Strategies for improving radical cure in *P. vivax* malaria

A thesis submitted for the degree of Master of Science by Research in Clinical Medicine

George Kunnackal John

St. Edmund Hall, University of Oxford

Trinity 2012

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Abstract

Plasmodium vivax can no longer be considered a benign cause of malaria with increasing reports of severe vivax malaria emphasising the importance of tackling this neglected parasite globally. Important differences in the lifecycle of *P. vivax,* in particular, its ability to relapse at varying periods after the primary infection make its control more difficult than *P. falciparum*. Prevention of relapse is dependent upon effective radical cure of blood and liver stage parasites with primaquine being the only current licensed drug effective against *P. vivax* hypnozoites. Despite half a century of its use in malaria treatment, there is still no consensus on how primaquine can be deployed safely and effectively. The aims of this thesis were to review the efficacy of different primaquine regimens in preventing *P. vivax* relapse, to quantify the risk of recurrence of *P. vivax* following *P. falciparum* infection and to determine risk factors for representation with *P. vivax*. Analyses of primaquine clinical trials conducted till date reveal the inadequacy of data for the recommendation of a uniform high dose regimen across the world. A review of clinical studies using artemether-lumefantrine (AL) for *P. falciparum* malaria conducted in *P. vivax* endemic regions demonstrated that *P. vivax* was paradoxically responsible for the majority of recurrent parasitaemia within 63 days of treatment. AL is highly active against both *P. falciparum* and *P. vivax* and hence these recurrences are likely to reflect relapse triggered by activation of dormant liver stages. To test the hypothesis that febrile illnesses other than malaria may be triggering relapses, risk factors for representation were assessed in Timika, Indonesia, an area endemic to the four main malarial species. The risk of representation with *P. vivax* was increased in patients initially presenting with non-malarial febrile illnesses (Adjusted Hazards Ratio 1.02-1.14). However, the odds were similar for representation with *P. falciparum,* suggesting an inherent risk of the patient population to cause representation rather than febrile illnesses. Patients presenting with *P. falciparum* and severe anaemia were at greatest risk of *P. vivax* representation. Further clinical studies are needed to ensure more pragmatic treatment regimens and reliable point of care testing for G6PD status to avoid haemolysis due to primaquine. In the interim, the target of primaquine radical cure should be widened to include these high risk groups.

Statement of contribution

This thesis consists of two review chapters and an analysis of surveillance data collected at Rumah Sakit Mitra Masyarakat (RSMM) hospital in Timika, Indonesia. For the review chapters, I was solely responsible for the review of literature, data extraction and analysis under the supervision of Professor Ric Price and Dr Brian Angus. Chapter 2 is modified from a manuscript recently published in the Malaria Journal; for which I wrote the first draft and am the first author. I received editorial comments from co-authors which have been incorporated into the chapter. The results of Chapter 3 have been submitted as a part of a manuscript in press in the journal Advances in Parasitology, for which I am the second author.

The data used in Chapter 4 was collected by the staff of Rumah Sakit Mitra Masyarakat (RSMM) hospital as a part of routine surveillance. I was responsible for formulating the research questions, collating the data and designing the analytical plan for the study. I assisted in the process of cross validating the raw data in excel. This data was merged along with pharmacy, mortality and laboratory data into a single database by Professor Ric Price and Dr. Nick Douglas. I conducted all the statistical analysis presented in all three chapters and drafted the presentation of results.

Published articles

John, GK, Douglas NM, et al. (2012). "Primaquine radical cure of Plasmodium vivax: a critical review of the literature." Malaria Journal **11**(1): 280.

Articles in submission

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Secondly, my co-supervisor, Dr. Brian Angus, without whom this entire project could not have been possible. I am extremely thankful for your input at crucial times which have guided me in the conceptualization and materialization of this project.

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Abbreviations

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95% CI	95% confidence interval
ACT	Artemisinin combination therapy
AHR	Adjusted hazard ratio
AL	Artemether-Lumefantrine
AM	Artemether
AQ	Amodiaquine
AS	Artesunate
AV	Atovaquone
CQ	Chloroquine
DHA	Dihydroartemisinin
DHP	Dihydroartemisinin-Piperaquine
G6PD	Glucose-6-phosphate dehydrogenase
G6PDd	Glucose-6-phosphate dehydrogenase deficiency
Hb	Haemoglobin
HR	Hazard ratio
IQR	Interquartile range
LUM	Lumefantrine
MQ	Mefloquine
N	Number
OR	Odds ratio
Pf	*Plasmodium falciparum*
PG	Proguanil
PIP	Piperaquine
Pm	*Plasmodium malariae*
PNG	Papua New Guinea
Po	*Plasmodium ovale*
PQ	Primaquine
Pv	*Plasmodium vivax*
Q	Quinine
RBC	Red blood cell
Ref	Reference category
RSMM	Rumah Sakit Mitra Masyarakat
SE	Standard error
SP	Sulfadoxine+pyrimethamine
Std dev	Standard deviation
$t_{1/2}$	Elimination half-life
URTI	Upper respiratory tract infection
vs.	Versus
WHO	World Health Organization

Chapter 1

1 Introduction

Malaria is one of the oldest diseases known in man, the *Plasmodium* species responsible for infection diverging from those infecting primates around 4 to 10 million years ago.[1] Over the ages, malaria has been one of the most deadly diseases in history. It is estimated that in the last hundred years, malaria accounted for approximately 150 million to 300 million deaths.[1] The name Malaria originated from Italian '*Mal Aria'*, meaning bad air, in the 15th Century when the Romans thought the disease was caused by vapours evaporating from the stagnant water of marshes. However, the first documentation of periodic fevers (most likely attributable to malaria) were reported as early as 2700 B.C in Chinese literature.[2] The symptoms of malaria have been recorded in the ancient Indian treatise *Sushtruta Samhita* of 6th Century B.C. and in various other texts from the Greek, Assyrian, Egyptian, Arabic and Roman civilizations.[2] The first detailed account of malaria was given by Hippocrates in the 5th Century B.C who described the different types of malaria depending on the periodicity of fever and observed splenomegaly in patients with malaria.[3] In 1880 Alphonse Laveran, a French army surgeon, gave the first description of the malaria parasite. Seventeen years later Ronald Ross, a British officer in the Indian Medical Service, identified mosquitoes as the vectors in transmission of infection.[4] Classically, four species of the apicomplexan parasite genus *Plasmodium* are known to cause malaria in humans: *P. falciparum*, *P. vivax*, *P. malariae* and *P. ovale.* More recently, *P. knowlesi,* known to cause malarial infection in monkeys and experimentally transmissible to humans, was found to be a significant cause of natural human malaria on the island of Borneo in Malaysia.[5 6] There are also recent observations by Sutherland et al suggesting that *P. ovale* may include two sympatric species, *P. ovale curtisi* and *P. ovale wallikeri*. Hence the ranks of species causing human malaria may have increased to six.

Among the *Plasmodium* species causing human infection, *P. falciparum* and *P. vivax* predominate with over half of the world's population estimated to be at risk of acquiring infection due to either species in 2010.[7] Of the two, infection due to *P. falciparum* has always been considered more dangerous due to its propensity to cause cerebral and complicated malaria. Malaria due to *P. vivax*, classically termed 'benign tertian malaria' has been considered less dangerous. However, the relapsing nature of *P. vivax* and propensity to cause disease in infancy has led to a renewed focus on this species.[8 9] The following sections of this chapter discuss the origin of *P. vivax*, the life cycle and uniqueness of the parasite, current burden of infection and public health problems specific to *P. vivax*.

1.1 Origin of *P. vivax*

Plasmodium vivax originated about 2 million years ago, diverging from the closely related simian parasite *P. cynomolgi*.[1 10] However, the geographical origin of *P. vivax* remains unclear. The "Malaria Hypothesis" first proposed by Haldane in 1948 suggests that malaria exerted a strong pressure on recent evolution and led to the development of various genetic polymorphisms affecting red blood cells.[11] One such polymorphism is the Duffy antigen, a chemokine receptor on the surface of red blood cells which is required for *P. vivax* merozoites to invade erythrocytes. Its absence in Duffy negative individuals protects against the development of *P. vivax* infection. The high prevalence of the Duffy negative blood group in West Africa has been proposed to be a protective evolutionary development against *P. vivax,* and the reason for the virtual absence of *P. vivax* in this region today.[1 12] Hamblin et al estimate that selection for the Duffy antigen took place between 6,500 and 97,200 years ago.[13]

However *P. vivax* infection in Duffy negative individuals has been reported recently in the West African countries of Angola and Equatorial Guinea.[14] This suggests the existence of

alternative mechanisms for *P. vivax* merozoites to invade erythrocytes or the evolution of *P. vivax* to develop such mechanisms. If these mechanisms have been in existence since the beginning, the theory of *P. vivax* originating in Africa is questionable. These reports however, may be indicative of an underestimation of the actual burden of *P. vivax* in Africa, which warrant further investigation.

Comparison of parasite isolates from around the world suggests that Asian isolates of *P. vivax* are the oldest. [15] The phylogenetic relationship between *P. vivax* and ten other *Plasmodium* species infecting primates were studied by Escalante et al using genotyping methods.[16] The authors concluded that the current *P. vivax* is a derivative of ancient parasites of macaque monkeys when hominoids colonized Southeast Asia. This view has also been supported by Mu et al who arrived at the same conclusion of an Asian origin from macaque monkeys using Bayesian and likelihood methods in conjunction with cophylogeny mapping.[17] In his paper titled *Speculations on the origin of Plasmodium vivax malaria*, Richard Carter has proposed that *P. vivax* originated around 2 million years ago in the Old World, somewhere in the combined Eurasia and Africa landmass. During the Ice Ages, Carter hypothesises that pockets of *P. vivax* malaria existed in relatively warm regions in Africa and the Mediterranean, from where there was further dispersion of the infection. Therefore, it is concluded that the extant *P. vivax* is a descendant of these parasites which survived the last Ice Age between 100,000 and 20,000 years ago.[10] These speculations provide a reasonable explanation for the presence of Duffy Negativity in Africa and the results of the phylogenetic analysis which indicates an Asian origin for *P. vivax*. The Duffy negativity in the African population may very well have been an evolutionary protective mechanism against infection by *P. vivax*, but why Duffy negativity isn't equally prevalent in other *P. vivax* endemic areas like Southeast Asia and the Indian subcontinent remains unclear.

1.2 Biology of *P. vivax*

1.2.1 Life cycle of the malarial parasite

The life cycle of the malarial parasite in humans begins when an infected female *Anopheles* mosquito bites an individual. In doing so, it transmits sporozoites of *Plasmodium* contained in the salivary glands of the mosquito into the human bloodstream. Sporozoites are motile forms which find their way to the liver in the host as early as one hour following a mosquito blood meal. In the liver, the sporozoites invade hepatocytes through the sentinel Kupffer cells.[18] Infected hepatocytes then become the site of asexual replication of the parasite, in a process termed exoerythrocytic schizogony or merogony to form non-motile forms called merozoites. The intrahepatic stage lasts for a median of 5.5 days in *P. falciparum,* 8 days in *P. vivax*, 9 days in *P. ovale* and about 15 days in *P. malariae*.[19] The infected hepatocyte then ruptures, releasing large numbers of merozoites into the host's blood stream. In *P. vivax* and *P. ovale* infections, non-replicating dormant forms called hypnozoites occupy some hepatocytes. These hypnozoites then re-awaken from dormancy to cause relapses of infection after varying periods from the primary infection. The trigger that initiates these relapsing infections is unknown. The number of merozoites released from each infected hepatocyte varies according to the species of *Plasmodium*, with *P. vivax* releasing 10,000 merozoites per hepatocyte to 30,000 in *P. falciparum* infection. [19]

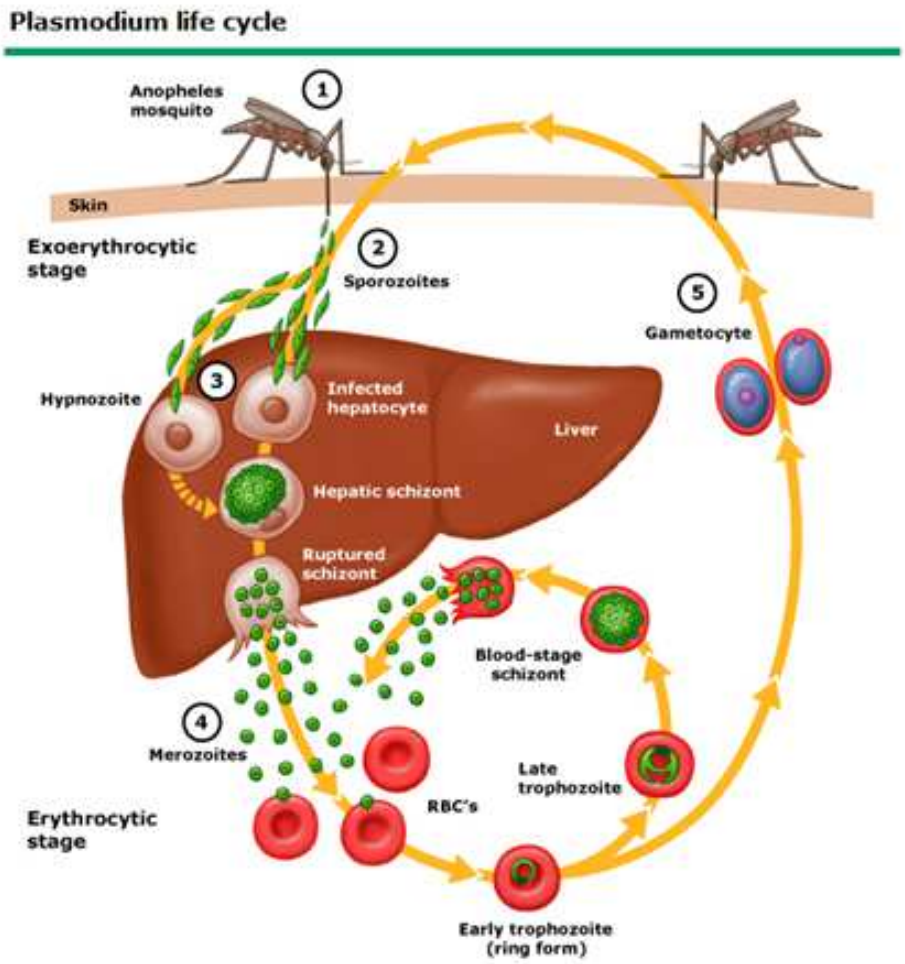


Figure 1.1. **Life cycle of the malarial parasite**. From Danny A Milner, J. (2012). Pathogenesis of Malaria. UptoDate. J. Daily. Reproduced with permission.

