	208 (74)	35 (87.5)	100 (68.5)	73 (76.8)	**0.07**
No	60 (21.4)	5 (12.5)	39 (26.7)	16 (16.8)	
Don't know	13 (4.6)	0	7 (4.8)	6 (6.3)	
If Yes, why? (n=208)*					
I want to make my community free from malaria	127 (61.1)	29 (82.9)	53 (53)	45 (61.6)	**0.008**
I want to help my community	85 (40.9)	14 (40)	56 (56)	15 (20.5)	**<0.001**
Malaria is a big problem in my community	6 (2.9)	2 (5.7)	1 (1)	3 (4.1)	0.26
Other	14 (6.7)	0	5 (5)	9 (12.3)	**0.036**
Don't know	21 (10.1)	1 (2.9)	6 (6)	14 (19.2)	**0.005**
Have your ever heard about MDA before?					
Yes	257 (91.5)	36 (90)	128 (87.7)	93 (97.9)	**0.028**
No	16 (5.7)	2 (5)	14 (9.6)	0	
Don't know	8 (2.8)	2 (5)	4 (2.7)	2 (2.1)	
Would you take part in MDA in future?					
Yes	198 (70.5)	39 (97.5)	91 (62.3)	68 (71.6)	**<0.001**
No	82 (29.2)	1 (2.5)	55 (37.7)	26 (27.4)	
Don't know	1 (0.4)	0	0	1 (1.1)	
*Multiple answers were possible and percentage does not add to 100, analyses were made against "yes" and "no".					

Part III-Community engagement

Chapter 7-Factors associated with population coverage of targeted malaria elimination (TME) in southern Savannakhet Province, Lao PDR

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Abstract

Introduction

Targeted Malaria Elimination (TME) in Laos included three rounds of Mass Drug Administrations (MDA) against malaria followed by quarterly blood surveys in two villages in Nong district at Savannakhet province. The success of MDA largely depends upon the efficacy of the anti-malarial drug regimen, local malaria epidemiology and the population coverage. In order to explore the reasons for participation in TME, a quantitative survey was conducted after the completion of the three rounds of MDA.

Methods

The survey was conducted in two villages with a total of 158 households in July and August 2016. Among the 973 villagers eligible for participation in the MDA, 158 (16.2%) adults (>18 years) were selected, one each from every household for the interviews using a quantitative questionnaire.

Results

150/158 (94.9%) respondents participated at least in one activity (taking medicine or testing their blood) of TME. 141/150 (94.0%) respondents took part in the MDA and tested their blood in all 3 rounds. 17/158 (10.7%) were partial or non-participants in three rounds of MDA. Characteristics of respondents which were independently associated with completion of three rounds of MDA included: attending TME meetings [AOR=12.0 (95% CI 1.1 to 20.5) (p=0.03)], knowing that malaria can be diagnosed through blood tests [AOR=5.6 (95% CI 1.0 to 32.3) (p=0.05)], all members from household participated [AOR=4.2 (95% CI 1.3 to 14.0) (p=0.02)], liking all aspects of TME [AOR=17.2 (95% CI 1.6 to 177.9) (p=0.02)] and the perception that TME was important [AOR=14.9 (95% CI 1.3 to 171.2) (p=0.03)].

Conclusion

Complete participation in TME was significantly associated with participation in community engagement activities, knowledge that the blood tests were for malaria diagnosis, family members' participation at TME and perceptions that TME was worthwhile. A responsive approach to community engagement that includes formative research and the involvement of community members may increase the uptake of the intervention.

Key words: Malaria, elimination, community, engagement, acceptability, knowledge, trust

Introduction

The spread of multidrug resistant *Plasmodium falciparum* in the Greater Mekong sub-Region (GMS) has added urgency to malaria elimination efforts [1-5]. Targeted Malaria Elimination (TME) has been proposed as a multi-pronged strategy to accelerate elimination in the GMS. The approach comprises: 1) the strengthening of village malaria workers (VMWs) to provide appropriate case management and distribute long-lasting insecticide treated bed nets (LLINs) and 2) mass drug (anti-malarial) administration (MDA) and quarterly blood survey (Figure 1). To date, this strategy is being evaluated in the Thai-Myanmar border area, Cambodia, Vietnam and Laos [6].

The pilot TME studies aim to interrupt local falciparum malaria transmission [6]. The probability of accomplishing this through MDA depends on the dynamics of local malaria transmission, the efficacy of the antimalarial regimen and coverage in the target populations [7]. Achieving a sufficiently high uptake in the target population – estimated at around 80% of all residents – is challenging for several reasons [7, 8]. For example, target communities in the GMS, where malaria transmission persists, are often isolated with limited healthcare infrastructure; apparently healthy, participants must adhere to the complete treatment regimen [9]; and concerns about potential and real side effects can discourage uptake and adherence [10].

To maximize coverage in target populations, community engagement often accompanies MDA [6-8]. This entails a range of activities to support and facilitate the uptake of an intervention and adherence, such as providing health education during community meetings or house-to-house visits [7, 11]. Community engagement is also a means of promoting sustainable change through increasing the health literacy and building local capacity [11-13].

To date, several questionnaire-based studies have examined the factors that influence coverage of mass anti-malarial administration in the GMS [9, 14]. These studies found that investments in providing information to villagers through trustworthy informants were essential to increase participation. No research has so far focused on the uptake of MDA in Laos, where this strategy has also been evaluated. In light of the specific social, cultural, health system and epidemiological circumstances in Laos, with a view to informing current and future malaria elimination campaigns, this article explores the factors associated with participation in MDA as a part of TME.

Methods

Study setting

Intervention villages

In 2016, MDA took place in two TME intervention villages (PhounMakMee: PMM; and Thathay: TT), located in remote Nong District, Savannakhet Province close to the Vietnam border (Figure 1). These villages were selected according to a 2015 malaria prevalence survey, which was conducted in two districts of Savannakhet Province [15]. Villagers were given anti-malarials as directly observed therapy (DOT). The anti-malarial regimen consisted of three rounds of 3 days of dihydroartemisinin piperaquine (DHAP) and a single low dose of primaquine (PQ) at monthly intervals (Figure 2). Blood samples were collected before the mass antimalarial administration and then every three month for 12 months to detect and quantify parasitaemia [6].

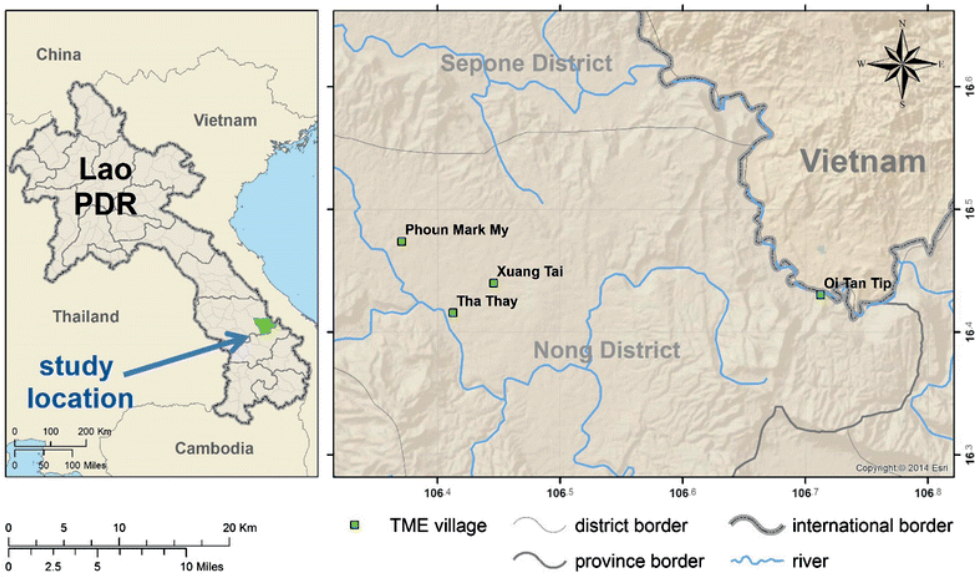

Fig. 1. TME study sites in Savannakhet Province of Laos

The residents of the intervention villages are mostly (96.8%; 153/158) from the *Lao Theung* ethnic group, who are Mon-Khamer speaking aboriginals whose oral language is incomprehensible to the majority (*Lao Lum*) ethnic group in Laos (Table 1). About one-third

of villagers are literate and the majority (90.5%) attended less than five years of school education. The majority (93%) of villagers are farmers and practice swidden cultivation of staple foods, mainly rice. Income generation is mostly based on rearing of domestic animals such as pigs, cows, buffaloes, chicken and goats, which are also a source of emergency cash [16].

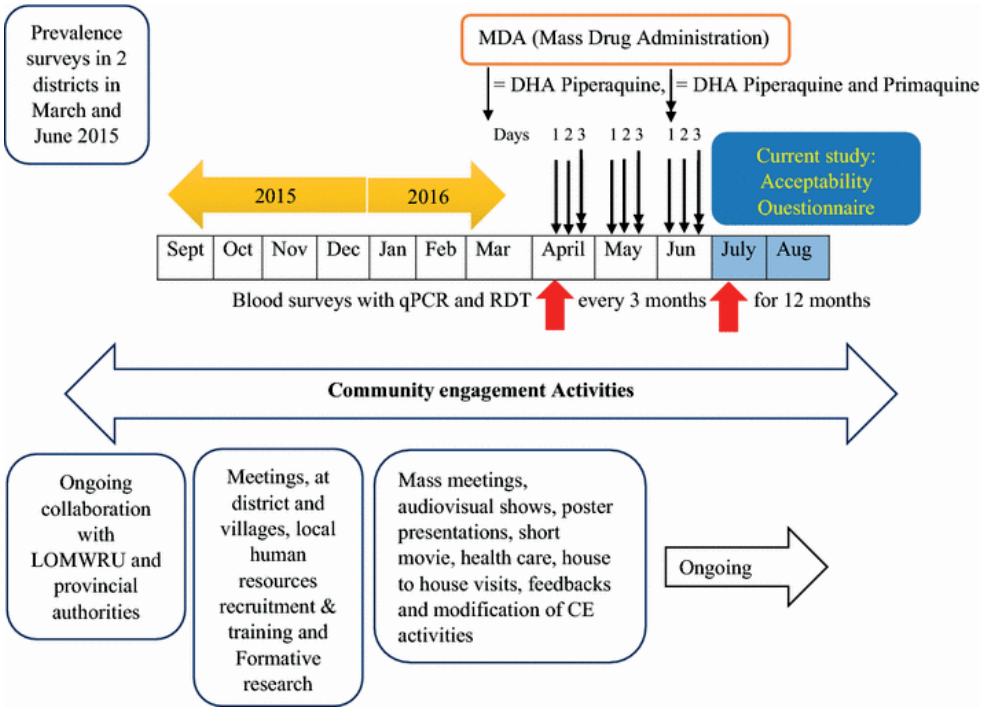

Fig. 2. Schematic diagram of MDA, CE, blood survey and acceptability questionnaire interviews.

Alongside TME in Laos, community engagement comprised five key elements. 1) The study entailed stepwise process that involved meetings with authorities at various levels before initiating village-level activities [16]. 2) Formative research (knowledge, attitudes and perceptions towards malaria and MDA) was conducted to formulate an appropriate approach at village level e.g. designing material that used pictorial explanations for TME because of villagers' low levels of literacy. 3) With the assistance of local leaders, villagers were selected and trained as volunteers who coordinated village-level meetings to inform villagers about the TME. 4) These meetings were part of the responsive approach whereby volunteers listened to and recorded their concerns so as to be able to adapt subsequent activities, for example, by conducting house-to-house visits, to respond to emerging rumors. 5) Community meetings –

and all TME activities at the village level – were jointly decided upon by TME staff and village volunteers. This shared leadership and decision making [16] is a core element of community-directed interventions and recognized as important to garner villagers' trust and participation [17, 18].

The health education tools, which were used during mass meetings and one-to-one community engagement, including videos about TME and MDA made by the study team, a malaria guide book with pictorial representation of the concept of TME, and a T-shirt with a message about malaria elimination. The study team made use of these to explain malaria transmission, prevention, treatment and elimination. These activities were intended to improve villagers' understanding of the MDA, the blood draws and of malaria in general – issues that have been recognized as barriers to participation [19, 20].