Merozoites are immotile forms which are programmed to invade red blood cells. Merozoites of *P. vivax* and *P. ovale* prefer immature red blood cells called reticulocytes, whereas merozoites of *P. malariae* prefer older red blood cells. Merozoites of *P. falciparum* can invade red blood cells of any age, but preferentially invade younger cells.[19] These merozoites then enlarge in size in the red blood cell, using up cell cytoplasm and haemoglobin to form trophozoites. Although early trophozoites are similar in appearance as ring forms in red blood cells, mature trophozoites vary in morphology and their appearance on peripheral blood smear is used to differentiate between the different species of *Plasmodium*. In *P.*

falciparum infections, only ring forms are seen in the peripheral blood during the erthrocytic schizogony phase. In *P. vivax*, irregularly shaped large ring forms and trophozoites are seen with brick red spots called Schuffner's dots in the red blood cells. These dots are also seen in *P. ovale* infected red blood cells and, on electron microscopy, have been shown to consist of caveolavesicle complexes and erythrocyte plasmalemma.[20] The infected erythrocytes are enlarged, oval and tufted in *P. ovale* infection. In *P. malariae* infection, band forms and rectangular trophozoites are seen. Pigment also becomes visible at this stage on peripheral smear and differs in colour among the different species. The erythrocytic stage, also called erythrocytic schizogony, lasts for 48 hours in *P. falciparum*, *P. vivax* and *P. ovale* but goes on for 72 hours in *P. malariae*. During this time the trophozoite undergoes nuclear division without cytokinesis and grows in size to form the schizont, which almost occupies the entire red blood cell.[21] Each schizont then gives rise to 6-30 merozoites which are released into the bloodstream as the red blood cell ruptures. These merozoites go on to infect more red blood cells, thus propagating the infection. The paroxysms of fever seen in the host coincide with the destruction of red blood cells by the parasite, with the periodicity depending on the length of the erythrocytic cycle.

Alongside the development of schizonts, a varying number of parasites also develop into sexual forms of macrogametocytes and microgametocytes. These are formed immediately after release from the liver in all species of *Plasmodium* except for *P. falciparum*, where sexual stages are formed only after a number of asexual cycles.[19] These gametocytes are taken up by female *Anopheles* mosquitoes when the infected host is bitten.

In the mosquito gut, favourable factors such as low temperature, mosquito metabolites and increased carbon dioxide induce gametogenesis resulting in a zygote.[21] This zygote matures into a motile ookinite which invades mosquito gut epithelial cells to develop into an oocyst. Asexual replication, termed sporogony, then takes place in the oocyst resulting in the

formation of motile sporozoites. These sporozoites have an affinity for the salivary glands of the mosquito and travel through the haemolymph to reach the salivary glands, thus completing the life cycle of the parasite.

1.2.2 Uniqueness of *P. vivax*

There are some features in the biology of *P. vivax* which set it apart from other *Plasmodium* species. Some of these features contribute to the difficulty in controlling *P. vivax* as a public health problem compared to other species. Perhaps the most important feature is the ability of *P. vivax* to attain dormancy in the liver by forming hypnozoites, a feature this species shares with *P. ovale*.[22] Not much is known regarding the biology of this liver stage. Re-awakening of these hypnozoites at varying intervals leads to a relapse of *P. vivax* infection in the host. The ability of *P. vivax* to relapse is likely to play a crucial role in the cumulative episodes of recurrent febrile illness, with substantial morbidity, mortality and economic consequences.[23]

Unlike *P. falciparum* gametocytes, *P. vivax* gametocytes are susceptible to all blood stage schizontocidal agents, and hence are rapidly cleared from the blood following initiation of treatment. However gametogenesis in *P. vivax* infection occurs early, usually prior to the development of symptoms, hence ingestion by the mosquito vector often occurs prior to drug treatment, increasing the chances of transmission of infection.[24] Erythrocyte invasion by merozoites in *P. vivax* differs from other species of the *Plasmodium* family. The invasion of reticulocytes by *P. vivax* is mediated through the Duffy antigen and leads to a large amoeboid trophozoite stage during which there is an increase in erythrocyte size and deformability.[24] This deformability is seen not only in infected red blood cells, but also in uninfected red blood cells. An experiment by Handayani *et al* mimicked the conditions of passage of red blood cells through the endothelial slits of splenic sinusoids by passaging *P. vivax* infected red blood cells through a 2μm microfluidic channel after which almost 70% of infected red cells

recovered a normal appearance.[25] Despite this increased deformability and the hypothetical ability of the flexible red blood cells to avoid destruction when passing through splenic sinusoids, *P. vivax* malaria has been associated with significant anaemia. In highly endemic areas, *P. vivax* infection is a major risk factor for severe anaemia in young children. The degree of anaemia in *P. vivax* is similar to that seen in *P. falciparum* , with red blood cell destruction sharing many of the same general processes but mediated through different cellular mechanisms.[26] In *P. vivax* infection, approximately 34 non-infected red blood cells are cleared for every infected cell compared to 8:1 ratio in *P. falciparum* malaria.[26] Although simple haemolysis of recurrent infections over a period of time would lead to substantial reduction in haemoglobin levels, the exact mechanism of anaemia in *P. vivax* malaria is still unclear and is hypothesized to be mediated through cytokine signalling pathways, oxidative damage, direct toxic effect on the marrow or through other immune mechanisms.[8 26]

P. vivax has an ability to generate fever in the host at much lower parasitaemia (pyrogenic threshold) than P. *falciparum*.[8 27] The restriction of merozoite invasion to immature erythrocytes in *P. vivax* infection translates to a lower peripheral parasitaemia. This is of significance in the detection and screening for infection, which may require multiple testing and highly skilled microscopy. The level of organ specific inflammation has also been shown to be higher in *P. vivax* infections compared to *P. falciparum*, but the exact mechanisms remain to be elucidated.[28 29]

1.3 Current burden of *P. vivax* malaria

In 2009, an estimated 2.85 billion people (42% of the total world population), were at risk of developing malaria due to *P. vivax*.[30] Although the World Health Organization (WHO) reported 2.01 million cases of *P. vivax* in 2010[31], alternate estimates place this figure

substantially higher, at anywhere from 71-80 million to 132-391 million infections each year.[23]

It is estimated that *P. vivax* causes 6-15 million infections each year in Africa, 11-28 million infections in South and Central America and 12–34 million infections in the East Mediterranean region.[7 23 32] However, the greatest burden of *P. vivax* malaria is in Southeast Asia (42.1-248 million) and the Western Pacific region (20-77 million) which together account for approximately 80% of all infections in the world.[23] Malaria due to *P. vivax* not only takes a huge toll on the overall health of its victims, but also affects their ability to lead a productive life. The total economic burden of *P. vivax* malaria, accounting for loss of productivity, healthcare costs and transport to clinics, is estimated to be approximately 1.4 to 4 billion US dollars every year.[23]

These numbers make *P. vivax* a globally important infection, affecting a large proportion of the world population and causing substantial financial costs. Although, *P. vivax* has been neglected due to its reputation of causing relatively "benign" infections compared to *P. falciparum,* this view is increasingly under threat, following *P. vivax* infection increasingly being identified as the cause of severe and fatal malaria in poorly resourced endemic communities.

1.3.1 Severe *P. vivax* malaria

Traditionally, *P. falciparum* has been considered the more dangerous or "malignant" malaria, and as a result generates the greatest public health concern. A fundamental property of *P. falciparum* is its ability to cause microvascular injury by cytoadhesion and sequestration resulting in the manifestations of severe malaria. These pathological processes are far less prevalent in *P. vivax* which is generally regarded as a "benign" malaria. However, recent reports suggest that in some locations *P. vivax* infections contribute significant risk of morbidity and mortality. There have been reports of severe malaria in *P. vivax* monoinfections from India[33 34], Brazil[35], Indonesia[36] and Papua New Guinea[37] which suggest

that the clinical spectrum of *P. vivax* infection may have been overlooked or is changing. Severe malaria due to *P. vivax* is particularly important in children less than 5 years of age where it has been demonstrated to cause severe anaemia and respiratory distress, as well as reports of renal dysfunction and cerebral malaria.[36-38] In a study investigating the spectrum of malarial infections in the high endemic region of Papua, Indonesia, there was a higher risk of severe malaria in *P. vivax* infections compared to *P. falciparum*.[36] Among the severe infections, severe anaemia with haemoglobin less than 5 g/dl was the major complication followed by respiratory distress, coma and death.[36]

Acute lung injury has been reported in several case reports from regions with high *P. vivax* endemicity.[39] [40] The exact mechanism of acute lung injury is not known, however, it is potentially caused by sequestration of *P. vivax* infected red blood cells in the pulmonary vasculature and inflammatory responses in the lung which causes progressive alveolar capillary dysfunction.[29]

The occurrence of *P. vivax* infection in pregnancy is an important cause of maternal anaemia and low birth weight.[41] A study looking at the effects of *P. vivax* in pregnancy on the western border of Thailand demonstrated significantly higher odds of low birth weight in women infected during pregnancy compared to uninfected mothers.[42] There was a mean birth weight difference of 108 g in babies born to the women with *P. vivax* infections compared to uninfected women and there was no association of maternal *P. vivax* infections with miscarriage, stillbirth or preterm deliveries.[41] [42] However, a recent study of 17,613 pregnant women conducted on the Thai Burmese border demonstrated increased risk of miscarriage in symptomatic and asymptomatic infections in the first trimester, the risk being similar in *P. vivax* and *P. falciparum*[43]. A study of 25 pregnant women infected with *P. vivax* in India also observed an increased risk of preterm delivery with 8 out of the 25 women delivering prematurely with 80% of all infants born to infected mothers having low birth weight.[44]

1.3.2 Chloroquine resistance in *P. vivax*

Chloroquine has been the most widely used antimalarial drug for the treatment of *P. vivax*. However, Chloroquine resistance in *P. vivax* has emerged as an important public health problem over the last three decades necessitating the use of second line antimalarials and artemisinin based combination therapy (ACTs) for the treatment of *P. vivax*.

Chloroquine resistance in *P. falciparum* was identified in the late 1950s[45]. In comparison, the first report of chloroquine resistant *P. vivax* was only in 1989 when two Australian soldiers returning from Papua New Guinea had persistent parasitaemia and symptoms despite high plasma concentrations of chloroquine. [46] The later development of resistance to chloroquine in *P. vivax* compared to *P. falciparum* is attributed to lower selective drug pressure in *P. vivax* and transmission before drug selection due to the early appearance of gametocytaemia.[9 47]

Resistance to chloroquine in *P. vivax* has now emerged in most vivax endemic regions of the world with high rates of failure reported in Papua New Guinea and eastern Indonesia and lower level resistance in Vietnam, South Korea, Burma, Malaysia, western Indonesia, eastern India, Turkey, South America, Madagascar and Ethiopia.[9 48] Chloroquine appears to retain efficacy against vivax in parts of the Indian subcontinent such as Pakistan, Afghanistan, most parts of India and in Iran and Azerbaijan.[9 48]

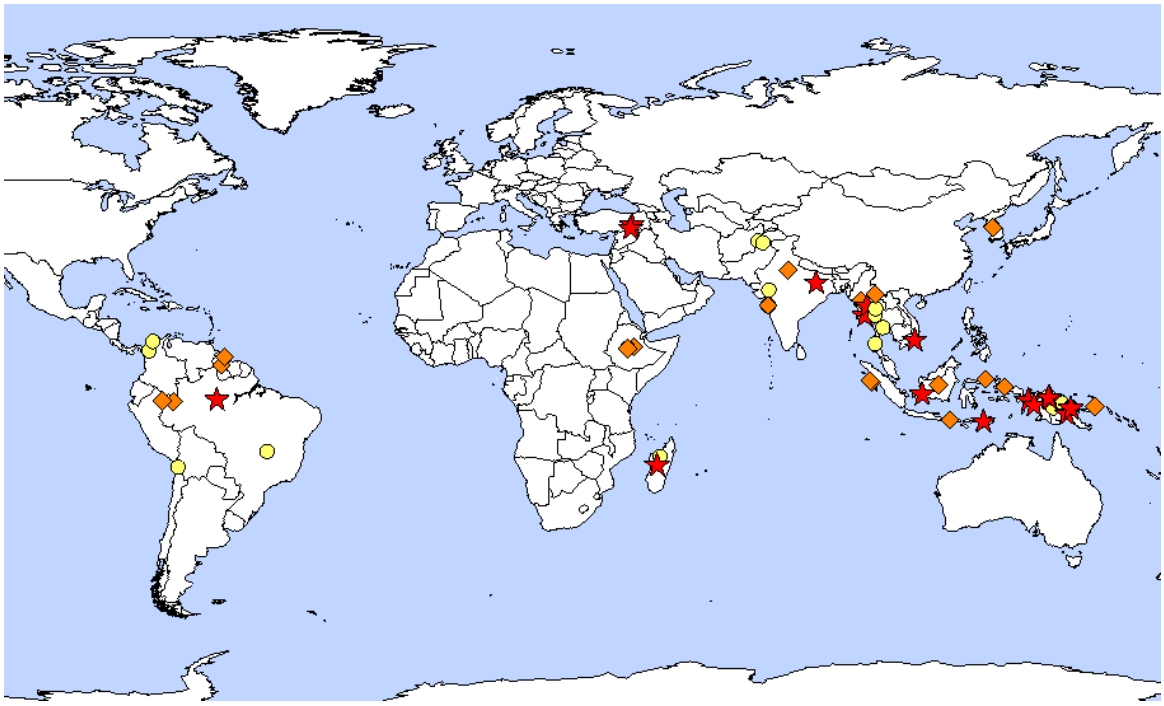


Figure 1.2. **Reports of chloroquine-resistant *Plasmodium vivax* by 2009.** From Douglas, N. M., N. M. Anstey, et al. (2010). "Artemisinin combination therapy for vivax malaria." The Lancet Infectious Diseases **10**(6): 405-416. Reproduced with permission.
Red stars represent greater than 10% recurrence (and more than five absolute failures) by day 28 with or without measurement of chloroquine concentrations. Orange diamonds represent less than 10% recurrence (or fewer than five absolute failures) by day 28, with measurement of chloroquine concentrations. Yellow circles represent less than 10% recurrence (or fewer than five absolute failures) by day 28, without measurement of chloroquine concentrations.

1.4 The origin of *P. vivax* relapses

As mentioned above, the exoerythrocytic cycle of *P. vivax* and *P. ovale* in the human host is distinct from other members of the Plasmodium genus in its ability to remain dormant in hepatocytes as a hypnozoite. The ability of *P. vivax* to cause fever many months after the primary infection was known as early as 1897. However, little was known about the mechanism behind this occurrence for the next half decade. In 1946, Shute proposed the existence of the "x body", sporozoites held up in tissue cells which could survive in host cells for a prolonged period of time.[49] This was followed by the discovery of liver schizonts in rhesus monkeys in 1948 by Shortt and Garnham 102 days after inoculation which led to a new hypothesis that successive cycles of development in the liver was responsible for

relapses of *P. vivax* malaria.[22 50] However, after further work this theory was rejected by the authors who proposed the existence of a dormant stage as suggested earlier by Shute. In 1980, Krotoski and Garnham *et al* identified possible dormant bodies using immunofluorescence and Giemsa staining of the liver of rhesus monkeys inoculated with *P. cynomolgi* which is known to be closely related to *P. vivax*. These parasite forms varied in size from 5.7 μm to 7 μm, were uninucleate and were present in the cytoplasm of hepatocytes.[51] These were called hypnozoites, a term which could possibly have been in existence earlier.[52] This term is derived from the Greek words *hypnos* (sleep) and *zoon* (animal). In 1982, Krotoski *et al* demonstrated the existence of hypnozoites in the liver of chimpanzees inoculated with *P. vivax*.[22] This discovery ended a long period of speculation about the existence of a latent stage in the life cycle of *P. vivax* and solved the mystery behind the reason for relapses in *P. vivax* malaria. However, despite 30 years since the discovery of the hypnozoite stage, the biology behind the dormancy and re-activation of *P. vivax* still remains an enigma.

1.4.1 Public health importance of relapse

Relapse due to *P. vivax* needs to be addressed with priority. It has been demonstrated that intense anti-malarial efforts are often successful in reducing cases of *P. falciparum* malaria but less so against *P. vivax*.[53 54 55] Moreover, in regions with co-endemicity of *P. falciparum* and *P. vivax* such as Southeast Asia and South America, an increase in *P. vivax* malaria has been observed following decline of *P. falciparum* malaria.[54-56] An important factor contributing to this increase in cases after anti-malarial programs is the re-awakening of dormant hypnozites leading to recurrence and renewed transmission of malaria in the region. Even if the majority of the population in a region is successfully treated for malaria, unless a radical curative treatment is administered, there always exists the possibility of re-emergence of malaria due to relapse. Individuals attain immunity against *P. vivax*, and hence this increases the likelihood that many of these relapses, especially in adults, are asymptomatic resulting in the

individual not seeking treatment. However, the morbidity of recurrent infection is often borne by children who are less likely to be immune and can get repeated symptomatic relapses leading to a significant drop in haemoglobin levels.[26].

Relapse of malaria can also be a difficult problem in countries closing in on elimination of the disease. For example, Sri Lanka which is in the pre-elimination phase faces the challenge of identifying individuals with asymptomatic *P. vivax* who may be responsible for re-emergence of malaria in the region. It is also difficult for these countries to screen for malaria in people returning from endemic regions, since no tests can identify the presence of hypnozites in the liver.

1.4.2 Reducing the burden of *P. vivax* associated with relapse

The vast majority of individuals with *P. vivax* parasitaemia are asymptomatic. In areas of high co-endemicity of *P. vivax* and *P. falciparum* such as the island of Papua, the population attains significant immunity to malarial infection. A community household survey in 2005 covering 800 households in Timika, Papua demonstrated that 71% of patients with *P. vivax* were afebrile.[57] These asymptomatic individuals continue to transmit *P. vivax* even at subpatent levels of parasitaemia and are likely to be important sources of transmission. Each episode of *P. vivax* malaria without radical cure results in the establishment of hypnozoites in the liver which remain dormant to be activated at a later stage. The reduction of relapse in *P. vivax* can only be brought about by reducing the total parasitaemia associated with inoculation and by ensuring radical cure in all infections of *P. vivax*. The first step should be effective treatment of individuals presenting to health facilities with symptoms following which mass screening and treatment options may be considered to reduce the burden in asymptomatic individuals.

1.4.3 Radical cure of *P. vivax*

Although antimalarials such as atovaquone-proguanil are effective against hepatic schizonts, only the 8-aminoquinoline agents have shown hypnozoitocidal properties. Currently, primaquine is the only licensed 8-aminoquinoline effective against hypnozoites of *P. vivax*. Primaquine has been in use for over 50 years, yet there is still no consensus on the optimal regimen to effect radical cure of *P. vivax*, mainly due to the wide variation of its effect derived from clinical studies from around the world. Infections acquired in Southeast Asia and Oceania are particularly prone to relapse after a standard 14-day 15mg daily regimen. Taking this into consideration, the WHO has revised its recommendations to allow for the use of a higher 30 mg daily regimen for 14-days for use in Southeast Asia and Oceania while retaining the original lower dose for other regions. Experiments conducted on volunteers reveal that the effectiveness of primaquine is related to the total cumulative dose of primaquine administered and not the daily dose. Therefore, 30mg of primaquine administered twice a day for a week (total 420mg) would have the same effect in preventing relapse as administering it once a day for two weeks.

Current guidelines only advocate that antirelapse treatment be administered to patients with *P. vivax* (either alone or mixed with other species). However, it is likely that a majority of the population in regions of many endemic areas harbour heavy loads of silent hypnozoites. It is essential that this reservoir of infection be targeted to ensure reduction in *P. vivax*. In the absence of a reliable and cheap screening tool to detect G6PD deficiency, the focus of effective antirelapse treatment should be on patients at greatest risk of developing *P. vivax* relapse. Efforts need to be made to identify high risk groups in whom administration of antirelapse treatment will reap maximum benefit, while balancing the risks of side effects of treatment.

1.5 Aims and chapters of this thesis

As national malaria control programs move towards eliminating malaria from their borders the relative importance of *P. vivax* is set to increase. If elimination of *P. vivax* is to be achieved then its radical cure needs to be optimised and deployed in a safe and effective manner. My thesis aims to investigate the efficacy of primaquine treatment regimens and identify exposed populations at greatest risk of relapse and thus potential candidates for targeted antirelapse therapy.

My specific aims are:

- To review the evidence for the efficacy of different primaquine regimens in preventing *P. vivax* relapse.
- To quantify the risk of recurrence of *P. vivax* following the treatment of *P. falciparum* infection.
- To determine the risk factors for representation with *P. vivax* malaria in patients presenting for medical care at a rural hospital in southern Papua.

Chapters 2 and 3 present review of the literature. Chapter 2 reviews all primaquine clinical trials published since 1950. Chapter 3 reviews clinical trials using artemether-lumefantrine for treatment of uncomplicated *P. falciparum* focusing on the risk of recurrence of *P. vivax* parasitaemia following treatment. In Chapter 4 data gathered from the Rumah Sakit Mitra Masyarakat Hospital in southern Papua province of Indonesia is used to quantify risk factors for representation to the hospital with *P. vivax* and investigate the interactions between malaria and other medical conditions. In this chapter the hypothesis that high febrile illness is associated with a greater risk of representation with *P. vivax* is explored. A general discussion and conclusion is presented in Chapter 5.

Chapters 2 and 3 in this thesis have been adapted from manuscripts published and in press in peer-reviewed journals. Chapter 4 is currently being prepared for submission. Co-authors of these articles are gratefully acknowledged at the end of each chapter.

Chapter 2

2 Primaquine for radical cure of *Plasmodium vivax*

2.1 Introduction

Over 40% of the world's population is at risk of infection with *P. vivax*, with an annual global burden estimated to be between 71 to 391 million clinical cases [23 30 58]. Vivax malaria causes significant morbidity and associated mortality, much of which is attributable to the chronic relapsing nature of the infection. As malaria control efforts intensify in malarious areas co-endemic for *P. vivax* and *P. falciparum*, the relative proportion of *P. vivax* tends to rise when compared to that of *P. falciparum*, reflecting the greater challenge for the control and elimination of this parasite. This can be explained by the ability *P. vivax* to develop hypnozoite stages which can lie dormant for months or even years. The number and frequency of *P. vivax* relapses vary with a number of factors including the geographical region where the infection is acquired, host immunity and the sporozoite load at the time of infection. The geographical differences in relapse patterns have been attributed to the existence of different strains of *P. vivax* which vary in the proportion of sporozoites that are committed to dormancy and the duration of dormancy and the propensity of dormant stages to re-awaken [59]. The frequent relapse phenotype is epitomised by the Chesson strain isolated originally from the island of New Guinea and thought to be prevalent in tropical regions such as Southeast Asia, Oceania and parts of the Indian subcontinent. These infections demonstrate the shortest relapse interval of approximately 3 weeks. In contrast North Indian, Madagascar & St. Elizabeth (United States and Southern Europe) strains follow a pattern of early relapses followed by a long interval before subsequent relapses, whereas the first relapse of the Hibernans strains (Korea, Netherlands, Scandinavia, Central Russia) is delayed by 8-12 months [59]. Such variation in relapse patterns, the absolute risk of recurrence

and the delay in clinical manifestations represent major confounding factors undermining the interpretation of primaquine efficacy and making comparison of regimens across geographical regions extremely difficult.

The antimalarial properties of primaquine, an 8 aminoquinoline, were first documented in 1946. The drug showed activity against both asexual and sexual blood stages of the parasite as well against the liver stage schizonts and hypnozoites[60-62]. Since the 1950s, clinicians and policy makers have relied on primaquine as the only licensed agent for the radical cure of *P. vivax*. However, despite over 60 years of continuous use there is still neither evidence nor consensus on the optimal dose or dosing regimen. Although primaquine pharmacokinetics have been well characterized in studies of healthy subjects and adult males with malaria, there are virtually no data available in young children or pregnant women, the most vulnerable populations in urgent need of an effective radical cure.

Primaquine can result in significant haemolysis particularly in those with glucose-6-phosphate dehydrogenase deficiency (G6PDd) [63 64]. G6PD deficiency is the most common heritable enzymopathy in the world, with a prevalence ranging from 2% to 40% [65]. Generally, the more severe the enzyme deficiency, the greater the severity of haemolysis; individuals with less than 10% of normal enzyme activity being at risk of life-threatening haemolysis even after a single dose of primaquine [66]. Individuals with milder variants may have negligible effects on exposure to primaquine [67]. In view of the risk of adverse reactions, primaquine dosing strategies are influenced more by concerns over toxicity than by their absolute efficacy. These concerns are particularly important in poorly resourced settings where routine G6PDd testing is not available. Early clinical studies demonstrated that the predominant determinant of therapeutic efficacy was the total dose of primaquine administered rather than the daily dosage or duration of therapy [68]. Some policy makers therefore attempt to mitigate the risk of serious harm by opting for a lower daily dose spread

over 14-days or a single weekly dose for 8 weeks for G6PDd patients[69]. Ultimately the effectiveness of such regimens is dependent upon adherence which is a function of a range of factors, particularly tolerability, duration of therapy and supervision of dosing.

The World Health Organization (WHO) guidelines for the radical cure of vivax malaria currently recommends the use of 0.25 mg/kg/day (3.5 mg/kg total dose) primaquine taken with food once daily, co-administered with chloroquine or artemisinin combination therapies (ACTs) depending on chloroquine sensitivity in the region [70] [71]. In South East Asia and Oceania the same guidelines recommend a total dose increase to 0.5 mg/kg in view of the high proportion of relapses. However there is a lack of data regarding the benefits of the higher dose, particularly from studies with prolonged follow up. In this chapter a systematic review of the literature is presented to highlight the diversity of methodologies that have been applied to anti-relapse clinical trials and to summarise the evidence of primaquine efficacy for different regimens across a range of epidemiological and geographical locations.

2.2 Methodology

2.2.1 Literature Search

A MEDLINE search was conducted for all studies on malaria using the search terms "malaria or *Plasmodium*" and all possible antimalarial drugs; Figure 2.1. This database was then filtered for clinical studies and studies containing "vivax" and "primaquine" in the title or abstract. In addition, the Cochrane Central Register of Controlled Trials [72] was searched using the terms "(malaria or vivax) and primaquine". Reference lists of previously published reviews and papers on primaquine were also screened for relevant studies in English.

MEDLINE	(*malaria* OR *plasmodium*)	AND	(*amodiaquine* OR *atovaquone* OR *artemisinin* OR *arteether* OR *artesunate* OR *artemether* OR *artemether* OR *artemotil* OR *azithromycin* OR *artekin* OR *chloroquine* OR *chlorproguanil* OR *cycloguanil* OR *clindamycin* OR *coartem* OR *dapsone* OR *dihydroartemisinin* OR *duo-cotecxin* OR *doxycycline* OR *halofantrine* OR *lumefantrine* OR *lariam* OR *malarone* OR *mefloquine* OR *naphthoquine* OR *naphthoquinone* OR *piperaquine* OR *primaquine* OR *proguanil* OR *pyrimethamine* OR *pyronaridine* OR *proguanil* OR *quinidine* OR *quinine* OR *riamet* OR *sulphadoxine* OR *tetracycline* OR *tafenoquine*)
CENTRAL	(*malaria* OR *vivax*)	AND	*primaquine*

Figure 2.1: **Search terms**

2.2.2 Data extraction

Data regarding study characteristics, recurrence and side effects were systematically extracted from the articles and entered into an EpiInfo™ 3.5.1 database and analysed using SPSS® Statistics vs19. Details of the study methodology including study site, inclusion and exclusion criteria, identification of clinical setting, randomization and blinding were extracted from all studies. The details of the dosing regimen such as co-administered drug, primaquine dosing, frequency, duration and supervision of treatment were extracted for each arm of included studies. The dose of primaquine was calculated according to the total dose per kg assuming a 60 kg adult, and categorized as very low dose (≤2.5 mg/kg), low dose (>2.5mg/kg-<5.0 mg/kg) and high dose (≥ 5.0 mg/kg). Study sites were identified using Google Earth™ and GPS co-ordinates presented using GPS visualizer (http://www.gpsvisualizer.com/) and are represented in Figure 2.2.

2.2.3 Statistical Analysis

The risk of recurrence was calculated according to a per protocol analysis using the number of patients with recurrent *P. vivax* during follow up, divided by the number of patients followed until the study endpoint. The analysis of the risk of recurrence was recorded only once for each treatment arm, at the maximum period of follow up. Recurrence rates were then presented according to the total dose of primaquine stratified by geographical regions. Studies carried out in the USA recruited a mix of soldiers returning from the war in Korea, Vietnam and Somalia as well as induced malaria using Chesson and West Pakistan strains; for the purposes of analysis these were categorized according to origin of the infecting strain. Recurrence rates following antirelapse treatment were categorized into three groups: "Good Responders" if there were less than 10% recurrence in studies with greater than 6 weeks follow up and "Poor responders" for studies with recurrence rate greater than or equal to 10% at any time during follow up. Studies with recurrence rates less than 10% but of short duration (≤1 month) were categorized as indeterminate. Studies with a control arm without primaquine were compared by Mantel-Haenszel odds ratios (OR), calculated using Review Manager (RevMan version 5.1. Copenhagen). Recurrence data after primaquine regimens at 1 month, 2-3, 4-6 months and 6 months were calculated using the Mann-Whitney U test, using IBM SPSS Statistics for Windows, Version 19.0. Armonk, NY: IBM Corp.