Data collection

To assess villagers' socio-demographic characteristics, their knowledge, attitudes, perceptions and experiences regarding TME, a questionnaire-based survey was conducted in July and August 2016 following three rounds of MDA. All households (n=158) within the intervention villages were included in the survey. One adult (above 18 years) from each house was interviewed. One of two trained social scientists approached the household head at his/her residence and asked his/her consent to participate in the survey. If the household head was not present, the interviewer sought consent from and interviewed any other adult household member. If consent was given, the questionnaire was administered face-to-face at the respondent's household. The majority of the questionnaires were administered in *Lao Theung* (127/158; 80.4%) with the assistance of trained local volunteers who could translate between *Pasha Lao* and *Lao Theung*. Each survey lasted about 20 to 30 minutes.

The questionnaire was adapted from a version used to assess the same factors in diverse settings, including The Gambia [20], Thai-Myanmar border [9] and Vietnam [14]. The questionnaire was translated, pre-tested and checked for clarity, language and comprehensibility with Laotian researchers at Laos-Oxford Mahosot Wellcome Trust Research Unit in Vientiane, then with 20 respondents in Vientiane, and finally at the Nong District headquarter with local household heads (n=6). After each round of pre-testing minor revisions were made.

The questionnaire contains five sections (Section I: consent, interviewer's initials, date, language of interview and participation in TME, Section II: socio-demographic characteristics of the study participants, Section III: knowledge about malaria and MDA, Section IV: experience on TME and Section V: Perceptions on TME). All variables broadly representing these sections were analyzed with the outcome variable "participation in TME".

Data management and analysis

The questionnaires were single entered into a Microsoft Excel spreadsheet. Consistency and outlying data were cross-checked against the paper questionnaire, which was used to collect data. Participation in MDA was re-categorized into 1. Complete participation and 2. None/Partial participation. Complete participation referred to respondents who took all nine doses of MDA with DHA Piperaquine and partial or none referred to respondents who took fewer than nine doses or did not participate at all. Initial analysis included frequency and percentage of socio-demographic variables in relation to participation. Comparisons were made using Chi squared test or Fisher exact test as appropriate. Significant associations were considered if p value ≤ 0.05. For logistic regression, all significant predictor including outcome variable were recoded into dichotomy, "0" representing "absence or No" and "1" representing "Presence or Yes". Considering the high correlation of the variables under a similar theme, variables representing a question or a theme relevant to research question were selected for univariate and multivariate analysis. A logistic regression model was used to test the association between the predicting variables and the outcome variables (0=partial/none participation and 1=complete participation). Variables, thematically relevant to research question, such as participation in meetings, knowledge about MDA, experience of participating in MDA and perceptions towards MDA, were explored and included in the final logistic regression model adjusting the effect of confounders. The fitness of the model was assessed using Omnibus Test of model coefficients (p value ≤ 0.05) and Hosmer and Lemeshow Test (with p value ≥ 0.05). Data were analyzed using IBM SPSS version 24.

Ethics

Ethical approval for the study was received from the Lao National Ethics Committee for Health Research (Ref. No. 013-2015/NECHR), Government of the Lao PDR and the Oxford Tropical Research Ethics Committee (1015-13).

Results

Participation in MDA and TME

The questionnaire was administered in two villages, with a combined population of 1017 (according to the TME census conducted in July 2016). Of these villagers, 973 were eligible for MDA, after excluding infants under six months, pregnant women and severely sick people. Of 973 residents, 855 (87.8%) participated in TME (blood survey and three rounds of MDA, based on the preliminary analysis). The questionnaire was administered to 158/1017 (16.2%) adults from 158 households in the intervention villages. Most respondents (150/158; 94.9%) participated in TME with 141 complete participants (141/150; 94%), who took the anti-malarial and had their blood tested in all three-monthly rounds and seventeen (17/158; 10.7%) partial or non-participants. Among these 17 partial or non-participants, nine (9/17; 52.9%) took part in at least one round of MDA and blood testing, eight (47.1%) did not participate at all (**Table 1** and **Appendix 1**). The complete non-participants, did not take part in MDA and blood test for several reasons including "fear of the blood test". Nine other respondents, could not complete the participation because s/he "was travelling", "was busy", "was pregnant" and "developed adverse events due to the medicine" (not shown in table).

Socio-demographic characteristics

Most respondents were from the *Lao Theung* ethnic group (153/158; 96.8%) (**Table 1**). Respondents reported limited education (143/158; 90.5% had <5 years of education), high illiteracy and low socio-economic status (140/158; 88.6% had monthly income of <60USD). Only a few (21/158; 13.3%) had access to a latrine at home and most defecated in the forest/fields. None of these socio-demographic characteristics were associated with participation in TME.

Table 1: Socio-demographic and economic characteristics of the respondents in relation to participation (n=158)

	Participation			
Characteristics	Partial/none (n=17)	Complete (n=141)	Total (n=158)	p-value
	Number (%)	Number (%)	Number (%)	
Respondent status				
Family Head	12 (70.6)	88 (62.4)	100 (63.3)	0.35
Other	5 (29.4)	53 (37.6)	58 (36.7)	
Age group				
≤29 years	6 (35.3)	47 (33.3)	53 (33.5)	0.72

30-40 years	7 (41.2)	48 (34)	55 (34.8)	
≥41 years	4 (23.5)	46 (32.6)	50 (31.6)	
Sex				
Female	5 (29.4)	29 (20.6)	34 (21.5)	0.28
Male	12 (70.6)	112 (79.4)	124 (78.5)	
Ethnicity				
Lao Lum	1 (5.9)	1 (0.7)	2 (1.3)	0.16
Lao Theung	16 (94.1)	137 (97.2)	153 (96.8)	
Other	0	3 (2.1)	3 (1.9)	
Religion				
Animist	16 (94.1)	138 (97.9)	154 (97.5)	0.36
Buddhist	1 (5.9)	3 (2.1)	4 (2.5)	
Marital Status				
In relationship	17 (100)	129 (91.5)	146 (92.4)	0.24
Not in relationship	0	12 (8.5)	12 (7.6)	
Literacy				
Illiterate	14 (82.4)	101 (71.6)	115 (72.8)	0.26
Literate	3 (17.6)	40 (28.4)	43 (27.2)	
Education in years				
≤5 years	16 (94.1)	127 (90.1)	143 (90.5)	0.5
≥5.1 years	1 (5.9)	14 (9.9)	15 (9.5)	
Occupation				
Farmer	16 (94.1)	131 (92.9)	147 (93)	0.66
Other	1 (5.9)	10 (7.1)	11 (7)	
Monthly income				
≤500,000 kip	16 (94.1)	124 (87.9)	140 (88.6)	0.72
≥500,001 kip	1 (5.9)	15 (10.6)	16 (10.1)	
Don't know	0	2 (1.4)	2 (1.3)	
Presence of Toilet Facility at home				
Yes	3 (17.6)	18 (12.8)	21 (13.3)	0.4
No	14 (82.4)	123 (87.2)	137 (86.7)	
Migrated from other village				
Yes	6 (35.3)	45 (31.9)	51 (32.3)	0.48
No	11 (64.7)	96 (68.1)	107 (67.7)	
Distance between forest and house in km				
≤1 km	10 (62.5)	82 (59.9)	92 (60.1)	0.53
≥1.1 km	6 (37.5)	55 (40.1)	61 (39.9)	
Frequency of visit to forest				
Everyday	9 (52.9)	87 (61.7)	96 (60.8)	0.77
≥ Every alternate day	7 (41.2)	48 (34)	55 (34.8)	
NA	1 (5.9)	6 (4.3)	7 (4.4)	

*Multiple answers were possible; percentage exceeds 100; analysis was made between "Yes" and "No"

Knowledge about MDA and malaria, experience of and perceptions towards TME

Several factors were associated with complete participation in TME. Respondents who attended TME meetings and had knowledge of malaria symptoms, diagnosis of malaria in TME (through blood test) were more likely to complete all three rounds of MDA (**Table 2**). Respondents were more likely to complete participation if their household members participated and had fewer complaints (**Table 3**). Respondents who felt that they have received enough information about TME, and had understood the study rationale and had positive impression about TME were more likely to participate in all rounds of MDA (**Table 4**).

Table 2: Knowledge about TME and Malaria of the respondents in relation to participation (n=158)

	Participation			
Characteristics	Partial/none	Complete	Total	p-value
	Number (%)	Number (%)	Number	
Heard about the current malaria elimination project				
Yes	17 (100)	141 (100)	158 (100)	NA
Heard through/from*				
District health team/village health	13 (76.5)	137 (97.2)	150 (94.9)	0.005
Neighbor	0	1 (0.6)	1 (0.6)	0.89
Village Head	10 (58.8)	109 (77.3)	119 (75.3)	0.089
Don't know	1 (5.9)	4 (2.8)	5 (3.2)	0.43
Attended meetings/events conducted by TME				
Yes	11 (64.7)	138 (97.9)	149 (94.3)	<0.001
No	6 (35.3)	3 (2.1)	9 (5.7)	
TME was explained to you by*				
Village Head	10 (58.8)	124 (87.9)	134 (84.8)	0.005
Volunteers	9 (52.9)	117 (83)	126 (79.7)	0.008
TME staffs	9 (52.9)	132 (93.6)	141 (89.2)	<0.001
Frequency of explanation about TME by study staffs				
Up to 30 times	8 (47.1)	128 (90.8)	136 (86.1)	<0.001
Can't remember/Don't know	9 (52.9)	13 (9.2)	22 (13.9)	
Frequency of explanation about TME by non-study staffs				
Up to 20 times	10 (58.8)	137 (97.2)	147 (93)	<0.001
Can't remember/Don't know	7 (41.2)	4 (2.8)	11 (7)	
We get malaria from*				
Forest	2 (11.8)	2 (1.4)	4 (2.5)	0.058
Mosquito	14 (82.4)	139 (98.6)	153 (96.8)	0.009
Signs and symptoms of malaria*				
Fever	8 (47.1)	115 (81.6)	123 (77.8)	0.003
Headache	7 (41.2)	105 (74.5)	112 (70.9)	0.007
Muscle pain	1 (5.9)	14 (9.9)	15 (9.5)	0.5
Vomiting	1 (5.9)	7 (5)	8 (5.1)	0.6

Chills/Shivering	8 (47.1)	116 (82.3)	124 (78.5)	0.003
Diarrhea	1 (5.9)	7 (5)	8 (5.1)	0.6
Don't know	6 (35.3)	15 (10.6)	21 (13.3)	0.013
Diagnosis of malaria*				
Through blood test	10 (58.8)	128 (90.8)	138 (87.3)	0.002
That person will have fever, chills and	0	7 (5)	7 (4.4)	0.44
Went to health worker	14 (82.4)	117 (83)	131 (82.9)	0.58
Went to forest before	2 (11.8)	0	2 (1.3)	0.011
An asymptomatic villager can have malaria parasite				
Yes	3 (17.6)	60 (42.6)	63 (39.9)	0.04
No	1 (5.9)	19 (13.5)	20 (12.7)	
Don't know	13 (76.5)	62 (44)	75 (47.5)	
Ways to eliminate malaria from the village*				
By Giving medicine to all the villagers	6 (35.3)	117 (83)	123 (77.8)	<0.001
By using mosquito nets	1 (5.9)	6 (4.3)	7 (4.4)	0.55
By Cleaning the surrounding	0	2 (1.4)	2 (1.3)	0.79
Don't know	9 (52.9)	18 (12.8)	27 (17.1)	<0.001

*Multiple answers were possible; percentage exceeds 100; analysis was made between "Yes" and "No"

Table 3: Experiences of TME of the respondents in relation to participation (n=158)

	Participation			
Characteristics	Partial/none (n=17)	Complete (n=141)	Total (n=158)	p-value
	Number (%)	Number (%)	Number (%)	
Provided blood for test during MDA				
Yes	9 (52.9)	141 (100)	150 (94.9)	<0.001
No	8 (47.1)	0	8 (5.1)	
If Yes, reasons (n=150)				
I want to check malaria	4 (44.4)	53 (37.6)	57 (38)	0.8
I am scared of malaria	1 (11.1)	27 (19.1)	28 (18.7)	
I am scared of illness	0	10 (7.1)	10 (6.7)	
I want to be free from malaria	2 (22.2)	19 (13.5)	21 (14)	
I want to have a good health	1 (11.1)	25 (17.7)	26 (17.3)	
Other	1 (11.1)	7 (5)	8 (5.3)	
Took medicine for mass drug administration				
Yes	9 (52.9)	141 (100)	150 (94.9)	<0.001
No	8 (47.1)	0	8 (5.1)	
If Yes, reasons (n=150)				
I want to be free from malaria	5 (55.6)	61 (43.3)	66 (44)	0.39
I want to have a good health	1 (11.1)	54 (38.3)	55 (36.7)	
I am scared of malaria	1 (11.1)	15 (10.6)	16 (10.7)	
I am scared of illness	1 (11.1)	5 (3.5)	6 (4)	
Other	1 (11.1)	6 (4.3)	7 (4.7)	
If Yes, location of the MDA (n=150)				
Village hall	7 (77.8)	101 (71.6)	108 (72)	0.56
Village center	2 (22.2)	18 (12.8)	20 (13.3)	