2.2.4 Nomenclature

Relapse refers to the phenomenon of activation of dormant *P. vivax* hypnozoites in the liver leading to a malarial attack. Recurrence, on the other hand, is a blanket term encompassing a repeat malarial infection which can be caused by either recrudescence, relapse or re-infection [73 74]. There is currently no reliable way of distinguishing between the three alternatives causes of recurrence[75]. In this chapter results are reported with regard to recurrences of *P. vivax* malaria.

2.3 Results

2.3.1 Details of included studies

A total of 120 clinical trials of primaquine were identified, of which 33 studies were excluded for the reasons listed in Figure 2.3. Of the 87 clinical trials included in the analysis, 43 (49%) were conducted on or after 2000 (supplementary table T2, Appendix). The greatest number of trials came from Thailand (24 studies) followed by India (17 studies); see Figure 2.2. Three studies in Germany, Israel and Taiwan enrolled travellers to endemic countries returning with imported *P. vivax* malaria. Studies conducted in the United States recruited soldiers returning from wars in Korea (n=3), Vietnam (n=2) and Somalia (n=2) or artificial inoculation of *P. vivax* in inmates of state penitentiaries (n=7). Details of the studies are provided in Supplementary table T1 and T2 included in the appendix.

Figure 2.2. **Map of study sites of primaquine antirelapse studies.** Footnote: One study (yellow icon); Two studies (orange icon), Three or more studies (red icon).

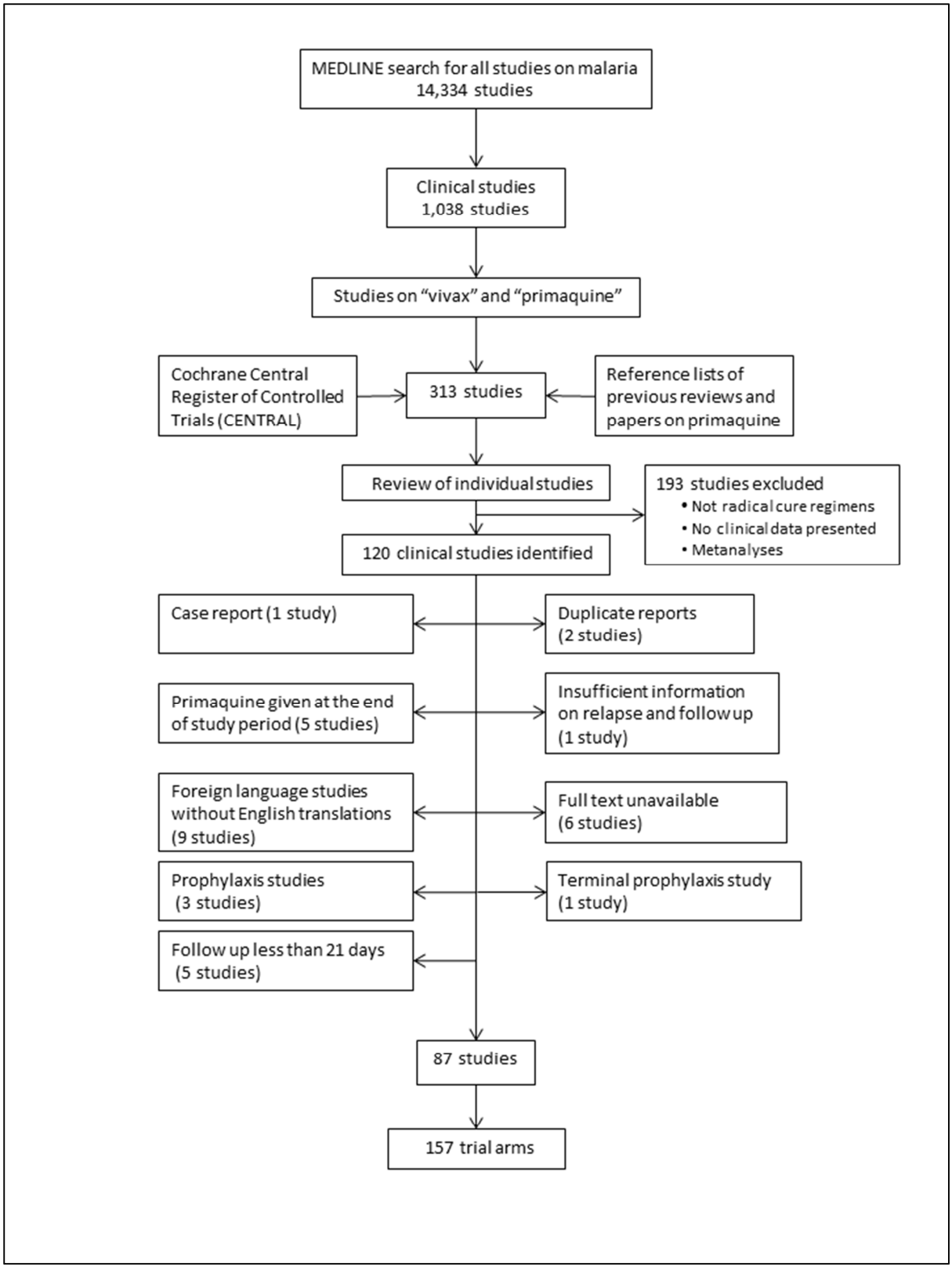


Figure 2.3. **Profile of clinical studies included in the analysis**

2.3.2 Design of the reviewed trials

There was marked heterogeneity in the design of the clinical studies (table 2.1) and dosing strategies of primaquine investigated (table 2.2). In total 54 studies reported comparative drug trials, of which 30 (56%) were randomized. The median number of treatment arms in these comparative studies was 3 (range 2-9), with 18 studies having a control arm in which patients did not receive antirelapse therapy. The remaining studies were either single arm cohort studies (n=28) or retrospective (n=5).

Table 2.1: Characteristics of selected studies

Year of publication	No. of studies
≤1960	8
1961-70	3
1971-80	10
1981-90	4
1991-00	24
2001-10	36
2011	2

Study setting and participants	No. of studies
Community setting	19
In-hospital setting	27
Out-patient setting	24
Soldiers	7
Travellers	3
Volunteers (induced)	7

Follow up period	No. of studies
< 30 days	27
31-120 days	11
121-179 days	3
180 days	13
181-364 days	14
365 days	8
>365 days	11

Table 2.2: Dosing Strategies

Duration of primaquine treatment	Very Low Dose ≤2.5mg/kg	Low Dose >2.5 to <5mg/kg	High Dose (≥5mg/kg)	Total
1 day	4	0	0	4
3 days	4	1	0	5
5 days	30	0	0	30
6 days	1	0	0	1
7 days	3	11	4	18
>7 to <14 days	0	3	1	4
14 days	2	64	11	77
>14 days	1	4	12	17
Total	45	83	28	156

Age-related inclusion criteria were recorded for 59 studies, of which 33 (56%) actively excluded children less than 14 years old. Parasitaemia criteria were applied for enrolment in 13 (15%) studies, the threshold for inclusion varying from greater than 40 asexual parasites/ μl to less than 100,000 parasites/ μl. Only 23 (26%) studies reported actively testing and excluding patients with G6PD deficiency. In 31 studies, patients with a history of recent treatment were excluded, the interval since prior treatment varying from 48 hours to 2 months.

The median duration of follow up was 180 days, ranging from 28 to 2,555 days (supplementary table T2, Appendix), and differed markedly with the location of the studies. The majority of studies conducted in Southeast Asia and Oceania (Thailand, Indonesia, Vietnam and Papua New Guinea) were generally of short duration, 70% (21/30) with no more than a month of follow up. Conversely follow up was continued for 6 months or more in 80% (9/12) of the South American studies and 90% (19/21) of studies on the Indian subcontinent.

2.3.3 Primaquine Dosing Regimens

Very low total dose primaquine was used in 44 (28%) of 156 treatment regimens, low dose in 82 regimens (53%) and high dose in 28 regimens (18%). In two studies the dose of primaquine was not stated; see Table 2-2 and supplementary Table T3 in appendix. Primaquine was administered within 15 days in 140 (90%) treatment regimens. The remaining 16 treatment arms were administered weekly or on alternate days over 21 to 98 days in studies conducted in Indonesia (4), Pakistan (1), soldiers returning from Vietnam or Somalia (3) or induced malaria with Chesson strain (8). The supervision of administration was recorded in 44 (51%) studies, with 81% (60/74) of treatment arms fully supervised, 15% (11) part supervised and 4% (3) unsupervised. In 114 (73%) treatment arms chloroquine was given as the accompanying schizontocidal therapy. Other schizontocides used included artesunate (n=17), artemether-lumefantrine (n=1), amodiaquine (n=2), atovaquone/proguanil (n=2), azithromycin (n=1), sulfadoxine-pyrimethamine (n=1), halofantrine (n=1), mefloquine (n=1), quinine (n=4) and rifampicin (n=1) and DB289 (n=1). In one study the schizontocidal drug was not stated, and in another with (2 treatment arms) patients received either chloroquine or quinine regimens. Primaquine was used as monotherapy in 7 studies.

2.3.4 Risk of recurrence

The risk of recurrence according to geographical location for different periods of follow up are presented in Figures 2.4, 2.5, 2.6 (maps) and Figure 2.7 (scatter plot), with further details in supplementary Table T3 in appendix. Studies using the very low dose regimen (figure 2.4 and 2.7A) were conducted mostly in the Indian subcontinent (19/44) and South America (16/44), with longer follow up (median 220 days; Range 28-1440). Across all regions, the median recurrence rate following very low dose primaquine was 15% (range 0-16%) at 1 month, 11.5% (range 3-20%) at 2-3 months, 25% (range 0-90) at 4-6 months, but fell to

9.2% (range 0-100%) at 7-12 months, reflecting almost exclusive representation of studies originating on the Indian subcontinent.

Figure 2.4. **Map of studies documenting the effectiveness of very low dose primaquine.**

Footnote: Adequate response (yellow icons): Recurrence rate < 10% in studies with greater than 6 weeks follow up; Poor response (red icons): recurrence rate >10% at any time during follow up. Studies of returning US soldiers are placed at country of origin. Indeterminate studies and studies of induced malaria have been excluded.

Low dose primaquine was assessed in 82 treatment arms with a median recurrence of 0.9% (range 0-30%) at 1 month, 6.8% (range 0-15%) at 2-4 months, 6.7% (range 0-59%) at 4-6 months and 17% (range 0-90%) at 7-12 months (Figure 2.5 and 2.7 B). In total 27 (33%) treatment arms were categorized as having a good response, 36 (44%) having a poor response, 19 (23%) studies being indeterminate (See Figure 2.5). In Thai studies low dose primaquine following chloroquine was associated with a median recurrence rate of 0% [Range: 0-1.5] at one month, significantly lower than in patients who initially received artesunate; (median = 4.8%, range: 4-11; p=0.009).

Figure 2.5. **Map of studies documenting the effectiveness of low dose primaquine.**

Footnote: Adequate response (yellow icons): Recurrence rate < 10% in studies with greater than 6 weeks follow up; Poor response (red icons): recurrence rate >10% at any time during follow up. Studies of returning US soldiers are placed at country of origin. Indeterminate studies and studies of induced malaria have been excluded.

High dose primaquine was administered in 28 treatment arms from 17 studies. In the 16 treatment arms in which primaquine was administered within 15 days the median risk of recurrence was 0% (Range: 0-6%) at 1 month and 0.6% (0-1.9%) at 7-12 months. These figures compared to 12% (Range: 0-15%) at 1 month and 15% (0-29%) at 4-6 months in the 11 studies in which primaquine was administered on alternate days or weekly (figure 2.6 and 2.7 C; and Supplementary table T3 in appendix).

Figure 2.6. **Map of studies documenting the effectiveness of high dose primaquine.**

Footnote: Adequate response (yellow icons): Recurrence rate < 10% in studies with greater than 6 weeks follow up; Poor response (red icons): recurrence rate >10% at any time during follow up. Studies of returning US soldiers are placed at country of origin. Indeterminate studies and studies of induced malaria have been excluded.

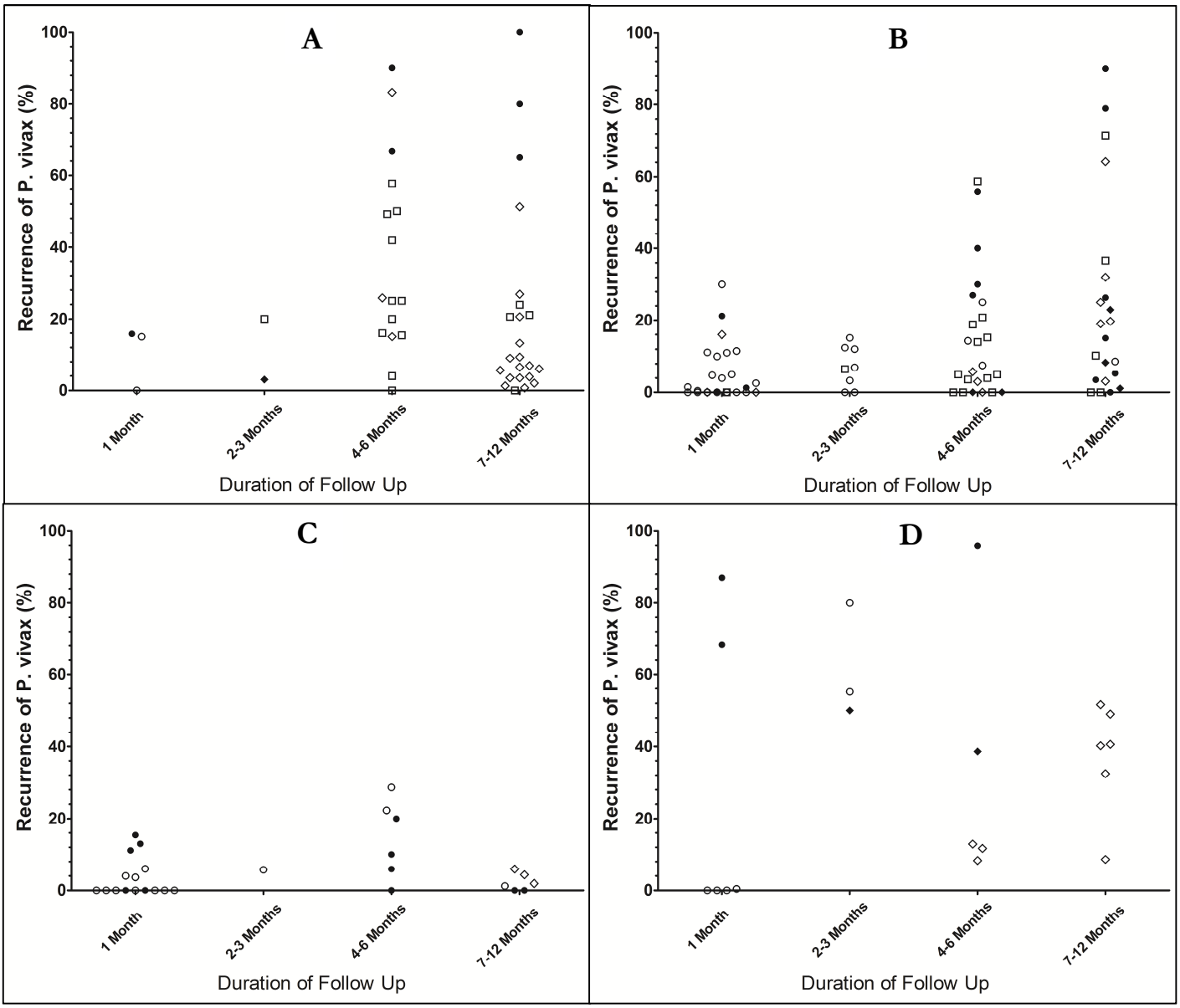


Figure 2.7. **Risk of recurrence at the end of the study following primaquine a)Very low dose: ≤2.5 mg/kg total dose. b) Low dose: >2.5 mg/kg to <5.0 mg/kg total dose. c) High dose: ≥5.0 mg/kg total dose. d) Control Arms in which no primaquine was given.**

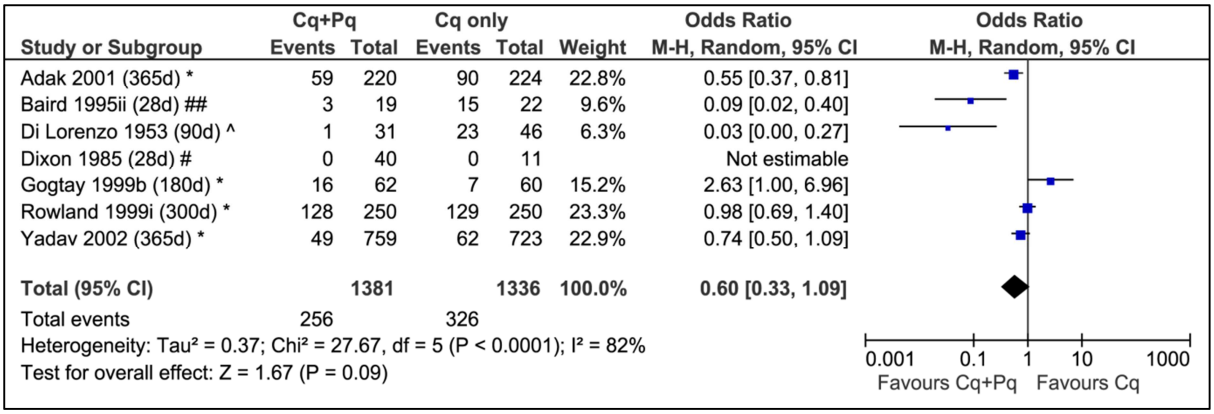
Footnote: Indonesia and PNG [Closed Circles]; Thailand and Vietnam (Open Circles), South and Central America (Open Squares); Indian Subcontinent, Middle East and Horn of Africa (Open Diamonds); Korea and China (Closed Diamonds). USA studies of induced malaria and returning soldiers categorized according to origin of infecting strain. Full details are given in supplementary tables T2 and T3 in appendix.

2.3.5 Odds of *P. vivax* recurrence after treatment

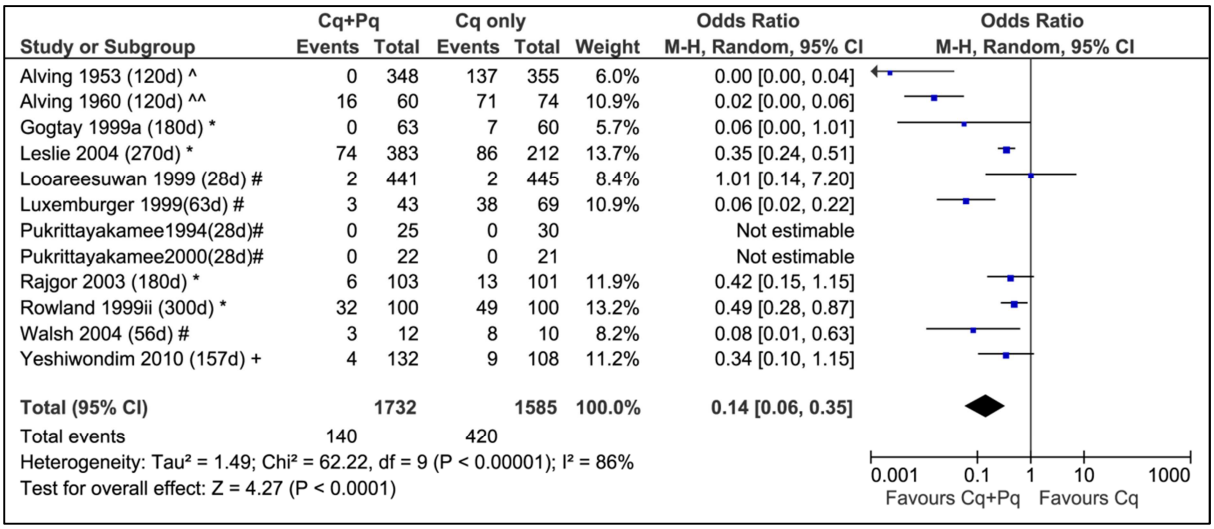
A total of 18 studies included patients recruited into control arms in which no primaquine was given; two of these studies had two control arms each; see supplementary Table T3 in

appendix and figure 2.8 A-C. All but one of the 6 comparative studies of very low dose primaquine were conducted in the Indian subcontinent; the overall effectiveness in preventing recurrence did not differ significantly with patients receiving no primaquine (OR=0.60, 95%CI 0.33-1.09, p=0.09), although there was marked heterogeneity between studies (I^2=82%). Low dose primaquine regimens were significantly better than control regimens in 50% (6/12) of studies assessed (OR=0.14, 95% CI 0.06-0.35; p<0.001). Two studies compared high dose primaquine with a control arm (OR=0.03, 95%CI: 0.01-0.13; p<0.0001).

A



B



C

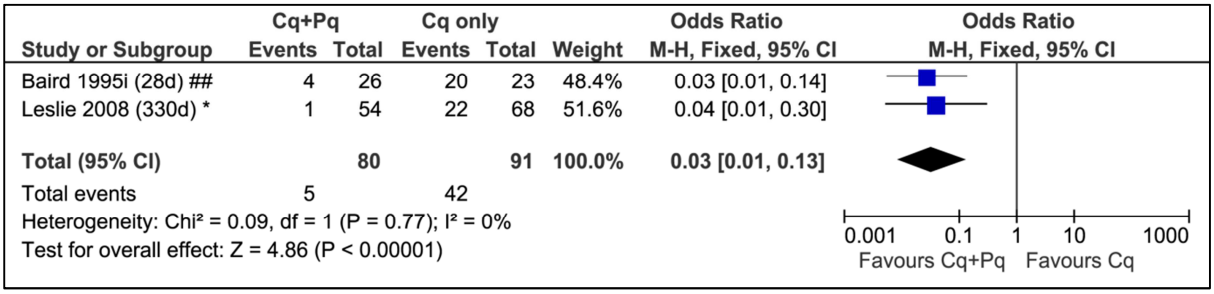


Figure 2.8. **Forest plot of the effectiveness of primaquine in studies with a control arm. a) Very low dose primaquine compared to no primaquine. b) Low dose primaquine compared to no primaquine. c) High dose primaquine compared to no primaquine**

* Indian subcontinent; ^ USA (Korea), ^^ USA (Chesson), # Thailand, ## Indonesia, +Ethiopia

2.3.6 Safety data

Overall, 39 (48%) studies reported monitoring for adverse effects of treatment recruiting a total of 11,466 patients (6,986 with very low dose, 3,322 with low dose, 961 treated with high dose and 197 with the dose not stated). A total of 10 serious adverse events (SAE) were reported potentially related to primaquine toxicity. The overall rate of SAE was therefore 8.7 (95% CI: 4.2-16) per 10,000. Assuming denominators were all G6PD normal, the conservative estimated rate of SAEs in G6PD normal individuals was 3.0 (95% CI: 0.1-16.8) per 10,000 following low dose primaquine and 10.4 (95% CI: 0.3-58) per 10,000 following high dose primaquine. No deaths were reported in any study.

2.4 Discussion

Despite the recognition that *P. vivax* constitutes a major threat to human health and the goal of malaria elimination, knowledge of the optimal treatment strategies for this parasite has changed little over 60 years. Newer drugs with activity against liver stages of the parasite are under development [76], but these are, at best, 5 to 10 years from reaching the global market. In the meantime better strategies need to be devised for delivering safe and effective antirelapse therapy using primaquine.

The 2010 WHO guidelines recommend a total primaquine dose of 3.5 mg/kg dose for the radical cure of *P. vivax*, whilst acknowledging that in Southeast Asia and Oceania a higher dose (7.0mg/kg total dose) may be warranted [70]. However, the evidence from which these recommendations are derived is limited. Malaria control programs prioritise acute antimalarial policy towards eliminating the initial threat to the patient (i.e. from blood stage parasites), rather than preventing future recurrences from hypnozoites. The most important factor for primaquine treatment policy is safety rather than efficacy [71]. However the benefits of radical cure may not be appreciated. Early studies in soldiers returning from the Korean War demonstrated that African-Americans were particularly prone to primaquine induced

haemolysis. This risk could be mitigated by reducing the dose and, in the Korean strains of *P. vivax* tested; this appeared to be feasible without compromising efficacy.

The plots presented in Figures 2.7 and 2.8 highlight the wide range of primaquine efficacy presented by published clinical trials. Both very low dose and low dose primaquine were vulnerable to high rates of recurrence of *P. vivax* (Figure 2.7A and 2.7B), whereas the use of high dose primaquine was more often associated with acceptable efficacy [71]. Our analysis also suggests that the treatment efficacy of high dose regimens is significantly better when regimens are given over 14 days rather than spread out over alternate days or weekly dosing. However such comparisons need to be interpreted with caution, since they are confounded by marked heterogeneity in study design, dose regimens, and idiosyncrasies of the endemic setting. The frequency and the timing of relapses are determined by sporozoite inoculum and parasite relapse phenotype and this results in huge regional variation in the absolute risk of recurrence independent of primaquine efficacy [59 77]. Goller et al applied a multivariate model to compare relapses in studies conducted in Brazil, India and Thailand demonstrating that after controlling for year of study, study design and dose of primaquine, the overall risk of relapse in Thailand was 10 times that in India and twice that in Brazil [78]. Alternatively primaquine efficacy can be gauged by comparison with a control arm in which patients receive no primaquine, thus controlling for background reinfection and relapse patterns. In their 2007 Cochrane review, Galappaththy and colleagues used such an approach to control for differences in the background rates of infection, concluding that the 5-day primaquine regimen was ineffective and that a 14-day low dose regimen was 13-fold better compared to the 5 day regimen [79].

Age is a major determinant of recurrent infection the risk of recurrence falling significantly as age increases. Although this may reflect exposure to new infections it is also a surrogate marker of immunity. Young patients are at greatest risk of symptomatic recurrent infections

and adverse morbidity associated with repeated vivax parasitaemia [9 80], and hence should be a priority for optimizing therapeutic interventions; in practice however they are often excluded from clinical trials. Pharmacokinetics can differ markedly in children and simply extrapolating dosing strategies from adults can result in systematic underdosing as was shown convincingly with the use of antifolates in *P. falciparum* [81]. Furthermore studies in adult populations can overestimate true primaquine efficacy since these patients are more likely to control vivax parasitaemia, remain asymptomatic or even clear the infection without treatment.

Based on the current analysis, the key elements required for the design of informative clinical studies of relapse prevention are presented in Table 2.3.

Table 2.3. Key components in the design of studies of relapse prevention by primaquine in vivax malaria

Essential	**Combination of primaquine with a blood schizontocide known to have high efficacy in the study population,** to minimise the risk of recrudescent infections. Primaquine efficacy may vary with partner drug and this must also be confirmed (see Box 3).
	Prolonged duration of follow-up (12 months) – Since relapses can occur over a 12 months period it is important to conduct studies with long duration of follow-up.
	Inclusion of a control arm – The risk and timing of *P. vivax* relapse varies with the location of the study site, hence the efficacy, safety and risk-benefit of primaquine needs to be gauged where ethically acceptable by comparison with a control arm (given no antirelapse medication).
	Assessment of adverse events with particular attention to haemolysis. Primaquine can cause haemolysis particularly in patients with G6PD deficiency and varies with the study population.
	Multicentre assessment. The risk of relapse varies between *P. vivax* strains and the intensity of sporozoite inoculation. The risk of haemolysis is related to the G6PD deficiency genotypes prevalent. Both vary regionally. Risk and benefit should be assessed in a range of locations.
Desirable	**Retreatment and following patients through multiple recurrences** with same treatment regimen. The clinical consequences of relapsing infections and the benefits of their prevention require characterisation of the total number of relapses following an initial infection.
	Quantification of haematological recovery. Both malaria and primaquine result in haemolysis, and thus risk benefit needs to be confirmed through close monitoring of haematological recovery.

The duration of follow up is a crucial component of the study design. In Southeast Asia and Oceania, early relapse occurs in a high proportion of patients and marked differences in antirelapse efficacy can be observed with short duration of follow up. Only 5 studies from Southeast Asia and Oceania (Thailand, Indonesia, Vietnam and Papua New Guinea) continued follow up beyond 3 months. In areas with a more temperate climate the risk of relapse is lower and relapse tends to occur late, often months after the initial infection. In such circumstances, short term follow up will fail to pick up treatment failures, falsely elevating efficacy estimates. Such a situation occurred in India, where a 5 dose primaquine regimen was long heralded as an appropriate treatment strategy, but actually reflected an intrinsic low absolute risk of relapse; the majority of relapses in adults being subclinical [81 82].