Other	0	20 (14.2)	20 (13.3)	
No Response	0	2 (1.4)	2 (1.3)	
Medicine distribution center was convenient				
Yes	9 (100)	138 (97.9)	147 (98)	0.83
No	0	3 (2.1)	3 (2)	
Distance between the medicine distribution center and your house				
≤100 meter	4 (40)	99 (70.2)	103 (68.2)	0.055
≥101 meter	6 (60)	42 (29.8)	48 (31.8)	
Number of people in your household				
≤6	10 (58.8)	80 (56.7)	90 (57)	0.54
≥7	7 (41.2)	61 (43.3)	68 (43)	
Everyone in my house participated in TME				
Yes	4 (23.5)	81 (57.4)	85 (53.8)	0.008
No	13 (76.5)	60 (42.6)	73 (46.2)	
I had complaints after taking medicine				
Yes	3 (33.3)	27 (19.1)	30 (20)	0.25
No	6 (66.7)	114 (80.9)	120 (80)	
If Yes, complaints started after				
Round 1	1 (33.3)	24 (88.9)	25 (83.3)	0.041
Round 2	1 (33.3)	2 (7.4)	3 (10)	
Round 3	1 (33.3)	1 (3.7)	2 (6.7)	
Household members had complaints after taking medicine (n=153)				
Yes	3 (23.1)	36 (25.7)	39 (25.5)	0.012
No	9 (69.2)	103 (73.6)	112 (73.2)	
No one took the medicine	1 (7.7)	0	1 (0.7)	
Don't know	0	1 (0.7)	1 (0.7)	

Table 4: Perceptions on TME of the respondents in relation to participation (n=158)

	Participation			
Characteristics	Partial/none	Complete	Total	p-value
	Number (%)	Number (%)	Number (%)	
Received enough information about the TME				
Yes	9 (52.9)	137 (97.2)	146 (92.4)	<0.001
Don't know	8 (47.1)	4 (2.8)	12 (7.6)	
Purpose of the medicine given to villagers*				
To kill malaria parasite in our body	8 (47.1)	132 (93.6)	140 (88.6)	<0.001
To protect from malaria	10 (58.8)	111 (78.7)	121 (76.6)	0.068
Gives me strength/energy	5 (29.4)	1 (0.7)	6 (3.8)	<0.001
Don't know	3 (17.6)	4 (2.8)	7 (4.4)	0.028
MDA medicine caused many illness in your village				
Yes	0	4 (2.8)	4 (2.5)	0.013
No	9 (52.9)	113 (80.1)	122 (77.2)	
Don't know	8 (47.1)	24 (17)	32 (20.3)	
Other villagers thought that medicine caused illness				
Yes	0	4 (2.8)	4 (2.5)	0.08
No	9 (52.9)	105 (74.5)	114 (72.2)	
Don't know	8 (47.1)	32 (22.7)	40 (25.3)	

Purpose of the blood test*				
To test for malaria parasite	7 (41.2)	121 (85.8)	128 (81)	<0.001
To test for all the diseases	0	5 (3.5)	5 (3.2)	0.56
To check if we were healthy	0	1 (0.7)	1 (0.6)	0.89
Don't know	10 (58.8)	19 (13.5)	29 (18.4)	<0.001
Disliked about TME				
Blood test	2 (11.8)	4 (2.8)	6 (3.8)	0.31
Unable to go to work	0	1 (0.7)	1 (0.6)	
Inadequate incentive	0	1 (0.7)	1 (0.6)	
Other	15 (88.2)	135 (95.7)	150 (94.9)	
If other, specify				
I like all	8 (53.3)	134 (99.3)	142 (94.7)	<0.001
I did not participate	7 (46.7)	0	7 (4.7)	
I did not like any	0	1 (0.7)	1 ((0.7)	
I think TME is important				
Yes	8 (47.1)	135 (95.7)	143 (90.5)	<0.001
Don't know	9 (52.9)	6 (4.3)	15 (9.5)	
Reason for current participation in TME*				
Because I wanted to get rid of malaria	8 (80)	109 (77.3)	117 (77.5)	0.6
Because I wanted to be healthy	5 (50)	83 (58.9)	88 (58.3)	0.4
Other	0	2 (1.4)	2 (1.3)	0.87
I would recommend TME to others				
Yes	5 (41.7)	38 (27)	43 (28.1)	0.004
No	4 (33.3)	63 (44.7)	67 (43.8)	
Don’t Know	2 (16.7)	40 (28.4)	42 (27.5)	
No response	1 (8.3)	0	1 (0.7)	
Ways a villager can help in the TME program				
I don’t know how to help	5 (41.7)	29 (20.6)	34 (22.2)	0.35
I will help by participating in the	2 (16.7)	45 (31.9)	47 (30.7)	
We all have to participate	4 (33.3)	58 (41.1)	62 (40.5)	
Other	1 (8.3)	9 (90)	10 (6.5)	
*Multiple answers were possible, therefore percentage exceeds 100; analysis were made between "Yes" and "No"				

Factors affecting participation in TME using a logistic regression model

Variables relevant to the research question underwent univariate and multivariate logistic regression analysis. Among these, five variables were found to influence participation independently: 1) Attending TME meetings [AOR=12.0 (95% CI 1.1 to 20.5) (p=0.03)]. Those who attended meetings or events, such as audio-visual shows and poster presentations were categorized as those attending meetings or events of TME. 2) Understanding that blood tests were for the diagnosis of malaria [AOR=5.6 (95% CI 1.0 to 32.3) (p=0.05)]. Respondents had multiple options (such as through blood test, through the symptoms such as fever, chills and

headache, through health worker and from the history of visiting forest) in response to how they could identify a person with malaria. TME's health messages were focused on diagnosis of malaria using blood test, also one of the main components of TME. 3) Coming from households in which all members participated [AOR=4.2 (95% CI 1.3 to 14.0) (p=0.02)]. Respondents were asked if everyone in their household participated in MDA. Respondents were more likely to complete the MDA rounds, if all family members participated. 4) Liking all aspects of the MDA [AOR=17.2 (95% CI 1.6 to 177.9) (p=0.02)]. Respondents were asked if there were any aspects of MDA that they disliked, such as blood test, taking medicine, lack of adequate health services provided by TME, loss of work while engaged in MDA, inadequate incentive, long waiting time in queue and other dislikes. Respondents who answered "I liked all" were classified as "liking all aspects of MDA". 5) The perception that MDA was worthwhile [AOR=14.9 (95% CI 1.3 to 171.2) (p=0.03)] (**Table 5**). Respondents were asked if they thought that MDA was important. Follow up questions were asked to provide the reasons; most respondents who described the importance of MDA provided reasons such as the health benefits of taking medicine, specifically to cure the disease and to avoid malaria in future.

Table 5: Logistic regression on association between covariates with complete participation

Covariates	Participation		Univariate analysis		Multivariate analysis	
	Partial/none (n = 17)	Complete (n = 141)	Crude OR (95% CI)	p value	AOR (95% CI)	p value
	Number (%)	Number (%)				
Sensitization by District Health Team/Village Health Workers/Study Staffs	13 (8.7)	137 (91.3)	10.53 (2.35–47.14)	*0.002*	0.98 (0.04–20.54)	0.99
Attended meetings of TME	11 (7.4)	138 (92.6)	25.09 (5.51–114.24)	*< 0.001*	12.01 (1.14–125.99)	*0.03*
Village head explained TME to you	10 (7.5)	124 (92.5)	5.1 (1.71–15.19)	0.003	4.54 (0.94–21.75)	0.058
Study staffs explained TME up to 30 times	9 (6.4)	132 (93.6)	11.07 (3.65–33.61)	*< 0.001*	2.97 (0.58–15.19)	0.19
We get malaria from mosquito	14 (9.2)	139 (90.8)	9.26 (1.21–70.63)	0.032	0.12 (0.002–7.60)	0.32
Fever is the sign and symptoms of malaria	8 (6.5)	115 (93.5)	4.97 (1.75–14.12)	*0.003*	2.21 (0.46–10.63)	0.32
Malaria can be diagnosed through blood test	10 (7.2)	128 (92.8)	6.89 (2.24–21.16)	*0.001*	5.68 (1.00–32.30)	*0.05*
A healthy looking person can have malaria	3 (4.8)	60 (95.2)	3.45 (0.95–12.56)	0.06	0.97 (0.18–5.15)	0.97
Malaria can be eliminated by giving medicine to all the villagers	6 (4.9)	117 (95.1)	8.93 (3.01–26.51)	*< 0.001*	3.87 (0.74–20.08)	0.1
Everyone from my house participated	4 (4.7)	81 (95.3)	4.38 (1.36–14.12)	*0.013*	4.27 (1.3–14.02)	*0.017*
Had complaints after round 1	1 (4)	24 (96)	3.28 (0.41–25.94)	0.26	3.01 (0.33–26.97)	0.32
Had complaints with my HH members	3 (7.7)	36 (92.3)	1.6 (0.43–5.88)	0.48	0.89 (0.21–3.73)	0.88
Received enough information	9 (6.2)	137 (93.8)	30.44 (7.68–120.62)	*< 0.001*	0.37 (0.01–11.89)	0.58
Medicine was given to kill malaria parasites	8 (5.7)	132 (94.3)	16.5 (5.13–53.02)	*< 0.001*	6.77 (0.89–51.5)	0.06
Medicine did not cause many illnesses	9 (7.4)	113 (92.6)	0.27 (0.09–0.78)	0.016	1.16 (0.12–10.91)	0.89
Blood was taken to test for malaria parasite	7 (5.5)	121 (94.5)	8.64 (2.94–25.33)	< 0.001	0.76 (0.04–11.75)	0.84
I liked all about MDA	8 (5.6)	134 (94.4)	21.53 (6.36–72.82)	< 0.001	17.2 (1.66–177.99)	*0.017*
MDA is important	8 (5.6)	135 (94.4)	25.31 (7.21–88.81)	*< 0.001*	14.94 (1.3–171.27)	*0.03*
I will participate if MDA happens next year	8 (5.8)	130 (94.2)	13.29 (4.27–41.32)	< 0.001	2.34 (0.27–20.05)	0.43
I would recommend MDA to others	5 (11.6)	38 (88.4)	0.88 (0.29–2.68)	0.83	0.18 (0.03–1.05)	0.057

*AOR=Adjusted Odds Ratio for age and sex

Discussion

The majority of respondents participated in all three rounds of MDA, which is necessary to clear parasitaemia completely [6, 9]. This study demonstrates that contact with TME staff, particularly during the community engagement meetings, was key to participating in the MDA. Villagers were also likely to be complete participants if all other household members participated. Among the community engagement activities that accompanied the MDA, village meetings were one of the most frequent means of delivering health education to the villagers.

A minority of participants never took part in MDA (n=8) because of fears about the blood testing. Others who could not complete the participation (n=9), gave reasons such as travelling, busy due to work and adverse events due to the medicine. Such explanations are consistent with those offered for partial or non-participation in past MDAs in the Gambia [19-21], Vietnam [14] and the Thai-Myanmar border regions [9]. The villagers' reasons for partial or non-participation were discussed in meetings, and those who voiced concerns about MDA were sought out and provided with additional health education during house-to-house visits [16].

As has been highlighted elsewhere, the community engagement strategy played an important role in promoting MDA coverage. For example, in Vietnam, participation in TME was also more likely among villagers who could recall that someone had explained to them "what MDA is" [14]. In Vanuatu, community engagement activities provided a forum for sharing information about the study and resolving concerns raised. This ultimately contributed to the elimination of malaria [22].

Community meetings have been an integral part of MDAs in past [7]. In the Gambia, district level government officials led village meetings in which study objectives and methods were discussed and concerns and issues raised by villagers were addressed [23]. In Indonesia, villagers chose volunteers who held monthly meetings and conducted house-to-house health education [24]. In Kenya, meetings with authorities and trained volunteers were held at different community locations, such as schools and trading centres [25]. In Nicaragua [26], Liberia [27], Cambodia [28] and Sierra Leone [29] meetings were held as part of a stepwise process of community engagement for MDA.