Early recurrence of *P. vivax* (within 21 to 63 days) is a function of the efficacy of the initial schizontocidal treatment in clearing the initial blood stage biomass as well as the post treatment prophylaxis afforded by slowly eliminated drugs [83]. This is highlighted in studies from Thailand in which almost none of the patients receiving chloroquine plus primaquine had a recurrence within 1 month, a reflection of the high efficacy chloroquine in this location and its prolonged suppressive activity on relapsing parasites. In contrast in the same location patients receiving primaquine combined with short acting but effective artesunate regimens, had a risk of recurrence of between 4 and 11%, likely a better indication of the underlying primaquine efficacy. Short duration studies of primaquine with slowly eliminated schizontocidal treatments are therefore of limited benefit in defining antirelapse efficacy. Studies with long duration of follow up capture a greater proportion of relapses, but are vulnerable to confounding from heterologous new infections. The rise of chloroquine resistance adds an additional complication of partial clearance of the initial blood stage biomass, in which early recrudescence can be misclassified as a relapsing infection. Currently,

no reliable method exists to differentiate relapses from re-infections and recrudescent infections.

Current guidelines recommend a 14 day course of primaquine administered either once or twice daily to reduce the risk of haemolysis and improve tolerability from gastrointestinal disturbance. Advocating such prolonged courses of treatment results in significant problems with adherence. Two studies from Thailand found recurrence rates of *P. vivax* to be 3-14 fold higher in patients who self-administered primaquine compared with those given directly observed therapy [84] [85]. In contrast a study from Pakistan showed no difference between education and DOTS in the setting of a refugee camp. Both of these groups had significantly fewer relapses than the placebo group which received routine treatment; Figure 2.9 [86]. Better strategies for improving adherence are urgently needed.

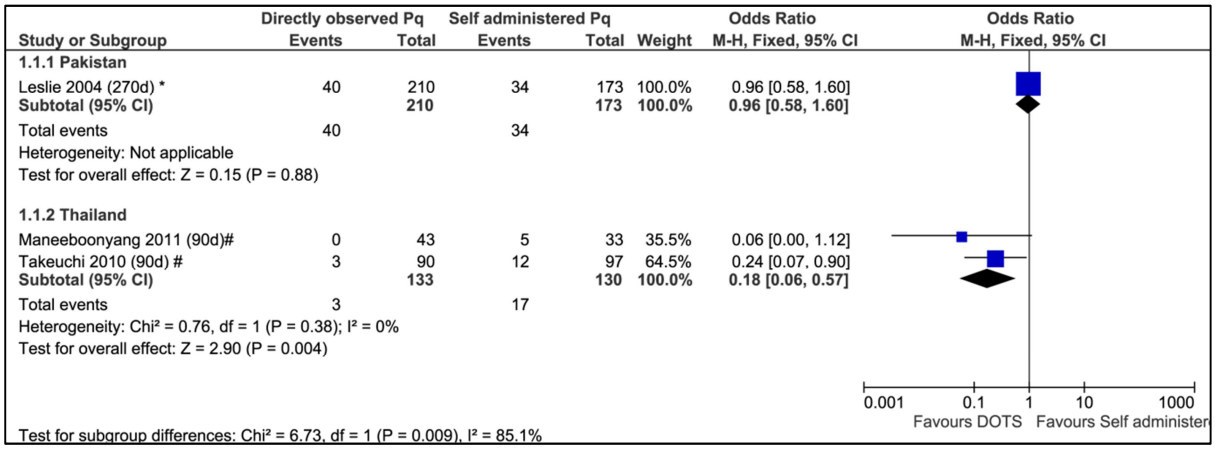


Figure 2.9. **Forest plot of the effectiveness of directly observed treatment in primaquine therapy**

Poor adherence to a 14 day course of unsupervised primaquine is likely to have a major impact on its public health benefit. Short-course, high dose regimens have potential to increase patient adherence and thus effectiveness [87], but appropriately powered prospective multi-centered randomized controlled trials are needed to assess the efficacy and effectiveness of such alternative radical curative regimens against current best practice. The

current methodology for primaquine treatment trials can be improved and should be harmonized to facilitate comparison between different sites. The factors highlighted in this review suggest that a minimum of 6 months and perhaps even 12 months follow up is required. Relapse is often a recurrent process rather than a single event; hence the analysis of such trials should focus on the incidence of treatment failure rather than the cumulative risk of the first event, which is the standard outcome measure for drug efficacy studies in *P. falciparum* malaria. Such cohort studies have a similar rationale as vaccine intervention studies in which efficacy is interpreted against a control intervention to account for new infections and variation in relapse patterns. The use of a control arm introduces a number of important ethical considerations. At some sites schizontocidal treatment alone is standard of care – governments fearing more harm than benefit by giving primaquine to patients without prior G6PDd screening. Although adverse effects were not reported consistently in the clinical trials reviewed, the data available suggested the rate of SAEs to be 3 per 10,000 exposures to low dose primaquine and was 10.4 per 10,000 exposures to high dose primaquine. These figures are comparable to a risk of 4.8 to 8.2 per 10,000 (1 per 2089 and 1 per 1217) severe neuropsychiatric reactions following 15 and 25mg/kg dose of mefloquine respectively.

Most endemic countries recommend, at least in principle, use of primaquine with expressed warnings on its potential toxicity in G6PDd patients. Several studies highlight that even in areas where primaquine is recommended, in practice it is prescribed rarely and even when given, adherence and thus effective are often poor [88 89]. Investigation of different primaquine regimens must also include appropriate measures of tolerability and be able to quantify the risk of severe adverse events, especially in patients with different variants of G6PDd. Here again a control arm is vital, since only then can the consequences of recurrent episodes of malaria on the health and well-being of the patient be assessed.

Table 2.4. **Clinical research priorities for optimising the radical cure of *P. vivax***

- Characterisation of true relapse rates in different geographic areas in the age group mainly affected.
- Assessment of efficacy and safety of short course, high dose primaquine treatment regimens.
- Development of robust point-of-care G6PD diagnostics.
- Investigation of the safety and efficacy of tafenoquine, a long acting 8 aminoquinoline.
- Defining the pharmacokinetic profile of primaquine in at risk populations.
- Confirmation of primaquine efficacy when combined with different blood schizontocidal partner drugs.
- Confirmation of effectiveness of primaquine regimens in clinical practice.
- Quantification of risk of clinically significant haemolysis according to primaquine dose and host susceptibility.
- Development of techniques to distinguish between relapse, reinfection and recrudescent infections.

Clinical research priorities for optimizing the radical cure of *P. vivax* are presented in Table 2.4. In populations with high rates of G6PDd, particularly those with variants at greatest risk of haemolysis, a critical hurdle for the safe deployment of primaquine will be the development of a rapid affordable point of care test. Cases of severe haemolysis following a single dose of primaquine can have a negative impact not only for the patient but also for public confidence in public health interventions [66] [90]. The development of newer and inexpensive methods for field-testing of G6PD deficiency should go hand in hand with studies of primaquine effectiveness. Reliable exclusion of patients at risk of severe harm opens the possibility for safer and more effective deployment of high dose regimens. If the elimination of vivax is to be achieved, a co-ordinated effort is needed to provide the evidence

across the spectrum of the vivax endemic world, from which to rationalize and deploy antirelapse therapy in a safe and effective manner.

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Chapter 3

3 *P. vivax* parasitaemia following artemether-lumefantrine treatment for *P. falciparum* malaria

3.1 Introduction

The previous chapter highlights the difference in primaquine efficacy around the world and establishes a need for standardized multi-regional studies to determine optimal radical treatment regimens. At present, radical cure of infection is directed only at individuals presenting with *P. vivax* malaria or mixed vivax infections. However, evidence from previous studies suggests the existence of groups such as individuals with *P. falciparum* malaria who have an increased risk of recurrent parasitaemia due to *P. vivax* within the first few months of effective blood schizontocidal treatment.

High rates of *P. vivax* recurrence after treatment for *P. falciparum* infection were first reported by Loowaresuwan *et al* from Thailand in 1987 where almost a third of patients treated with quinine, or mefloquine developed patent *P. vivax* malaria within six weeks.[91] Since the patients were admitted for the duration of follow up in an area outside *P. vivax* transmission, it can be confidently assumed that these recurrences were in fact due to either relapse from *P. vivax* hypnozoites or recrudescence rather than reinfection. Since both the quinine and mefloquine treatment courses were supervised and shown to be highly effective against *Plasmodium*, recrudescence was deemed to be unlikely. One explanation is that these recurrences represent patients co-infected with both *P. falciparum* and *P. vivax*, but in whom the *P. vivax* was suppressed and only recurs once the *P. falciparum* has been eliminated. A review of the existing literature on Anopheline mosquitoes found that only 6.6% of *P. falciparum* infected mosquitoes also carried *P. vivax* in Asia and Oceania, much lower than expected if these infections were from simultaneous inoculation from mosquitoes.[92] This

suggests that these mixed infections arise from separate inoculations from different mosquitoes within a 2-3 day time period. However, the entomological inoculation rate in most vivax endemic settings rarely exceed one infected bite per year and thus two infected bites within four days would occur rarely. A more plausible explanation is that the co-infection consists of an acute patent *P. falciparum* peripheral parasitaemia and a dormant brood of *P. vivax* hypnozoites acquired previous; the systemic derangement caused by the acute infection of *P. falciparum* activating the dormant hypnozoites of *P. vivax*. These *P. vivax* infections occur within 63 days, at a time one expects the prevalent *P. vivax* strains in this region to relapse.

Currently, the World Health Organization (WHO) recommends use of five artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated *P. falciparum* malaria: artemether plus lumefantrine, artesunate plus amodiaquine, artesunate plus mefloquine, artesunate plus sulfadoxine-pyrimethamine and dihydroartemisinin plus piperaquine.[93] The elimination of ACTs vary according to the half-life of the partner drug, ranging from 3-6 days in artemether-lumefantrine[94] to 23-28 days in piperaquine[95]. Artemether-lumefantrine (AL) is one of the most studied ACTs for the treatment of *P. falciparum* malaria. The six dose combination of artemether-lumefantrine (AL) contains 20mg of artemether and 120mg of lumefantrine. This drug regimen developed in China in the 1990s has become the most widely used ACT in the world today with over 400 million treatments delivered to developing countries by the manufacturer between 2001 and 2011.[96] AL is highly effective against chloroquine, amodiaquine and antifolate resistant strains of *P. falciparum*.[97] The initial rapid onset of action of artemether combined with the residual action of lumefantrine on parasites is thought to prevent the development of resistance to the combination. The PCR adjusted cure rates of AL in *P. falciparum* at 42 days were reported to be greater than 95% in

studies conducted in regions with a high burden of multidrug-resistant *P. falciparum* infection such as the Thai-Myanmar border[98] and Jalpaiguri district of West Bengal in India[99].

With the emergence of chloroquine resistance in most vivax endemic regions of the world[48], artemisinin based combination therapies(ACTs) are increasingly being employed for the treatment of *P. vivax*. The Solomon Islands, Vanuatu, Papua New Guinea use AL as a unified first-line therapy for both major species of malaria while dihydroartemisinin-piperaquine is used in Papua, Indonesia.[100] The combination of artemether and lumefantrine leads to faster clearance of initial parasitaemia and fever compared to chloroquine, chloroquine combinations and other non-artemisinin combinations.[101-104] However, the short half-life of lumefantrine (3-6 days)[94] while useful in preventing the development of resistance, ensures that 95% of the drug will be out of the blood stream within 16 days of the last dose and is likely to provide only minimal inhibition of the first relapse of *P. vivax* which would be predicted to occur at 21 days in this region.[59] This makes it an unsuitable candidate for the post treatment prophylaxis of *P. vivax* compared to long acting antimalarials agents such as chloroquine, mefloquine and piperaquine. For these reasons parasitological failure rates by day 28 and 42 in patients given AL without primaquine are significantly worse than that of chloroquine[105] and dihydroartemisinin-piperaquine[101]. Only one study conducted in Thailand compared the efficacy of AL administered along with primaquine to chloroquine-primaquine and demonstrated similar effectiveness in both groups.[103]

The observations by Loowaresuwan *et al* of high rates of *P. vivax* recurrence after treatment for *P. falciparum* infection were confirmed in a pooled analysis of 10,549 patients with pure *P. falciparum* (89%) or mixed infections (11%) enrolled in clinical trials on the Thai-Myanmar border from 1991 to 2005. In this meta-analysis the overall risk of recurrence of *P. vivax* at 63 days was 31.5%. [106] Mixed infection, male gender, younger age, lower haematocrit, higher *P. falciparum* parasite density and *P. falciparum* gametocytaemia were associated significantly

with a higher risk of *P. vivax* recurrence at 63 days. Patients treated with rapidly eliminated drugs had significantly higher risk of *P. vivax* at 63 days (51.1%) compared to treatment with drugs with intermediate or slow elimination (35.3% and 19.6% respectively). The high risk of parasitaemia following ACT does not constitute a failure of blood schizontocidal therapy, since the initial parasite is generally eliminated. What the study does highlight however is that the greatest risk of parasitaemia following the treatment of *P. falciparum* comes not from recurrent *P. falciparum* infection but *P. vivax* relapse. This has potentially very important public health implications for directing anti-relapse strategies towards patients presenting with *P. falciparum* malaria in co-endemic regions. Until now, this risk has not been well quantified in regions outside Thailand. This risk needs to be quantified in the various malaria endemic regions so that future antimalarial efforts can be directed at addressing the risk of *P. vivax* recurrence in these groups. In this chapter the focus is on AL due to its widespread use around the world for *P. falciparum* malaria and its lack of post prophylaxis effect which unmasks the phenomenon of recurrences of *P. vivax* following treatment with this drug. In studies using DHP it is likely that the first relapse is delayed or treated and thus this phenomenon would only be observed if study participants were followed for longer periods. The published literature is reviewed in this chapter to quantify the risk of recurrent *P. vivax* parasitaemia following AL treatment for *P. falciparum* in a variety of co-endemic regions around the world. The implication of reducing the burden of *P. vivax* relapses is then discussed.

3.2 Methodology

3.2.1 Literature Search

A MEDLINE search was conducted using the search terms "(artemether AND lumefantrine) OR coartem OR co-artemether OR riamet" resulting in 441 studies. The study

profile and details of excluded studies is given in Figure 3.1. Reference lists of previously published reviews and papers on artemether-lumefantrine were also screened for relevant studies in English.