The community engagement and other TME activities were coordinated with volunteers from each village. Through the volunteers, the villagers were able to take an active role in deciding on and executing TME activities. Such an approach has been recognized as a major element of

effective community engagement [7, 17, 18, 22] and community members taking more prominent roles in the design of community engagement had a positive impact in population coverage in a recent MDA in Cambodia [10].

In addition to the community engagement, villagers' experience of the TME study as a whole influenced their participation. Respondents who liked all the components of TME and thought that TME was a worthwhile activity participated in the MDA. Even though study staff made the distinction between community engagement and the clinical study, villagers tended to view the range of activities as part of one "project", which is understandable given the integrated nature of community engagement within TME. Similar findings were reported from a TME study in Myanmar where villagers and staff considered community engagement an integral part of TME [30]. Consistent with the findings from Laos, perceptions such as "MDA was important" that referred to the whole study was found to be associated with participation in The Gambia [20].

The results also indicate a role for social relationships in uptake of MDA. Villagers were more likely to be complete participants if all household members participated in the study. In Laos, a high value is placed on familial cohesion and integrity [31], and in the study villages, household hierarchies, usually led by a male household head, are important [32, 33]. There was also a tendency for conformism across households in TME villages, likely to be rooted in villagers' *Lao Theung* identity and the traditional system of mutual help between the households [32]. As previous ethnographic research has described, *Lao Theung* communities demonstrate a system of mutual support and labour exchange between households, for example work in the field, housing construction and other daily tasks. This is often termed *"aw wan sai wan"* (to take a day and to give a day) [34]. This interdependence was reflected in the communal community decision which villagers often expressed as *"If all participate, I will participate"*.

As well as raising awareness of the study, increasing villagers' familiarity with malaria, and addressing misconceptions, participation in village-wide meetings also generated pressure to conform and participate. Repeated home visits and interactions with TME staff and volunteers, gestures of commensality – sharing and eating food together – and participating in their rituals also strengthened social relationships. Developing ties of this kind, which went beyond the formal researcher-respondent relationship, prompted reciprocity and encouraged participation. In Myanmar, by following the social conventions (sharing traditional foods with the villagers,

participating in social activities, such as funerals and festivals), study staff were able to build social relationships and garner trust. Sometimes this meant that villagers participated in MDA in spite of lack of a clear understanding of the intervention [30]. In The Gambia, developing social relationships between researchers and participants, which were akin to familial bonds, has been recognized as key to building trust and for participation in clinical trials [35].

Strengths and limitations

This study took place alongside a clinical trial of TME, which entailed a carefully planned programme of community engagement that began six months before the MDA. Such intensive community engagement may not be possible for MDAs that are part of large-scale malaria control programmes. As part of large-scale implementation, it is also unlikely that blood surveys would accompany the MDA. Further research is needed to assess the factors that influence participation in large scale mass anti-malarial administrations.

The questionnaire used for this study, has been employed in locally adapted versions from several previous surveys of factors influencing participation in MDA. The questionnaire also underwent extensive pretesting. However, using a questionnaire alone limits the depth of information on villagers' reactions to TME, community engagement and nature of social relationships. Additional qualitative data collection will provide a more nuanced understanding of attitudes and behaviors when offered MDA in this context. Additional qualitative data collection, particularly using observations will provide insight into whether villagers' responses were influenced by desirability bias.

In this study, the low number of partial or non-participants limits statistical comparison and increases the likelihood of type 1 error. In addition, this low sample in one of the arms within outcome variable also affects the sensitivity and specificity of the model. Future studies with large sample size with comparable arms are required for robust statistical assessment.

Conclusion

Participation in MDA was associated with involvement in community engagement activities, knowledge that the blood test was for malaria diagnosis, family members' participation in TME and the perception that TME was worthwhile. The comprehensive community engagement strategy, which encompassed formative research, involved villagers in implementing the study and was responsive to the needs and preferences of the community contributed to uptake of

MDA in a remote population with low literacy and socio-economic status. Villagers' overall impression of the study also influenced their participation and this illustrates that community engagement cannot be easily extricated from the overall implementation of an intervention. Social relationships were also relevant to participation in MDA, suggesting that rapid implementation that leaves little time for developing such bonds may face additional challenges. Further research is needed to investigate these factors when malaria elimination activities are scaled up.

DECLARATIONS

Authors Contribution

BA, MM, AD, LvS, CP and PY designed the study. BA, XS, PK, KP, GH collected the data. BA analyzed the data and wrote the first draft. All authors read and approved the final proof.

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Competing interests

The authors declare that they have no competing interests.

Availability of data and material

All data pertaining to this study are within the manuscript and the supporting files.

Ethics approval and consent to participate

Ethical approval for the study was received from the Lao National Ethics Committee for Health Research (Ref. No. 013-2015/NECHR), Government of the Lao PDR and the Oxford Tropical Research Ethics Committee (1015-13). Verbal and written informed consent were sought with each participant before each interview.

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Appendix 1: Detailed analysis of the questionnaire

Table S1: Socio-demographic and economic characteristics of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
Respondent status				
Family Head	12 (70.6)	88 (62.4)	100 (63.3)	0.35
Other	5 (29.4)	53 (37.6)	58 (36.7)	
Age group				
≤29 years	6 (35.3)	47 (33.3)	53 (33.5)	0.72
30-40 years	7 (41.2)	48 (34)	55 (34.8)	
≥41 years	4 (23.5)	46 (32.6)	50 (31.6)	
Sex				
Female	5 (29.4)	29 (20.6)	34 (21.5)	0.28
Male	12 (70.6)	112 (79.4)	124 (78.5)	
Ethnicity				
Lao Lum	1 (5.9)	1 (0.7)	2 (1.3)	0.16
Lao Theung	16 (94.1)	137 (97.2)	153 (96.8)	
Other	0	3 (2.1)	3 (1.9)	
Religion				
Animist	16 (94.1)	138 (97.9)	154 (97.5)	0.36
Buddhist	1 (5.9)	3 (2.1)	4 (2.5)	
Marital Status				
In relationship	17 (100)	129 (91.5)	146 (92.4)	0.24
Not in relationship	0	12 (8.5)	12 (7.6)	
Literacy				
Illiterate	14 (82.4)	101 (71.6)	115 (72.8)	0.26
Literate	3 (17.6)	40 (28.4)	43 (27.2)	
Education in years				
≤5 years	16 (94.1)	127 (90.1)	143 (90.5)	0.5
≥5.1 years	1 (5.9)	14 (9.9)	15 (9.5)	
Occupation				
Farmer	16 (94.1)	131 (92.9)	147 (93)	0.66
Other	1 (5.9)	10 (7.1)	11 (7)	
Monthly income				
≤500,000 kip	16 (94.1)	124 (87.9)	140 (88.6)	0.72
≥500,001 kip	1 (5.9)	15 (10.6)	16 (10.1)	
Don't know	0	2 (1.4)	2 (1.3)	
Daily expense				
≤3000 kip	12 (70.6)	76 (53.9)	88 (55.7)	0.14

Table S1: Socio-demographic and economic characteristics of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
≥3001 kip	5 (29.4)	65 (46.1)	70 (44.3)	
Properties owned*				
House	16 (94.1)	141 (100)	157 (99.4)	0.1
Land	17 (100)	135 (95.7)	152 (96.2)	0.49
Motorbike	10 (58.8)	107 (75.9)	117 (74.1)	0.11
Tractor	6 (35.3)	48 (34)	54 (34.2)	0.55
Cars	1 (5.9)	4 (2.8)	5 (3.2)	0.43
Cattles	9 (52.9)	69 (48.9)	81 (51.3)	0.54
TV	1 (5.9)	31 (22)	32 (20.3)	0.1
Radio	0	8 (5.7)	8 (5.1)	0.39
Mobile	5 (29.4)	56 (39.7)	61 (38.6)	0.29
Material of the wall*				
Bamboo	6 (35.3)	29 (20.6)	35 (22.2)	0.14
Wood	13 (76.5)	113 (80.1)	126 (79.7)	0.46
Concrete	0	2 (1.4)	2 (1.3)	0.79
Plastic	0	4 (2.8)	4 (2.5)	0.63
Metal	0	3 (2.1)	3 (1.9)	0.7
Material of the roof*				
Bamboo	1 (5.9)	8 (5.7)	9 (5.7)	0.65
Wood	1 (5.9)	1 (0.7)	2 (1.3)	0.2
Plastic	1 (5.9)	3 (2.1)	4 (2.5)	0.36
Metal	13 (76.5)	105 (74.5)	118 (74.7)	0.56
Shingles	3 (17.6)	30 (21.3)	33 (20.9)	0.5
Material of the floor*				
Bamboo	2 (11.8)	11 (7.8)	13 (8.2)	0.42
Wood	16 (94.1)	130 (92.2)	146 (92.4)	0.62
Mud	0	3 (2.1)	3 (1.9)	0.7
Concrete	0	5 (3.5)	5 (3.2)	0.56
Presence of Toilet Facility at home				
Yes	3 (17.6)	18 (12.8)	21 (13.3)	0.4
No	14 (82.4)	123 (87.2)	137 (86.7)	
***If No*, you defecate at (n=137)**				
Field	0	2 (1.6)	2 (1.5)	0.8
Forest	14 (100)	121 (98.4)	135 (98.5)	
Migrated from another village				
Yes	6 (35.3)	45 (31.9)	51 (32.3)	0.48
No	11 (64.7)	96 (68.1)	107 (67.7)	

Table S1: Socio-demographic and economic characteristics of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
***If Yes*, years of living (n=51)**				
≤15 years	4 (66.7)	25 (55.6)	29 (56.9)	0.47
≥15 years	2 (33.3)	20 (44.4)	22 (43.1)	
Distance between forest and house in km				
≤1 km	10 (62.5)	82 (59.9)	92 (60.1)	0.53
≥1.1 km	6 (37.5)	55 (40.1)	61 (39.9)	
Distance between rice field and house in km				
≤2 km	12 (75)	89 (68.5)	101 (69.2)	0.414
≥2.1 km	4 (25)	41 (31.5)	45 (30.8)	
Frequency of visit to forest				
Everyday	9 (52.9)	87 (61.7)	96 (60.8)	0.77
≥ Every alternate day	7 (41.2)	48 (34)	55 (34.8)	
NA	1 (5.9)	6 (4.3)	7 (4.4)	
*Multiple answers were possible; percentage exceeds 100; analysis were made between "Yes" and "No"				

Table S2: Knowledge about TME and Malaria of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
Heard about the current malaria elimination project				
Yes	17 (100)	141 (100)	158 (100)	NA
Heard through/from*				
District health team/village health workers/study staffs	13 (76.5)	137 (97.2)	150 (94.9)	**0.005**
Neighbor	0	1 (0.6)	1 (0.6)	0.89
Village Head	10 (58.8)	109 (77.3)	119 (75.3)	0.089
Don't know	1 (5.9)	4 (2.8)	5 (3.2)	0.43
Discussed the information about TME with another person				
Yes	2 (11.8)	40 (28.4)	42 (26.6)	0.25
No	14 (82.4)	98 (69.5)	112 (70.9)	
Don't know	1 (5.9)	3 (2.1)	4 (2.5)	
Attended meetings/events conducted by TME				
Yes	11 (64.7)	138 (97.9)	149 (94.3)	**<0.001**
No	6 (35.3)	3 (2.1)	9 (5.7)	
TME was explained to you by*				
Village Head	10 (58.8)	124 (87.9)	134 (84.8)	**0.005**
Volunteers	9 (52.9)	117 (83)	126 (79.7)	**0.008**
TME staffs	9 (52.9)	132 (93.6)	141 (89.2)	**<0.001**
Frequency of explanation about TME by study staffs				
Up to 30 times	8 (47.1)	128 (90.8)	136 (86.1)	**<0.001**
Can't remember/Don't know	9 (52.9)	13 (9.2)	22 (13.9)	
Frequency of explanation about TME by non-study staffs				
Up to 20 times	10 (58.8)	137 (97.2)	147 (93)	**<0.001**
Can't remember/Don't know	7 (41.2)	4 (2.8)	11 (7)	
Causes of malaria				
Mosquito	15 (88.2)	139 (98.6)	154 (97.5)	0.058
Other/I don't know	2 (11.8)	2 (1.4)	4 (2.5)	
We get malaria from*				

Table S2: Knowledge about TME and Malaria of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
Forest	2 (11.8)	2 (1.4)	4 (2.5)	0.058
Mosquito	14 (82.4)	139 (98.6)	153 (96.8)	**0.009**
Signs and symptoms of malaria*				
Fever	8 (47.1)	115 (81.6)	123 (77.8)	**0.003**
Headache	7 (41.2)	105 (74.5)	112 (70.9)	**0.007**
Muscle pain	1 (5.9)	14 (9.9)	15 (9.5)	0.5
Vomiting	1 (5.9)	7 (5)	8 (5.1)	0.6
Chills/Shivering	8 (47.1)	116 (82.3)	124 (78.5)	**0.003**
Diarrhea	1 (5.9)	7 (5)	8 (5.1)	0.6
Don't know	6 (35.3)	15 (10.6)	21 (13.3)	**0.013**
Diagnosis of malaria*				
Through blood test	10 (58.8)	128 (90.8)	138 (87.3)	**0.002**
That person will have fever, chills and headache	0	7 (5)	7 (4.4)	0.44
Went to health worker	14 (82.4)	117 (83)	131 (82.9)	0.58
Went to forest before	2 (11.8)	0	2 (1.3)	**0.011**
An asymptomatic villager can have malaria parasite				
Yes	3 (17.6)	60 (42.6)	63 (39.9)	**0.04**
No	1 (5.9)	19 (13.5)	20 (12.7)	
Don't know	13 (76.5)	62 (44)	75 (47.5)	
Ways to eliminate malaria from the village*				
By Giving medicine to all the villagers	6 (35.3)	117 (83)	123 (77.8)	**<0.001**
By using mosquito nets	1 (5.9)	6 (4.3)	7 (4.4)	0.55
By Cleaning the surrounding	0	2 (1.4)	2 (1.3)	0.79
Don’t know	9 (52.9)	18 (12.8)	27 (17.1)	**<0.001**
*Multiple answers were possible; percentage exceeds 100; analysis were made between "Yes" and "No"				

Table S3: Experiences of TME of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
Provided blood for test during MDA				
Yes	9 (52.9)	141 (100)	150 (94.9)	**<0.001**
No	8 (47.1)	0	8 (5.1)	
***If Yes,* reasons (n=150)**				
I want to check malaria	4 (44.4)	53 (37.6)	57 (38)	0.8
I am scared of malaria	1 (11.1)	27 (19.1)	28 (18.7)	
I am scared of illness	0	10 (7.1)	10 (6.7)	
I want to be free from malaria	2 (22.2)	19 (13.5)	21 (14)	
I want to have a good health	1 (11.1)	25 (17.7)	26 (17.3)	
Other	1 (11.1)	7 (5)	8 (5.3)	
Took medicine for mass drug administration				
Yes	9 (52.9)	141 (100)	150 (94.9)	**<0.001**
No	8 (47.1)	0	8 (5.1)	
***If Yes,* reasons (n=150)**				
I want to be free from malaria	5 (55.6)	61 (43.3)	66 (44)	0.39
I want to have a good health	1 (11.1)	54 (38.3)	55 (36.7)	
I am scared of malaria	1 (11.1)	15 (10.6)	16 (10.7)	
I am scared of illness	1 (11.1)	5 (3.5)	6 (4)	
Other	1 (11.1)	6 (4.3)	7 (4.7)	
***If Yes,* location of the MDA (n=150)**				
Village hall	7 (77.8)	101 (71.6)	108 (72)	0.56
Village center	2 (22.2)	18 (12.8)	20 (13.3)	
Other	0	20 (14.2)	20 (13.3)	
No Response	0	2 (1.4)	2 (1.3)	
Medicine distribution center was convenient				
Yes	9 (100)	138 (97.9)	147 (98)	0.83
No	0	3 (2.1)	3 (2)	
Distance between the medicine distribution center and your house				
≤100 meter	4 (40)	99 (70.2)	103 (68.2)	0.055
≥101 meter	6 (60)	42 (29.8)	48 (31.8)	
Number of people in your household				
≤6	10 (58.8)	80 (56.7)	90 (57)	0.54
≥7	7 (41.2)	61 (43.3)	68 (43)	
Everyone in my house participated in TME				

Table S3: Experiences of TME of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
Yes	4 (23.5)	81 (57.4)	85 (53.8)	**0.008**
No	13 (76.5)	60 (42.6)	73 (46.2)	
I had complaints after taking medicine				
Yes	3 (33.3)	27 (19.1)	30 (20)	0.25
No	6 (66.7)	114 (80.9)	120 (80)	
***If Yes,* complaints started after**				
Round 1	1 (33.3)	24 (88.9)	25 (83.3)	**0.041**
Round 2	1 (33.3)	2 (7.4)	3 (10)	
Round 3	1 (33.3)	1 (3.7)	2 (6.7)	
Household members had complaints after taking medicine (n=153)				
Yes	3 (23.1)	36 (25.7)	39 (25.5)	**0.012**
No	9 (69.2)	103 (73.6)	112 (73.2)	
No one took the medicine	1 (7.7)	0	1 (0.7)	
Don't know	0	1 (0.7)	1 (0.7)	

Table S4: Perceptions on TME of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
Received enough information about the TME				
Yes	9 (52.9)	137 (97.2)	146 (92.4)	**<0.001**
Don't know	8 (47.1)	4 (2.8)	12 (7.6)	
Purpose of the medicine given to villagers*				
To kill malaria parasite in our body	8 (47.1)	132 (93.6)	140 (88.6)	**<0.001**
To protect from malaria	10 (58.8)	111 (78.7)	121 (76.6)	0.068
Gives me strength/energy	5 (29.4)	1 (0.7)	6 (3.8)	**<0.001**
Don't know	3 (17.6)	4 (2.8)	7 (4.4)	**0.028**
MDA medicine caused many illness in your village				
Yes	0	4 (2.8)	4 (2.5)	**0.013**
No	9 (52.9)	113 (80.1)	122 (77.2)	
Don't know	8 (47.1)	24 (17)	32 (20.3)	
Other villagers thought that medicine caused illness				
Yes	0	4 (2.8)	4 (2.5)	0.08
No	9 (52.9)	105 (74.5)	114 (72.2)	
Don't know	8 (47.1)	32 (22.7)	40 (25.3)	
Purpose of the blood test*				
To test for malaria parasite	7 (41.2)	121 (85.8)	128 (81)	**<0.001**
To test for all the diseases	0	5 (3.5)	5 (3.2)	0.56
To check if we were healthy	0	1 (0.7)	1 (0.6)	0.89
Don't know	10 (58.8)	19 (13.5)	29 (18.4)	**<0.001**
Purpose of the blood test thought by villagers*				
To test for malaria parasite	4 (23.5)	81 (57.4)	85 (53.8)	**0.008**
To check all the diseases	0	4 (2.8)	4 (2.5)	0.63
To check if we were healthy	0	3 (2.1)	3 (1.9)	0.7
To sell	17 (100)	140 (99.3)	1 (0.6)	0.89
To clean our blood from diseases	1 (5.9)	0	1 (0.6)	0.1
Don't know	12 (70.6)	54 (38.3)	66 (41.8)	**0.011**
Number of people with malaria will decrease this year				
Yes	7 (41.2)	90 (63.8)	97 (61.4)	0.18
No	0	2 (1.4)	2 (1.3)	
Maybe	0	3 (2.1)	3 (1.9)	
Don’t know	10 (58.8)	46 (32.6)	56 (35.4)	
***If yes,* reasons**				
Because we took medicine	6 (85.7)	82 (91.1)	88 (90.7)	0.31
We tested blood and took medicine	0	5 (5.6)	5 (5.2)	

Table S4: Perceptions on TME of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
Other	1 (14.3)	3 (3.3)	4 (4.1)	
If only a group of people take medicine, consequences are				
Medicated group will have less malaria	5 (29.4)	86 (61)	91 (57.6)	0.07
Both groups will have less malaria	0	1 (0.7)	1 (0.6)	
Don't know	12 (70.6)	53 (37.6)	65 (41.1)	
No Response	0	1 (0.7)	1 (0.6)	
Disliked about TME				
Blood test	2 (11.8)	4 (2.8)	6 (3.8)	0.31
Unable to go to work	0	1 (0.7)	1 (0.6)	
Inadequate incentive	0	1 (0.7)	1 (0.6)	
Other	15 (88.2)	135 (95.7)	150 (94.9)	
If other, specify				
I like all	8 (53.3)	134 (99.3)	142 (94.7)	**<0.001**
I did not participate	7 (46.7)	0	7 (4.7)	
I did not like any	0	1 (0.7)	1 ((0.7)	
I think TME is important				
Yes	8 (47.1)	135 (95.7)	143 (90.5)	**<0.001**
Don't know	9 (52.9)	6 (4.3)	15 (9.5)	
***Yes,* because (n=143)**				
It will make me/us healthy	4 (50)	81 (60)	85 (59.4)	0.54
They are here to treat us	1 (12.5)	12 (8.9)	13 (9.1)	
We can eliminate malaria	1 (12.5)	12 (8.9)	13 (9.1)	
To treat/prevent malaria	0	17 (12.6)	17 (11.9)	
Other	2 (25)	13 (9.6)	15 (10.5)	
I would participate in future TME				
Yes	8 (47.1)	130 (92.2)	138 (87.3)	**<0.001**
No	4 (23.5)	3 (2.1)	7 (4.4)	
Yes, only if	0	5 (3.5)	5 (3.2)	
Don’t know	5 (29.4)	3 (2.1)	8 (5.1)	
Reason for current participation in TME*				
Because I wanted to get rid of malaria	8 (80)	109 (77.3)	117 (77.5)	0.6
Because I wanted to be healthy	5 (50)	83 (58.9)	88 (58.3)	0.4
Other	0	2 (1.4)	2 (1.3)	0.87
I would not participate if not provided following items				
Free health care	3 (30)	65 (46.1)	68 (45)	0.62
T shirts	0	2 (1.4)	2 (1.3)	

Table S4: Perceptions on TME of the respondents in relation to participation (n=158)				
	Participation			
Characteristics	**Partial/none (n=17)**	**Complete (n=141)**	**Total (n=158)**	**p-value**
	Number (%)	**Number (%)**	**Number (%)**	
All of the above	2 (20)	34 (24.1)	36 (23.8)	
Even if nothing	5 (50)	38 (27)	43 (28.5)	
Other	0	2 (1.4)	2 (1.3)	
I would recommend TME to others				
Yes	5 (41.7)	38 (27)	43 (28.1)	**0.004**
No	4 (33.3)	63 (44.7)	67 (43.8)	
Don't Know	2 (16.7)	40 (28.4)	42 (27.5)	
No response	1 (8.3)	0	1 (0.7)	
***If Yes,* reasons**				
Because it will keep us healthy	3 (60)	20 (52.6)	23 (53.5)	0.43
I want to eliminate malaria	1 (20)	12 (31.6)	13 (30.2)	
I want to help them	0	3 (7.9)	3 (7)	
I am scared that they can transmit me	1 (20)	1 (2.6)	2 (4.7)	
Some people do not understand	0	2 (5.3)	2 (4.7)	
***If No,* reasons**				
I don't know how to say	3 (75)	30 (47.6)	33 (49.3)	0.46
Thy don't listen to me	0	14 (22.2)	14 (20.9)	
I don't want to talk	1 (25)	8 (12.7)	9 (13.4)	
Other	0	11 (17.5)	11 (100)	
Ways a village can help in the TME program				
I don't know how to help	5 (41.7)	29 (20.6)	34 (22.2)	0.35
I will help by participating in the project	2 (16.7)	45 (31.9)	47 (30.7)	
We all have to participate	4 (33.3)	58 (41.1)	62 (40.5)	
Other	1 (8.3)	9 (90)	10 (6.5)	
*Multiple answers were possible, percentage exceeds 100; analysis is based on a binary response "Yes" or "No"				

Part IV-Discussion of the main findings and perspectives

Chapter 8- Discussion of the main findings and perspectives about how to eliminate all malaria.

Discussion

Malaria elimination is one of the greatest challenges for national malaria control programmes. The global malaria community view that malaria elimination can be achieved in some countries and regions by scaling-up aggressively current available tools, but that global eradication is too difficult to reach [1]. However, the global goal is to eliminate all malaria species in 35 countries by 2030, including countries in the GMS. This goal seems to be very challenging to achieve as the current approach to malaria control are threatened by the emergence and spread of multidrug resistant *P. falciparum* parasites across the GMS, while the current tools do not target the asymptomatic reservoirs and *Plasmodium vivax* or its liver hypnozoites that require radical treatment with an 8-aminoquinoline. To avoid a reversal of the gains made over the last two decades, and to achieve the goal by 2030, mass drug administration, that targets both symptomatic malaria as well as asymptomatic reservoirs, has become a renewed center of interest and needs to be assessed.