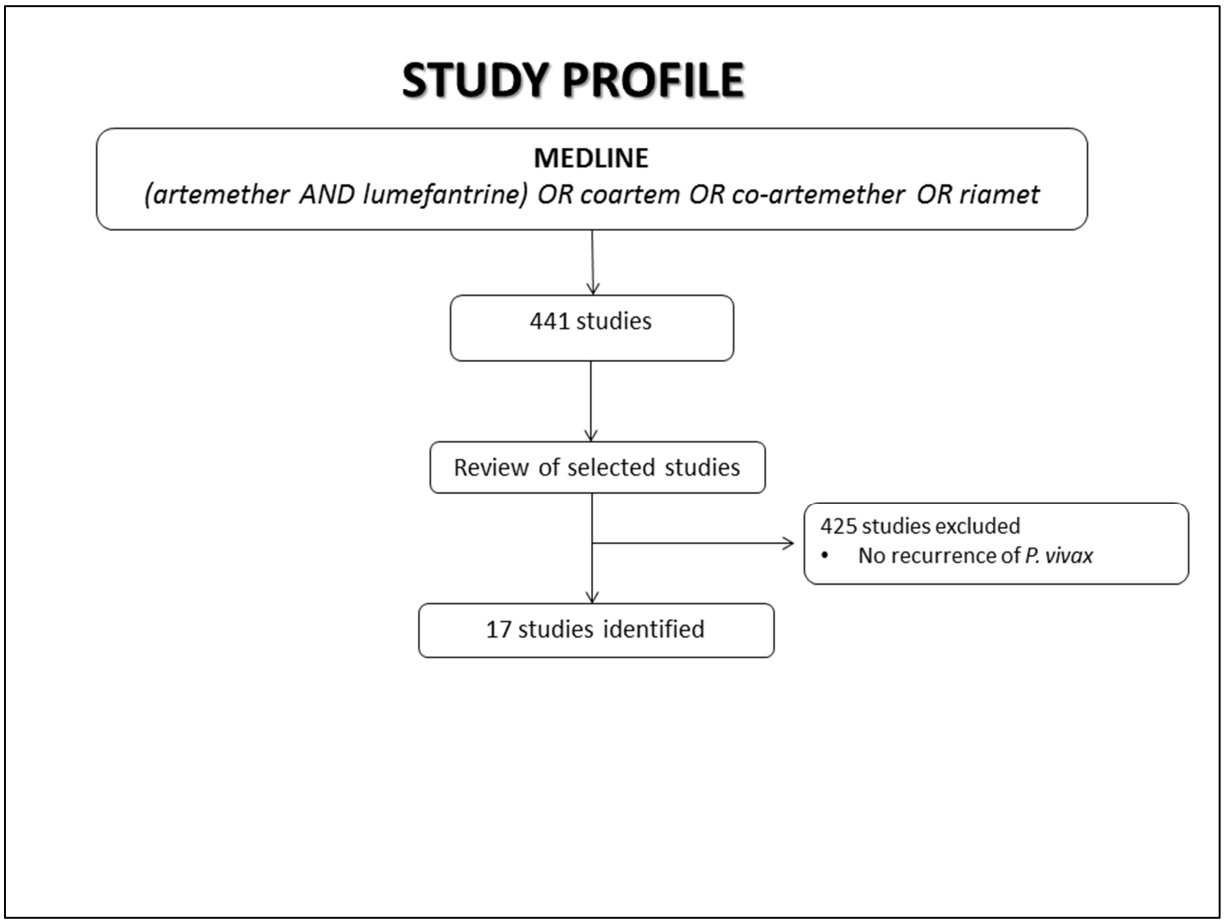

Figure 3.1. **Study Profile**

3.2.2 Data extraction and analysis

Data regarding study characteristics and recurrence of parasitaemia were systematically extracted from the articles and entered into an EpiInfo™ 3.5.1 database and analysed using IBM® SPSS® Statistics version 20. Study sites were identified using Google Earth™ and GPS co-ordinates presented using GPS visualizer (http://www.gpsvisualizer.com/).

The risk of recurrent parasitaemia was calculated according to a per protocol analysis using the number of patients with recurrent parasitaemia during follow up, divided by the number

of patients followed until the study endpoint. The risk was only calculated at the end of the follow up period. Recurrence rates following artemether-lumefantrine treatment were categorized into two groups: "Low recurrence" if there was less than 10% recurrence within 42 or 63 days and "High recurrence" for recurrence rates greater than or equal to 10% during follow up.

3.3 Results

3.3.1 Details of included studies

A total of 17 clinical trials with recurrence of *P. vivax* parasitaemia following artemether-lumefantrine treatment for *P. falciparum* were included in the analysis. Only two studies were conducted prior to 2000. A total of 2,378 patients were enrolled into AL treatment arms of which 11 studies were conducted in Southeast Asia and Oceania, four in the Indian subcontinent and two in the Horn of Africa.

All studies were prospective in nature, with 13 studies randomized. Two studies recruited two separate AL treatment arms. Mixed infections were included in six studies recruiting 344 patients (5.8%, total n=5881). Of the 19 trial arms, 13 (68%) were based in the out-patient setting, 5 (26%) in the in-patient setting and 1 (5%) study was based in the community. Drug treatment was fully supervised in 14 (74%) trial arms, and part supervised in 3 (16%) while only the first dose was administered under supervision in 2 (11%) trial arms.

3.3.2 Risk of recurrent parasitaemia

Across all studies a total of 835 (37.2%) patients were reported to have recurrent parasitaemia during follow up. The overall risk of recurrence was greater for *P. vivax* (23.8%, n=533) than for *P. falciparum* (13.5%, n=302). The risk of recurrent parasitaemia due to *P. falciparum* and *P. vivax* following treatment with artemether-lumefantrine in each study are

presented in Table 3.1. The proportion of recurrent parasitaemia due to *P. vivax* increased with longer follow up (Figure 3.2). In the studies conducted in South East Asia the median risk of recurrent parasitaemia due to *P. vivax* following artemether-lumefantrine treatment was 4.0% (range 1.3%-12.2%) in the four studies reporting the 28 day risk, compared to 30.2% (range 4.1%-64.9%) in the seven studies reporting 42 days risk and 40.4% (range 35.5% - 45.3%) in the two studies reporting the risk at 63 days. In comparison, the risk of recurrent parasitaemia due to *P. vivax* was similar at 28 and 42 days in the four studies conducted on the Indian subcontinent [13.6% at 28 days and 10.5% (range 5.5%-21.0%) at 42 days] and the two studies from the Horn of Africa (5.9% at 28 days and 7.1% at 42 days).

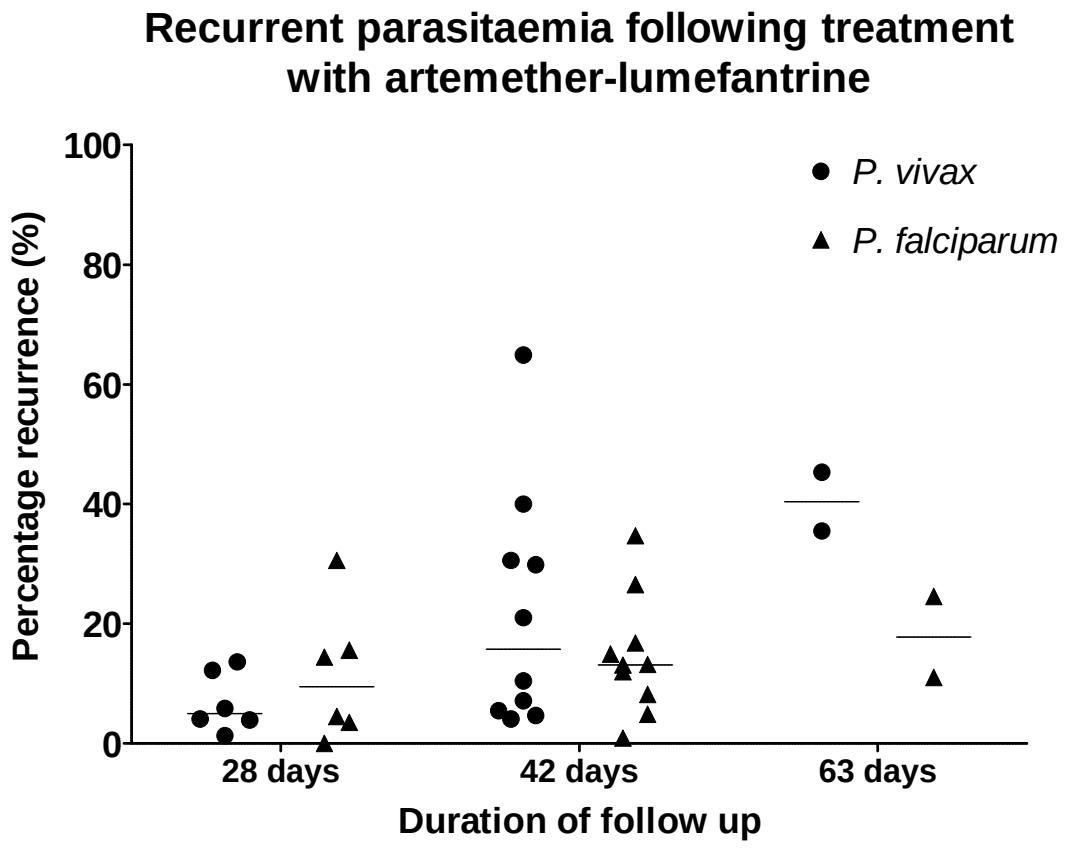

Figure 3.2. **Recurrent parasitaemia following treatment with artemether-lumefantrine**

Table 3.1. Recurrence of *P. vivax* parasitaemia following treatment of *P. falciparum* malaria with artemether+lumefantrine

Reference	Duration of follow-up (days)	Number of patients completing follow-up	Number (%) with recurrent *P. vivax*[1]		Number (%) with recurrent *P. falciparum* (PCR unadjusted)		Percentage of recurrences due to *P. vivax*
Southeast Asia and Oceania							
Thailand							
McGready et al [107]	42	124	37[a]	(29.8)	43	(34.7)	46.3%
Hutagalung et al [108]	42	225	90	(40.0)	27	(12.0)	76.9%
Lefevre et al [109]	28	155	6	(3.9)	7	(4.5)	46.2%
van Vugt et al [110#]	28	90	11	(12.2)	13	(14.4)	45.8%
van Vugt et al [110#]	28-63[b]	142	43		8		
van Vugt [111]	63	248	83	(35.5)	61	(24.6)	57.6%
Papua New Guinea							
Karunajeewa et al [112]	42	102	66	(64.9)	5	(4.9)	93.0%
Myanmar							
Smithuis et al [113]	63	137	62	(45.3)	15	(11.0)	80.5%
Laos							
Mayxay et al [114]	42	107	5	(4.7)	14	(13.1)	26.3%
Stohrer et al [115]	42	47	2	(4.1)	13	(26.5)	13.3%
Indonesia							
Ratcliff et al [116]	42	219	67	(30.6)	29	(13.2)	69.8%
Cambodia							
Denis et al [117##]	28	49	2	(4.1)	15	(30.6)	11.8%
Denis et al [117##]	28	77	1	(1.3)	12	(15.6)	7.7%
Indian Subcontinent							
Bangladesh							
Thriemer et al [118]	42	73	4	(5.5)	6	(8.2)	40%
van den Broek [119]	42	119	25	(21.0)	20	(16.8)	55.6%
Haque et al [120]	42	67	7	(10.4)	10	(14.9)	41.2%
Nepal							
Thapa et al [121]†	28	64	9[c]	(13.6)	0	(0.0)	100.0%
Africa							
Ethiopia							
Assefa et al [122]	28	85	5	(5.9)	3	(3.5)	62.5%
Hwang et al [123]	42	112	8	(7.1)	1	(0.9)	88.8%

[1] When patients were excluded due to recurrent *P. vivax* infection or *P. falciparum* re-infection during the follow-up period, these numbers were added to the eligible participants

[#] Evaluated 3 regimens of artemether+lumefantrine at 2 locations with variable follow-up

[##] Contained details of 2 separate studies

[a] Before delivery or day 42 if later

[b] Variable follow-up periods used for different participants

[c] *P. vivax* infection detected by PCR

† Includes 5 patients with mixed infection by PCR at enrolment.

Abbreviations: AM+LUM; artemether+lumefantrine, PCR; polymerase chain reaction

3.4 Discussion

Studies conducted by Imwong *et al* on *P. vivax* relapse using genotyping methods show that in infants, the first relapse in life is genetically homologous to the primary infection.[124] In comparison, in adults the authors demonstrate that new *P. vivax* populations are responsible for relapses following primary infection by a different population.[125] These findings point to two possibilities. One is that mosquitoes inject *P. vivax* sporozoites of different genotypes into hosts leading to the formation of hypnozoites with different genotypes in the liver. This however, is unlikely given the rarity of finding sporozoites with different genotypes in infected mosquito dissections. The second and more plausible explanation is that sporozoites from previous *P. vivax* infections remain dormant in the liver and get re-activated as a result of a new primary infection. It is likely that repeated attacks of *P. vivax* induce significant clinical immunity towards infection and that many of the previous *P. vivax* infections do not become clinically apparent. The demonstration of a high incidence of *P. vivax* malaria after treatment for *P. falciparum* malaria as shown in this chapter supports this possibility. This lends credence to the hypothesis that in areas of moderately high *P. vivax* endemicity such as that found in Southeast Asia, Oceania and India, hypnozoites exist in the livers of a large section of the population dormant from previous asymptomatic infections primed to re-activate through a yet unknown mechanism. The "activation of latent hypnozoites" hypothesis as put forth by Professor Nick White suggests that latency in *P. vivax* is indeed due to a biological clock which determines the interval length before the hypnozoite becomes susceptible to activation as previously considered, but that there is a separate sensing mechanism which determines whether or not this activation occurs.[59] The current data on the prevalence of mixed infections from endemic regions coupled with the low probability of individuals being inoculated with *P. vivax* in one or two days following *P. falciparum* inoculation do not explain high rates of *P. vivax* recurrence following treatment of *P. falciparum*. In addition, the finding of heterologous *P. vivax* hypnozoites being activated in

adults[125] suggests that primary infection with *P. vivax* may lead to the activation of pre-existing hypnozoites dormant in the liver. The finding of higher rates of relapses in studies conducted in endemic regions compared to artificially inoculated volunteers also supports the hypothesis that latent hypnozoites activated by the *P. falciparum* illness give rise to the *P. vivax* recurrences observed after *P. falciparum* infection.[59] Although there is no way to distinguish reliably between recrudescence, relapse and reinfection currently[125] the background rates of *P. vivax* infection in the study areas and the fact that these patients were treated with an effective blood schizontocidal drug also suggest that these recurrences are likely to be relapses of *P. vivax* malaria from previously acquired infections.