The impact of MDA with dihydroartemisinin-piperaquine and single low dose primaquine on the prevalence and incidence of *P. falciparum*

In light of the emergence of artemisinin resistant *P. falciparum* across the GMS and while available ACTs are still effective, targeted mass drug administration with the aim to eliminate *P. falciparum* was assessed in the four countries of the GMS [2]. In Laos (**chapter 5**), screening surveys looking for villages with high prevalence of asymptomatic *P. falciparum* infection were conducted in 18 villages in Thapangthong and Nong districts of Savannakhet province by using ultrasensitive qPCR. To our knowledge, this was also the first survey that showed the importance of asymptomatic malaria in Laos, and that these asymptomatic malaria infections are common in low-endemic areas where residents are considered to have limited protective or suppressive immunity [3]. *Plasmodium* parasites were detected in 175/888 individuals (20%). The prevalence of asymptomatic malaria varied between villages and districts, with the highest prevalence of mono-*P. falciparum* infection being 16% and mono-*P. vivax* 30%. Similar to clinical cases of malaria, asymptomatic *P. vivax* infection was more frequent than asymptomatic *P. falciparum* infection. Treatment of the asymptomatic reservoirs should be targeted because they lead to undetected and untreated malaria enabling further transmission, particularly asymptomatic *P. vivax* carriers who can infect mosquitoes through blood feeding, and those carriers harbour parasites over a potentially long period of time [4-6].

The impact of mass drug administration with three rounds of DP and single low dose primaquine on the prevalence and incidence of *P. falciparum* in Laos PDR was reported in **chapter 2**. This study highlighted that MDA was feasible and acceptable in the Lao context, with high levels of participation and adherence to treatment. This was established through intensive community engagement. The prevalence of asymptomatic *P. falciparum* infections after MDA decreased by 85% from 4.8% at baseline to 0.7% at month 12 (10 months after the last round of MDA). Although MDA could not interrupt malaria transmission, a sustained reduction of *P. falciparum* prevalence to less than 1% for at least one year is important for malaria elimination programmes. In the pooled data from the four countries participating in the TME study, the asymptomatic *P. falciparum* prevalence dropped from 5.1% at baseline to 0.4% at month 3 after which it rose gradually to 1.8%, 2.8% and 3.3% at month 6, 9 and 12, respectively. The impact of MDA varied by country [7], the greatest impact was recorded in Laos, followed by Cambodia and Myanmar, with little effect in Vietnam. There have been three other cluster-randomized control trials on the impact of MDA on the prevalence of *P. falciparum* so far. A study in Zanzibar, where *P. falciparum* was predominant (about 70% of all malaria cases), in which two rounds of DP and single dose of primaquine were administered, assessed the confirmed cumulative incidence and prevalence of all types of malaria. The study reported a short-term impact on all malaria infections at 3 months post-MDA but not thereafter, despite high MDA coverage [8]. An MDA study conducted in the low endemic area in Tanzania was underpowered to assess the impact of MDA due to prior decline in malaria intensity before the intervention. The study reported a *P. falciparum* prevalence by molecular technique prior to the intervention of 2.2-2.7%, but parasites were undetectable during follow-up in both the control and intervention villages [9]. An MDA study conducted in the highly endemic setting in the Gambia with *P. falciparum* prevalence being more than 40%, found no benefit of MDA, with *P. falciparum* incidence being even higher after two months in the MDA villages [10]. In contrast, there are reports from non-randomized controlled studies and uncontrolled before-after studies that do report an impact of MDA on the prevalence of *P. falciparum* extending beyond 6 months [11].

Uncontrolled before-and-after studies of MDA conducted on islands in Vanuatu showed a larger impact of MDA resulting in malaria-free areas. This successful MDA was not only attributed to compliance to MDA, and bed net use, but also to the isolation of the area which reduced the risk of imported malaria [12]. Findings from another uncontrolled before-and-after evaluation of mass drug administration with DP and a single primaquine dose conducted in 17

villages in Cambodia also showed a varied reduction in malaria by village. Eight of 17 villages experienced a drop of prevalence to 0% for at least three years of follow-up [13]. Areas of high malaria transmission experienced a return of malaria to the pre-MDA level as quickly as 4 weeks after MDA [14]. While MDA shows impact in low-endemic areas than in high endemic areas, MDA in low-endemic areas where imported cases or mobility of residents are expected to be high might also not be useful.

The (long-term) impact of an MDA intervention depends on several factors. The intervention needs to have a high coverage, otherwise it leaves too many residual infections in the non-participation group and people with contra-indications to drugs used in MDA. These individuals will then serve as a source for further transmission. Moreover, sustaining the reduction in malaria transmission post-MDA requires concomitant strengthening of existing malaria control measures including prompt diagnosis and treatment, effective vector control, and a strong surveillance system to prevent the reintroduction of malaria from malarious areas, and MDA should be scaled-up to neighbouring areas where malaria is also endemic.

Elimination of *P. falciparum* from the GMS should be accelerated before all available and still effective ACTs become ineffective. Meanwhile, alternative treatments should be investigated urgently for improved case management and further elimination.

In conclusion, MDA with three rounds of DP and single dose of primaquine was safe, feasible, acceptable and effective with high MDA coverage and reduction of *P. falciparum* infections for over one-year follow-up in research settings in Laos. However, logistical, financial, operational, and biological (emergence of drug resistant due to drug pressure) challenges remain if larger scale MDA is considered as a tool to accelerate malaria elimination, while the reintroduction of malaria is inevitable if the areas are highly malaria prevalent.

The concomitant impact of MDA with the aim to eliminate *P. falciparum* on the prevalence and incidence of *P. vivax*

While the goal of elimination of *P. falciparum* is set for 2025, the same goal for of *P. vivax* is set for 2030. The elimination of *P. vivax* is even more challenging than that for *P. falciparum* due to its numerous adaptive biological mechanisms. Currently, appropriate strategies to control and eliminate vivax malaria are weak, and there is a lack of robust evidence concerning the efficacy of specific tools [15]. In **Chapter 3**, we reported a post-hoc analysis of the concomitant effect of MDA with dihydroartemisinin-piperaquine (DP) plus single low dose primaquine for *P. falciparum* elimination on prevalence, incidence and frequency of recurrence

of *P. vivax* infection the Greater Mekong Subregion. The single low dose primaquine is to clear *P. falciparum* gametocytes but has no effect on hypnozoites of *P. vivax.* This study found no evidence of a sustained effect of MDA with DP on the prevalence, incidence and frequency of recurrence of *P. vivax* infection. The rebound in the prevalence of *P. vivax* infection after MDA was seen in all countries except Laos. The absence of the rebound in Laos is probably due to the lower incidence of asymptomatic and symptomatic *P. vivax* infections, resulting in a lower risk of relapse or reinfection. Generally, recurrent infection after treatment of the blood stage infection can be due to recrudescence of persistent infection, probably because of drug resistance, relapse due to activation of hypnozoites, or reinfection following a new infection by an infectious mosquito bite. Recrudescence was unlikely for our study as several uPCRs done within this period were negative [16]. The recurrences of *P. vivax* parasitaemia after three rounds of MDA were likely due to relapses and, to a lesser extent, reinfections. In areas where *P. falciparum* and *P. vivax* are co-endemic, MDA should include an 8-aminoquinoline to eliminate all malarias. Radical treatment to clear persistent *P. vivax* liver stages requires at least a 7 day-course of high-dose-primaquine (preferably 14 days standard dose) or single dose of tafenoquine. The use of 8-aminoquinolines is generally limited, particularly in affected areas due to their risk of acute haemolysis in people with G6PD deficiency, the severity of which is driven by the magnitude of the G6PD deficiency and the dose of primaquine. Therefore, administering primaquine is not recommended without prior G6PD testing. Despite the existing recommendation to use a weekly dose of primaquine in unknown G6PD status or deficiency, this recommendation is rarely followed. Large-scale distribution of primaquine has been conducted in several countries without G6PD testing like Azerbaijan, Tajikistan, North Afghanistan and Democratic People's Republic of Korea. This was in an attempt to control post-eradication malaria epidemics using a 14-day primaquine regimen with a dose of 0.25 mg/kg/day, resulting in significant reductions of vivax malaria [17]. In some areas in Azerbaijan where prevalence of G6PD deficiency is high, interrupted mass primaquine preventive treatment (MPPT) was adopted by stopping primaquine on day 5, 6, and 7 and restarting the treatment on day 8 until day 17 [17]. This interrupted regimen allows identifying the toxicity of primaquine and discontinuation of the treatment. Rapid diagnostic tests to detect G6PD deficiency are available but not practical in rural settings either due to their difficult interpretation, or their inability to detect G6PD deficiency in heterozygous women with intermediate enzyme activity [18].

The efficacy of 14-day primaquine at 0.5 mg/kg/day for reducing the incidence of recurrent asymptomatic *P. vivax* infection detected by ultrasensitive PCR was assessed and reported in **Chapter 4**. It is well known that *P. vivax* is an important contributor to morbidity in endemic areas due to its ability to cause relapses by dormant liver stage parasites weeks to months after the initial attack. In South-East Asia, relapses are usually at three weeks intervals. However, another determinant of the timing and number of relapses is the number of sporozoites inoculated by infected mosquitoes [19]. The 14-day regimen is recommended to mitigate the risk of haemolysis. However, in South-East Asia and Oceania where the vivax strain has been shown to be more resistant to primaquine resulting in more frequent relapses, a higher dose of primaquine (0.5 mg/kg for 14 days) has been recommended [19]. This prolonged course of treatment can result in poor adherence requiring directly observed therapy (DOT) to make sure patients are compliant with treatment. That is why many studies have tried to shorten the course. Trials to assess the efficacy of radical treatment of *P. vivax* require a large sample of participants to detect clinically relevant reductions in the number of recurrent clinical *P. vivax* malaria episodes. On the other hand, asymptomatic relapses are quite common after treatment of the infection and might be missed by conventional diagnostic procedures. We therefore assessed the efficacy of standard high dose primaquine on the incidence of recurrent asymptomatic *P. vivax* infection by using ultra-sensitive quantitative PCR with lower limit of detection at 22 parasites/ml as those asymptomatic recurrences can also be a potential source of transmission. This high dose given together with a three day-course of DP was well tolerated, safe, and effective for the prevention of recurrences over 12 month follow-up of asymptomatic *P. vivax* infection. In our study, there was no clinically relevant drop in haemoglobin concentration, despite the risk of haemolysis increasing with increasing doses of primaquine and potential misclassification of the G6PD status of G6PD-heterozygous females because of the use of the fluorescent spot test [20]. However, our sample size was too small to properly assess the safety of this high dose, so safety needs to be reconfirmed in much larger studies.

Five of the 20 participants (29%) in the placebo arm experienced asymptomatic, recurrent *P. vivax* infections. The median time to first recurrence of asymptomatic *P. vivax* was 178 days (range: 62-243 days). None of the participants with recurrent *P. vivax* infection was symptomatic. Our study shows the potential use of uPCR as a tool to detect the asymptomatic malaria as recurrent infections can also be asymptomatic resulting in a hidden infective reservoir. It also highlighted that a long follow-up for up to 12 months is required to capture

the majority of recurrences of asymptomatic malaria unlike the typical treatment of symptomatic cases, where relapses occur around six weeks after treatment [21, 22].

In the context of malaria elimination in settings where *P. vivax* is co-endemic, a full course of an 8-aminoquinoline should be added to MDA, in combination with reliable G6PD testing for safe use of 8-aminoquioline to eliminate all malarias. The current point-of-care G6PD tests in general misidentify women who are heterozygous for G6PD deficiency and put them at risk of haemolysis if a higher daily dose of primaquine is used. There is a need for the development of reliable quantitative point-of-care testing for G6PD activity with affordable cost, robust in tropical climate, and being user friendly [23]. There have been 14 fatal cases related to PQ treatment with severe haemolysis over the last six decades and they were likely due to overdosing of primaquine [24], but there have been no severe adverse events resulting in death reported from mass primaquine use without G6PD testing conducted in China, Democratic People's Republic of Korea, Azerbaijan, Afghanistan, Tajikistan [25]. Mass primaquine in 1951 in US troops without special medical supervision was also shown to be safe [26].

To conclude, MDA with three rounds of DP and single dose of primaquine had a short-term impact on *P. vivax* prevalence, incidence and recurrence. In the countries where *P. vivax* coexist with *P. falciparum* or is even more predominant, MDA with 8 aminoquinolines (radical treatment of hypnozoites) should be included as an appropriate method for elimination of all malaria species. However, in areas of low malaria transmission and low *P. vivax* asymptomatic reservoirs, carrying out MDA with a radical cure course of primaquine might put a large number of participants at risk of experiencing haemolysis. Moreover, elimination of *P. vivax* will be tremendously ambitious and costly and takes considerably longer than the elimination of *P. falciparum*. It requires strong political commitment, funding, strong health infrastructures to provide diagnosis and treatment, effective vector control, active surveillance, and cross-border collaboration.

Role of community engagement to promote the uptake of MDA to eliminate all hidden reservoirs

Participation of the community and compliance to the treatment regimen are key factors of any MDA programme. They are independent from other factors such as the treatment regimen, the number of MDA rounds and its intervals, the timing of the intervention, and the background epidemiology of the target areas. Encouraging community members without symptoms to take several doses of antimalarials is a considerable challenge.

The lessons learnt from our experience in implementing the MDA approach in Lao PDR are discussed in **Chapter 6 & 7**. Overall, the community engagement activities should comprise the key components as follows. First, **involving all stakeholders**. A good knowledge of social and administrative structures, and health services of the community are essential. We therefore involved all stakeholders right from the beginning of the project by holding several series of meetings with authorities or leaderships. The fact that our study was conducted in a very remote area of Laos made this relatively straightforward. However, if the intervention was to be conducted in urban settings, researchers should bear in mind the complexity of urban governance with unclear responsibilities and different interests of authorities, the heterogeneity of the population with regard to social and economic characteristics, and higher levels of mobility which can enhance misinformation, misconception and mistrust. Despite active efforts and careful planning, mass public health campaigns in urban settings can be sometimes undermined [27]. Second, **sharing responsibility and leadership**. At village level, committees including village volunteers were formed that were composed of key and respected persons from the target population. During the fight against lymphatic filariasis in Kenya, MDA coverage was affected because of poor communication and distrust between community and drug distributors and this reflects that drug distributors should be patient, well-mannered, respectful, and effective communicators [28]. Village volunteers, who were selected by the community and our team, were usually helpful, not only informing the community about MDA activities and encouraging them to participate, but also being first who knew all the fears, skepticisms and rumors around the villages. This helped us to respond to the problems and misconceptions right away. They also were trained to help us provide information using illustrative posters on asymptomatic malaria, the role and composition of MDA, side effects of medication used, and the need for blood draws. The messages were consistent, simple, and easy to understand by the community.

As shown by formative research in **Chapter 6**, asymptomatic malaria was a new concept among the community. We therefore created two different posters at our setting, one to raise awareness of asymptomatic malaria and its possibility to develop symptoms in the future and onward transmission, the other to educate about how people get malaria, how it is transmitted in the community and where to seek diagnosis and treatment. We discovered later that the posters were not explicit on their own and required village volunteers to explain them due to low literacy rate in the areas. Moreover, to counteract potential language barriers with the posters, we made a humorous video in the local Lao Theung language, with participation of

community members and health staff as actors and actresses showing malaria signs and symptoms, and proper care seeking. This video was repeatedly shown during community engagement activities. Third, understanding **the socio-cultural context of the community**. The community engagement activities were predesigned but also adapted to the findings from formative research during the intervention, like their needs, worries, and beliefs or perception. Individuals who participated in MDA and quarterly surveys got incentives both in cash and as gift, together with ancillary care as needed throughout the study. Borehole pump installations for the community and electricity supply to community centres were provided as requested by the villagers. Economic disadvantage was reported as barriers to engagement with research [29]. We therefore compensated for time lost in participation in Lao PDR as participants valued the ancillary care and financial compensation [30]. Without this, the coverage could be undermined as poverty, distance to the health centre and lack of transportation were barriers to health care seeking in the areas [31]. Nevertheless, absence of such a cash compensation did not negatively affect the willingness to participate when MDA was implemented in the Cambodian site, and neither did it in another intervention in Zanzibar [32, 33]. Building trust and determining all possible barriers in advance is complex but crucial when implementing MDA. In Laos, the community had experienced in the past that a development project did not keep its promise about providing electricity to the villages for their contribution to the project. This delayed our plan for MDA for a few months and made our team work harder by organizing more meetings with presence of district and provincial authorities to assure them that our goal was to improve health and benefit the community. Similarly, communities in Myanmar had negative past experiences with MDA against filariasis [32]. Reasons for non-participation or non-completion (as shown **in Chapter 7**) were fear of blood drawing, traveling during MDA, being busy with other work, and fear or experience of adverse events due to study drugs. Similar reasons were also found in neighbouring countries where MDA was implemented [32]. Other potential barriers that should be considered are negative rumours, spreading of misinformation and inadequate duration of engagement activities [34], as well as seasonal diseases that people might consider to be side-effects of MDA drugs [35]. During our community engagement activities, the community stated that the amount of blood in the body is fixed and cannot be replenished. Moreover, they believed that we took their blood for trading. In Myanmar, where HIV prevalence was high, people were worried that blood taken would be tested for HIV [36]. Problems due to perceptions about blood taking will not occur when MDA is implemented in a routine rather than experimental setting, as the former would not include any blood draw. As such, participation in an MDA strategy could be increased. We noticed

that participants complained of a lot of side effects of study drugs used in MDA during the first round, but less at the second third round of MDA (**Chapter 2**). This triggered deployment of additional health staff to deal with problems in the field resulting in reduced worries of the participants. Experience from around the world is that getting high uptake of MDA is challenging due to misconceptions about MDA and poor understanding of its rationale [37]. Our studies show the importance of community engagement by informing people about asymptomatic infections, building trust through active collaboration of government stakeholders, key community members and the wider community through training and devolving responsibility. In our interview of 158 households (**Chapter 7**) we found that complete participation in the MDA intervention was significantly associated with participation in community engagement activities, knowledge that the blood test was used for malaria diagnosis, family members' participation, and the perception that MDA was worthwhile.

In conclusion, community engagement activities will need to differ from site to site due to a variety of factors influencing the coverage of MDA. There is no "one size fits all". Community engagement activities to increase the uptake of MDA have to involve stakeholders at different levels, be adapted to study types and designed based on a conceptual framework, be adapted to feedback from the community through formative research, and involve shared leadership by having communities participate in decision-making, building trust and strengthening social relationships.

In conclusion

The elimination of malaria worldwide is one of biggest challenges but the ultimate goal for endemic countries to achieve. For several reasons, it is unrealistic to expect elimination in the near future. Although MDA is considered as a promising tool for elimination, with ACT resistant *P. falciparum* emerging across the GMS, its implementation in this region is still questionable. Our studies showed that MDA with DP and a single low dose of primaquine in Laos was safe, feasible, and acceptable by the community. But this required prolonged and intensive community engagement activities, and a lot of effort and coordination which took at least six months before MDA implementation, and continued until the end of the trial; the operation cost was also enormous. Despite a significant reduction of *P. falciparum* prevalence after three rounds of MDA over twelve months of follow-up in Laos, its impact on *P. falciparum* varied between countries with the resurgence or reintroduction of malaria infection over time. This highlights the heterogeneity of malaria transmission in different areas within

and between countries, likely due to biological, parasitological, social, and environmental factors that drive the success or failure of MDA. Moreover, elimination of malaria where *P. vivax* is predominant is even harder to achieve due to the parasite's biological adaptation. MDA without radical treatment for *P. vivax* resulted in the recurrence of vivax infection by month 6. Adding a 14-day course of primaquine to MDA prevented recurrence of asymptomatic infections. In areas where *P. falciparum* and *P. vivax* are co-endemic, MDA should include both schizontocidal and hypnozoitocidal drugs to eliminate all malaria. However; MDA will probably fail if it is conducted in areas with heterogeneity of malaria transmission (more prevalent in hard-to-reach areas, at international borders and at different epidemiological phases requiring different interventions), high rates of migration, inefficient vector control, lack of strong health infrastructure and qualified staff, and lack of continued financial, technical support, political commitment, as these factors favour reintroduction of malaria.

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Chapter 9-Summary

Summary:

There have been significant reductions in malaria morbidity and mortality globally over the last two decades. However, artemisinin resistant *P. falciparum* that first emerged in Cambodia has spread to many countries in the Greater Mekong Subregion. This will threaten malaria control programmes globally if further spread of this resistant *P. falciparum* occurs to countries where malaria is the most predominant, especially in Africa.

Chapter 1

In chapter 1, we provided the information about malaria control programmes in the past and present, and strategies that have contributed to the reduction of malaria morbidity and mortality, We paid specific attention to the drivers of the failures and successes of mass drug administration in malaria eradication activities. Furthermore, we described the challenges posed by the emergence of artemisinin-resistant *P. falciparum*, the prevalence of asymptomatic malaria, and *P. vivax*.

Part I – The impact of mass drug administration on *P. falciparum* & *P. vivax*

Chapter 2

This study was part of a multi-country cluster randomized clinical trial to evaluate the feasibility, safety, acceptability and impact of mass drug administration on asymptomatic *P. falciparum* infection with three rounds of a 3 day-treatment course of dihydroartemisinin-piperaquine plus single low dose of primaquine at one-month intervals. In Lao PDR, two villages were randomized to receive MDA and the other two served as control. There was a high MDA participation with coverage being 84% (872/1036), of whom 90% completed all three rounds of MDA (9 doses). This study found a significant reduction in *P. falciparum* prevalence and incidence following MDA, and MDA was safe and tolerated well by participants.

Chapter 3

Here we report a post hoc analysis of the concomitant effect of the MDA strategy used in the cluster randomized trial for *P. falciparum* elimination on *P. vivax* in the Greater Mekong Subregion. The main finding from this analysis was a transient impact of the MDA strategy on *P. vivax* infections. The prevalence of asymptomatic *P. vivax* infections dropped significantly

from 9.31% at baseline to 0.89% at month 3 (one month after the third MDA round), but rebounded to 5.81% at month 6. There was no evidence that the intervention reduced the cumulative incidence of asymptomatic *P. vivax* infection (95% confidence interval for the odds ratio: 0.29 to 1.36).

Chapter 4

This was an efficacy study to evaluate 14 days of high dose primaquine (0.5 mg/kg/day) on the prevention of recurrent *P. vivax* infection by using ultra-sensitive PCR as a diagnostic tool. The study was added to the randomized controlled trial on MDA in Lao PDR, and enrolled 20 participants in a primaquine group and a placebo group. All participants had normal G6PD status. We found that the 14-day course was safe, well tolerated, and without a clinically relevant drop in haemoglobin levels. This regimen was effective in the prevention of recurrences of clinical malaria and asymptomatic malaria infections over a period of 12 month follow-up. Five of 20 (29%) participants in the placebo group experienced recurrences, with a time of recurrence ranging from 62-243 days after the last dose of primaquine. None of the participants in the primaquine group experienced a recurrence. We noted that participants with the highest parasite load at enrolment had the shortest time to first recurrence and were found to be infected with *P. vivax* for a longer period.

Part II – Asymptomatic malaria

Chapter 5

Here we described the results of a cross-sectional study that was conducted in two districts of Savannakhet Province, Lao PDR. The aim of the study was to identify the villages that could be included in the MDA-trial as mentioned in **Chapter 2**, by performing a survey on the prevalence of asymptomatic malaria infections using ultra-sensitive quantitative PCR. This study showed a high prevalence of asymptomatic *Plasmodium* infection with *P. vivax* being the dominant species. Identified isolates of *P. falciparum* harboured a mutation in the kelch 13 gene, which is associated with reduced susceptibility to artemisinin derivatives in the remote area like Nong District. These findings reflect the need for rapid elimination of *P. falciparum* in Laos and the intervention should target those asymptomatic reservoirs.

Chapter 6

This chapter described the mixed-methods formative research conducted to understand knowledge, attitudes, perceptions and practices related to asymptomatic malaria infections and mass drug administration in Nong District, Savannakhet, Lao PDR. A questionnaire was administered to each household head or representative (n=281 household) in four villages. These topics were also discussed in 12 single-gender focus group discussions (FGDs) and each FGD consisted of eight to 10 participants selected by simple randomization during village meetings. We reported that most of the participants were not aware that healthy people could carry malaria parasites in their blood. Neither was there adequate knowledge that MDA can be used as a tool to eliminate malaria. This finding was not surprising as asymptomatic malaria is a new concept, not only among villagers but also health care providers. The findings suggest that community engagement activities should focus on improving the awareness of asymptomatic malaria and its potential source of transmission before implementation of any MDA strategy, to facilitate the participation in and compliance with MDA activities.

Part III – Community engagement

Chapter 7

To assess villagers' socio-demographic characteristics, their knowledge, attitudes, perceptions and experiences regarding MDA, we performed interviews following three rounds of MDA with a representative from each household participating in the MDA trial (a total of 158 households in the two MDA villages). This information was used to identify factors associated with the number of MDA treatments received. 150/158 (94%) respondents participated in at least one activity (taking medicine or testing their blood). 141/150 (94%) of participants participated in all three MDA rounds. Using logistic regression, the independent factors that increased the possibility to participate in three complete MDAs were attending TME meetings, understanding the rationale of blood draws, coming from a household in which all members participated, liking all aspects of the MDA, and the perception that MDA was worthwhile. The minority of participants who never took part in MDA gave reasons that they feared for the blood testing, drug adverse events, travelled away, and were busy with other work.

Addendum

Summary in Dutch

Acknowledgements

PhD portfolio

About the author

List of publications

Samenvatting:

De laatste twee decennia zag wereldwijd een significante reductie in de morbiditeit en mortaliteit van malaria. Echter, artemisinine-resistente *P. falciparum,* dat als eerste werd gerapporteerd in Cambodja, heeft zich verspreid naar meerdere landen in de *Greater Mekong Subregion*. Dit heeft een negatief effect op nationale malariabestrijdingsprogramma's als de verspreiding van deze resistente *P. falciparum* zich verspreidt naar landen met een hoge malaria-incidentie, zoals in Afrika.

Hoofdstuk 1

Dit hoofdstuk geeft een introductie over malariabestrijdingsprogramma's in het heden en verleden. Het beschrijft strategieën die hebben bijgedragen aan het terugdringen van malaria-geassocieerde morbiditeit en mortaliteit. Er is specifieke aandacht voor factoren die maken dat programma's met malariabehandeling op populatieniveau (*mass drug administration* [MDA]) succesvol zijn of niet. Verder worden de uitdagingen beschreven die gepaard gaan met de opkomst van artemisinine-resistente *P. falciparum*, de prevalentie van asymptomatische malaria en *P. vivax*.

Deel I – De impact van malariabehandeling op populatieniveau (MDA) op *P. falciparum* en *P. vivax*.

Hoofdstuk 2

Deze studie maakte deel uit van een cluster-gerandomiseerde studie in meerdere landen, met als doel het testen van de haalbaarheid, veiligheid, aanvaardbaarheid en impact van MDA voor asymptomatisch infecties met *P. falciparum*. De interventie bestond uit drie maandelijkse rondes van elk 3 dagen behandeling met dihydroartemisinine-piperaquine plus één lage dosis primaquine. In Laos werden vier dorpen geïncludeerd waarvan er twee gerandomiseerd werden naar de MDA-groep en twee naar de controlegroep. De participatiegraad was met 84% (872/1036) hoog. Van alle deelnemers voltooide 90% alle drie de MDA-rondes (9 doses). Deze studie vond een significante vermindering van de prevalentie en incidentie *van P. falciparum* na MDA, terwijl MDA veilig was en goed werd verdragen door de deelnemers.

Hoofdstuk 3

Dit hoofdstuk beschrijft een post-hoc analyse van het bijkomende effect van de MDA-strategie die wordt gebruikt in de cluster-gerandomiseerde studie voor eliminatie van *P. falciparum* op *P. vivax* in de *Greater Mekong Subregion*. De belangrijkste bevinding uit deze analyse was een voorbijgaande impact van de MDA-strategie op asymptomatische infecties met *P. vivax*. De prevalentie hiervan daalde significant van 9,31% bij aanvang tot 0,89% op maand 3 (een maand na de derde MDA-ronde), maar keerde terug naar 5,81% op maand 6. Er was geen bewijs voor een reductie van de cumulatieve incidentie van asymptomatische infecties met *P. vivax* door de interventie (95% betrouwbaarheidsinterval voor de Odds Ratio: 0,29 tot 1,36).

Hoofdstuk 4

Dit hoofdstuk beschrijft een studie naar de effectiviteit een 14-daagse behandeling met een hoge dosis primaquine (0,5 mg / kg / dag) ter voorkoming van recidiverende infecties met *P. vivax*, waarbij een ultragevoelige PCR als diagnostisch hulpmiddel werd gebruikt. De studie werd toegevoegd aan het cluster-gerandomiseerde studie naar MDA in Laos, waarbij 20 deelnemers werden gerandomiseerde naar de primaquine-groep en 20 naar de placebogroep. Alle deelnemers hadden een normale G6PD-status. De 14-daagse behandeling was veilig, werd goed verdragen en leidde niet tot een klinisch-relevante daling van de hemoglobineconcentratie. De behandeling was effectief in het voorkomen van recidieven van klinische malaria en asymptomatische malaria-infecties gedurende een periode van 12 maanden. Vijf van de 20 (29%) deelnemers in de placebogroep ondervonden tem mintse één recidief van een asymptomatische infectie, met een recidiefperiode variërend van 62-243 dagen na de laatste dosis primaquine. Geen van de deelnemers in de primaquine-groep kreeg een recidief. Deelnemers met de hoogste parasietenbelasting bij de start van de behandeling hadden de kortste tijd tot een recidief en een langere periode van infectie met *P. vivax*.

Deel II – Asymptomatische malaria

Hoofdstuk 5

Hier beschrijven we de resultaten van een cross-sectioneel onderzoek dat werd uitgevoerd in twee districten van de provincie Savannakhet, Laos. Het doel van de studie was het identificeren van de dorpen voor inclusie in de cluster-gerandomiseerde MDA-studie zoals deze is beschreven in hoofdstuk 2, door het meten van de prevalentie van asymptomatische

malaria-infecties met behulp van een ultragevoelige kwantitatieve PCR. Deze studie toont een hoge prevalentie van asymptomatische Plasmodium-infecties met *P. vivax* als de dominante soort. Geïdentificeerde isolaten van *P. falciparum* vertoonden een mutatie in het kelch 13-gen, wat geassocieerd is met verminderde gevoeligheid voor artemisininederivaten in het afgelegen gebied zoals Nong District. Deze bevindingen weerspiegelen de noodzaak van snelle eliminatie van *P. falciparum* in Laos met een interventie die gericht zou moeten zijn op reservoirs van asymptomatische infecties.

Hoofdstuk 6

Dit hoofdstuk beschrijft de resultaten van formatief onderzoek naar kennis, attitudes, percepties en gedrag met betrekking tot asymptomatische malaria-infecties en malariabehandeling op populatieniveau (MDA) in Nong District, Savannakhet, Laos. Elk gezinshoofd of elke gezinsvertegenwoordiger (n = 281 huishoudens) in vier dorpen werd geïnterviewd met behulp van een vragenlijst. De onderwerpen van de vragenlijst werden ook besproken in 12 focusgroep discussies, elk bestaand uit acht tot tien deelnemers (van hetzelfde geslacht) die door middel van eenvoudige randomisatie waren geselecteerd tijdens dorpsbijeenkomsten. Dit resulteerde in de observatie dat de meeste deelnemers niet wisten dat gezonde mensen malariaparasieten in hun bloed kunnen dragen. Evenmin was er voldoende kennis dat MDA kan worden gebruikt om malaria te elimineren. Deze bevinding zijn niet verrassend omdat asymptomatische malaria een nieuw concept is voor zowel dorpelingen als voor zorgverleners. De bevindingen suggereren dat activiteiten op het gebied van het betrekken van de lokale gemeenschap bij een MDA-interventie gericht moeten zijn op het verbeteren van de kennis van asymptomatische malaria als mogelijke bron van overdracht, voordat een MDA-strategie wordt geïmplementeerd. Zodoende kan de deelname aan en naleving van MDA-activiteiten worden ondersteund.

Deel III – Betrokkenheid van de gemeenschap

Hoofdstuk 7

Om de sociaal-demografische kenmerken van de dorpsbewoners, hun kennis, attitudes, percepties en ervaringen met betrekking tot MDA te beoordelen, hebben we een een vertegenwoordiger van elk huishouden dat deelnam aan de MDA-studie in Laos (in totaal 158 huishoudens in de twee MDA-dorpen) geïnterviewd na afloop van de drie MDA-rondes. Deze informatie werd gebruikt om factoren te identificeren die geassocieerd zijn met het aantal

ontvangen MDA-behandelingen. 150/158 (94%) respondenten namen deel aan ten minste één activiteit (het nemen van medicijnen of het testen van hun bloed). 141/150 (94%) van de deelnemers nam deel aan alle drie de MDA-rondes. Het bijwonen van TME-vergaderingen, begrip van de rationale van bloedafnames, wonend in een huishoudens waarin alle leden deelnamen aan MDA, waardering van alle aspecten van de interventie en de perceptie dat MDA de moeite waard is, verhoogden de kans op deelname aan de volledige MDA-interventie. De minderheid van de deelnemers die nooit aan MDA hebben deelgenomen, gaf als redenen dat ze opzagen tegen het bloedonderzoek en de bijwerkingen van de geneesmiddelen, als ook de afstand en drukke bezigheden door hun werk.

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AMC Graduate School for Medical Sciences

PhD Portfolio Summary of PhD training

Name of PhD student: Koukeo Phommasone PhD period: January 2016-April 2020 Name of PhD supervisors: Assoc. Prof. Frank van Leth, Prof. Arjen Dondorp, Prof. Frank Cobelens, Assoc. Prof. Mayfong Mayxay.	
PhD training:	
General courses	
Course & Organizer	Time
Clinical data management at AMC, the Netherlands	9-13 Oct. 2017
Systematic reviews at AMC, Amsterdam, the Netherlands	30 Oct.-1 Nov. 2017
Endnote at AMC, Amsterdam, the Netherlands	13 Nov. 2017
Specific courses	
Survival analysis: Kaplan-Meier, Cox regression, time-dependent covariates and competing risk, Bangkok, Thailand	24-26 May. 2017
Advanced course in epidemiological analysis, at London School of Hygiene and Tropical Medicine, London, UK	4th-15th Sep. 2017
Course Methods to handle Missing Data at Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam.	27th-29th Nov. 2019
Seminar, workshops (Oral presentation/(inter)national conferences	
Targeted malaria elimination in Nong, Savannakhet Province, Lao PDR. ECTMH Antwerp, Belgium	16-20 Oct. 2017

About the author

Dr. Koukeo Phommasone, is a senior research physician at Lao-Oxford-Mahosot-Wellcome Trust Research Unit (LOMWRU) in the Lao People's Democratic Republic. He was born in Pakse District, Lao PDR, on 10th June 1978, and obtained a baccalaureate at Thesaban High School, Pakse, in 1996. Following graduation with a Diploma in Medical Doctor in 2003 from the University of Health and Sciences, National University of Laos, he received an award to train in cardiovascular diseases at Haut-Leveque Hospital, University of Victor Segalen Bordeaux 2, France. In 2007, he obtained a Master's degree in Tropical Medicine and International Health, at the Institut Francophone de Médecine Tropicale, Laos. He worked as an infectious disease doctor at Champasack Provincial Hospital prior to joining LOMWRU in 2009. Since working at LOMWRU he has been involved as clinical liaison between the laboratories and the hospital wards, and has made a significant contribution to many medical research projects carried out at the Unit, including the clinical epidemiology of melioidosis and meningitis in Laos, and clinical trials of typhus treatment. He has 39 peer-reviewed publications to his name. He is also actively involved in teaching clinical and microbiological subjects to junior doctors working at LOMWRU and Lao medical residents.

Dr Phommasone's main research areas are the epidemiology of hand, foot and mouth disease in Vientiane and enterovirus carriage in kindergarten age children; targeted malaria elimination with mass drug administration; and coordinating the Expanded Fever Surveillance study in three provinces of Laos which look for the causes of febrile illnesses and acute respiratory infections in the rural populations of Laos.

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1. Stresman G, Sepúlveda N, Fornace K, Grignard L, Mwesigwa J, Achan J, Miller J, Bridges DJ, Eisele TP, Mosha J *et al*: **Association between the proportion of Plasmodium falciparum and Plasmodium vivax infections detected by passive surveillance and the magnitude of the asymptomatic reservoir in the community: a pooled analysis of paired health facility and community data**. *Lancet Infect Dis* 2020.

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4. Phommasone K, van Leth F, Imwong M, Henriques G, Pongvongsa T, Adhikari B, Peto TJ, Promnarate C, Dorda M, Sirithiranont P *et al*: **The use of ultrasensitive quantitative-PCR to assess the impact of primaquine on asymptomatic relapse of Plasmodium vivax infections: a randomized, controlled trial in Lao PDR**. *Malar J* 2020, **19**(1):4.

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