The triggers of subsequent relapses of *P. vivax* are unknown but strain variation, sporozoite load and host immunity appear to play key roles. The observation of high rates of *P. vivax* after *P. falciparum* malaria leads to another plausible hypothesis, that *P. falciparum* infection activates dormant parasites either by a specific parasite factor re-awakening hypnozoites or by a systemic imbalance such as fever associated with falciparum malaria. If the latter is correct then the possibility that relapse is triggered by high fever due to other systemic illnesses needs to be considered. This will be addressed further in Chapter 4.

An analysis of commonly used drugs against *P. vivax* at the Bangkok Hospital for Tropical Diseases in 2004 showed that artemisinin derivatives had the highest potency against *P. vivax* followed by chloroquine, mefloquine, quinine, halofantrine, primaquine, antibacterials clindamycin, azithromycin, tetracycline, doxycycline and lastly sulfadoxine-pyrimethamine.[126] Although drugs with higher potency clear parasitaemia faster than low potency drugs, the occurrence of early gametocytaemia in *P. vivax* prior to the appearance of symptoms means that just switching to drugs with higher potency will have a minimal effect on reducing transmission.

Several countries with high endemicity already employ a unified artemisinin-based treatment against both major species of malaria.[100] The ideal drug for a unified regimen against both *P. falciparum* and *P. vivax* should possess prolonged post treatment prophylaxis against recrudescence of parasites, relapse from hypnozoites of *P. vivax* and from new infections due to either species. Among the ACTs, Dihydroartemesinin-piperaquine (DHP) with the longer half-life of piperaquine seems to be a better candidate as a drug for unified treatment of malaria conferring a higher degree of protection from recurrent parasitaemia. An open labelled randomized study from Papua compared the effectiveness of AL with DHP in both *P. falciparum* and *P. vivax* and determined that the overall risk of failure in both treatment groups at 42 days was similar in *P. falciparum* but in *P. vivax* the risk was 28% higher in patients treated with AL than those treated with DHP.[116]

The findings in this chapter show that even in pure *P. falciparum* infections, recurrent parasitaemia is most often due to *P. vivax*, with rates of *P. vivax* parasitaemia being especially high in Southeast Asia and Oceania. Although adoption of DHP would effectively reduce recurrences at 42 days caused due to recrudescence of *P. falciparum* and early relapse of *P. vivax*, this will likely only delay rather than prevent subsequent relapse. Hence, in regions with high co-endemicity, later recurrences can only be prevented by the addition of anti-relapse treatment (primaquine and other members of the 8-aminoquinoline family) to a unified treatment regimen.

Chapter 4

4 Risk factors for representation to hospital with *P. vivax* malaria in Papua, Indonesia

4.1 Introduction

Several factors increase the risk of recurrence of *P. vivax*. Some of the factors which have been identified include primary infection with *P. vivax* or mixed infection with other species, younger age and lower immunity.[127] Residence in an area with high breeding of the *Anopheles* mosquito can also lead to repeated reinfections contributing to high recurrence after effective treatment. However, there are several incompletely understood factors which also contribute to recurrence such as high risk of *P. vivax* parasitaemia after pure *P. falciparum* as discussed in Chapter 3 and the wide variation of effectiveness in radical cure as discussed in Chapter 2. In this chapter, some of the risk factors for recurrence of *P. vivax* infection in Papua are further explored.

Papua is the westernmost province of Indonesia comprising of the eastern half of the island of New Guinea. This area is endemic to the four major species of *Plasmodium*. In this region, *P. vivax* is the predominant species causing malaria in infants and is responsible for 43% of malaria in the 1-4 year age group.[57] Multidrug resistant vivax malaria in Papua has been responsible for severe and fatal malaria, especially in children, with *P. vivax* having a similar case-fatality rate to *P. falciparum* in infants.[80] The major complication attributable to *P. vivax* in Papua is severe anaemia with 87% of patients with severe vivax malaria having a haemoglobin level less than 5 g/dl.[36] Infants with *P. vivax* had a higher risk of severe anaemia and thrombocytopenia compared to those with *P. falciparum* infection.[80] The spectrum of severe malaria due to *P. vivax* in Papua also included respiratory distress, coma and death[36] and in pregnancy, is associated with an increased risk of anaemia and low birth weight.[41]

The clinical epidemiology of malaria in this region highlights the public health importance of *P. vivax*, and raises important questions regarding the underlying pathological processes that result in severe disease[8]. Recent evidence suggests that increasing chloroquine resistance could be associated with the emergence of severe disease in *P. vivax*, a trend similar to that observed with severe infection and mortality in *P. falciparum* during the rise of chloroquine resistance in *P. falciparum*.[9] Chloroquine resistant strains of *P. vivax* have faster parasite growth rates[128] which are also observed in *P. falciparum* infections causing severe disease[129]. The region of Papua is unusual in harbouring high grade antimalarial resistance in both *P. falciparum* and *P. vivax*. Chloroquine resistant strains of *P. falciparum* were documented as early as 1974 [130] and in 1989 the first reports were made of chloroquine resistant *P. vivax*[131]. In 2003, failure rates by day 28 following chloroquine treatment exceeded 90% for both *P. falciparum* and *P. vivax*.[132] Resistance has also been demonstrated in both these species of malaria against second line drugs such as sulfadoxine-pyrimethamine and amodiaquine.[133-136]. Inadequately treated *P. vivax* infection led to recrudescence and relapses in up to 80% of patients within 4 weeks with repeated infections leading to the development of severe anaemia. Almost 20% of patients hospitalized with *P. vivax* in Papua were found have a haemoglobin level less than 7g /dl.[36]

In the lowland area the Rumah Sakit Mitra Masyarakat (RSMM) hospital is the main hospital for the Timika district. Its extensive electronic data system has allowed detailed analyses of the malaria attributable burden of *Plasmodium* and how these vary between patient groups in this region. The risk of representation to the hospital after initial presentation with vivax malaria has been described in a previous study.[127] This found that almost a third of patients with vivax malaria represented within a year with recurrent infections. Younger age and highland Papuan ethnicity were risk factors for greatest recurrence. Furthermore recurrent

infections particularly in the first year of life were associated with high rates of severe anaemia. Importantly, representation did not differ between patients prescribed unsupervised primaquine for radical cure and those just receiving schizontocidal therapy. Hence even when radical cure is prescribed for patients with *P. vivax*, its effectiveness is poor, a likely consequence of poor adherence from unsupervised treatment and unknown efficacy of low dose primaquine in this regimen.

The Chesson strain of *P. vivax*, endemic in Papua, is known to relapse early with reactivation of hypnozoites occurring as early as one month after the primary infection. This strain has been studied extensively, both in soldiers returning from endemic regions and in experiments using artificial inoculation in volunteers and has required high doses of primaquine (≥5.0mg/kg/day) for effective prevention of relapse.[60 68 69 137-139] The high rates of recurrence of *P. vivax* emphasise the difficulties of achieving radical cure. However in endemic settings this phenomenon is not limited to just those presenting with an initial episode of vivax malaria. As shown in the previous chapter, most patients in an endemic setting will have had prior exposure to infection and thus be at significant risk of harbouring dormant asymptomatic infections for long periods of time. The finding of higher rates of relapses in studies conducted in endemic regions compared to artificially inoculated volunteers also supports the hypothesis that latent hypnozoites activated by the *P. falciparum* illness give rise to the *P. vivax* recurrences observed after *P. falciparum* infection.[59] One theory recently advocated by Professor White, is that activation of latent hypnozoites may be caused due to any systemic illness capable of causing high fever similar to that seen in a primary attack of *P. falciparum* or *P. vivax*.[59] If this hypothesis is valid, patients presenting with illnesses causing high fever in regions with high *P. vivax* endemicity may be at risk of developing a relapse immediately following the febrile episode.

The aims of the current analysis is to identify risk factors for representation with *P. vivax* in all patients presenting to RSMM, not just those presenting with an initial *P. vivax* infection, the goal being twofold. Firstly, to test the hypothesis that high grade fever due to non-malarial causes can lead to reactivation of hypnozoites of *P. vivax* and early relapse. Secondly, to identify high risk patients at greatest risk of *P. vivax* relapse who can be targeted for radical cure with high dose primaquine to reduce morbidity from and transmission of *P. vivax*.

4.2 Methods

4.2.1 Study Site

4.2.1.1 Geography

The data used in this study were collected at the Rumah Sakit Mitra Masyarakat (RSMM), the largest health care facility in Timika. Timika is a semi-urban municipality located in Mimika district in the southern lowlands of the province of Papua in Indonesia. Papua is the largest and easternmost province of Indonesia, comprising of the western half of the island of New Guinea (Figure 4.1). The Mimika district covers a land area of 21,522 square-kilometres of largely forested coastal, mountainous and lowland areas with 12 sub-districts and 85 villages. Timika is situated in the dense, lowland tropical rainforest along the banks of the Mimika River and is the most populous municipality in the district.

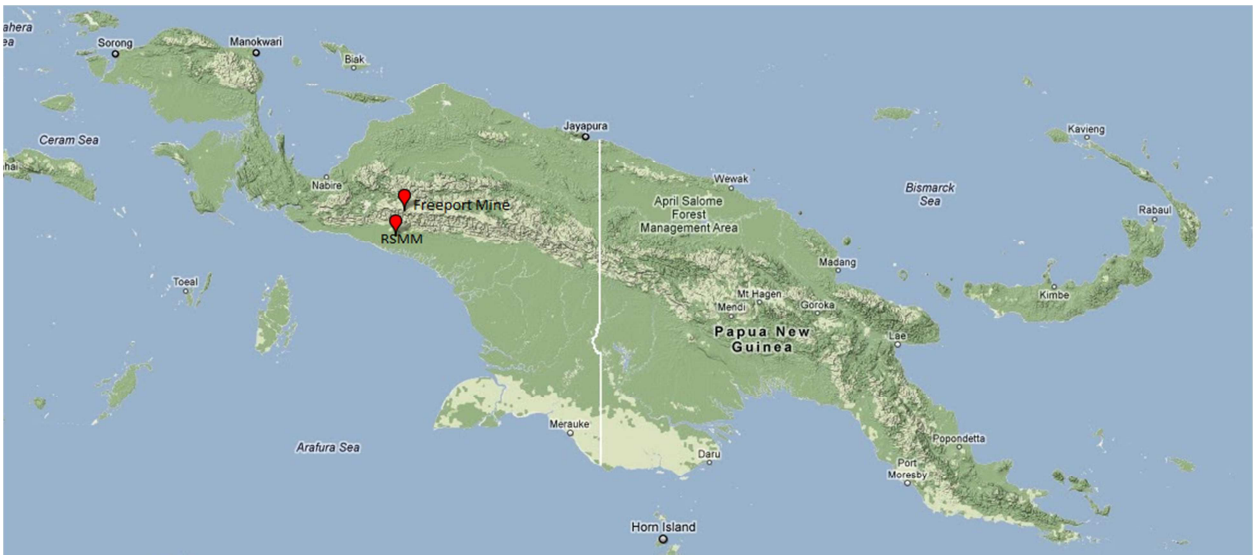

Figure 4.1. **Study Site**

The Grasberg mine operated by PT Freeport Indonesia is located on the highlands in Timika (Figure 4.1). The establishment of the mine in 1973 and the discovery of enormous copper and gold deposits in 1988 had a major impact on the population. Thousands of indigenous Papuans were displaced from the highlands to areas surrounding Timika. The mine has also attracted thousands of Indonesian transmigrants seeking potential employment which has resulted in Timika having one of the highest population growth rates for any urban region in Indonesia.

4.2.1.2 Demographics

Timika has a population of approximately 200,000 people with 28% of the population estimated to be Highland Papuan, 26% Lowland Papuan and 45% of non-Papuan origin, the vast majority being Indonesians from other provinces. The highland tribes, such as the Amungme, have traditionally been cultivators of sweet potato and practiced pig husbandry whereas the lowland tribes, such as the Komoro, have been hunters and gatherers. Historically, different Papuan tribes had relatively little interaction with each other, but

displacement due to the Freeport mine and newer economic opportunities have brought them living in close proximity to each other.

4.2.1.3 Climate

There is little seasonal variation in the climate in Timika with temperatures ranging from a maximum of 32 ºC in the hottest month of January to a minimum of 22 ºC in July, the coldest month of the year. There is heavy rainfall of approximately 5,000mm/year, mostly in the afternoon and evening in the lowlands with a mean humidity of 89% through the year.

4.2.1.4 General health indicators

The health indicators of Papua highlight a maternal mortality rate of 1,145 deaths per 100,000 live births and an infant mortality rate of 68 deaths per 1,000 live births.[140] The prevalence of HIV is estimated to be 2.4% in individuals aged 15-49 years.[141]

4.2.1.5 Epidemiology of malaria in Timika

All the major Plasmodium species which infect humans are endemic in Papua. Local mosquito vectors include three main species (*Anopheles punctulatus*, *An. koliensis*, *An. farauti)*, with other species such *An. longirostris*, *An. karwari* and *An. bancrofti* also reported.[142] The entomological inoculation rates for *P. falciparum, P. vivax* and mixed *P. falciparum* / *P. vivax* infections have been estimated at 0.50, 0.28 and 0.06 infectious bites per year respectively.[57] The presence of splenomegaly was estimated to be 44.0% in a survey of various communities in Timika in 1992 with a parasitaemia prevalence of 60% in children between one to two years of age.[142] In 2005, a house to house survey of 800 households conducted in Timika and the surrounding region documented the overall prevalence of asexual parasitaemia to be 16.3 % with 46% of infections due to *P. falciparum*, 39% due to *P. vivax*, 4% due to *P. malariae* and 11% due to mixed infections.[57] The median age of *P. falciparum* parasitaemia was 19 years compared to 13.5 years in *P. vivax*. The peak prevalence of *P. falciparum* parasitaemia was 10% in the 15-24 years age group whereas the peak prevalence in the *P. vivax* group was seen in

the 1-4 year age group (9.5%). In the same year the incidence of parasitaemia was estimated to be 876 episodes per 1,000 people per year with the species breakdown being 512 per 1,000 for *P. falciparum,* 322 per 1,000 for *P. vivax*, 15.7 per 1,000 for *P. malariae* and 26.3 per 1,000 for mixed infections.[57]

4.2.1.6 Treatment and control of malaria in Timika

The Public Health Malaria Control Program and Dinas Kesehatan (Indonesian Health Department) conduct a range of malaria control activities in Timika. Indoor residual spraying, distribution of insecticide treated bed nets and larvicidal treatment have been regularly conducted in Timika from 2004 to 2011.

Antimalarial treatment guidelines in Timika have been greatly influenced by clinical trials conducted locally. The local administration has been quick to implement evidence based changes in the treatment of malaria. Until 2006, patients with uncomplicated malaria due to any species were treated with either 3-day oral chloroquine or 7-day oral quinine, both without supervision. Following a series of clinical trials, in March 2006 the treatment of uncomplicated malaria due to any *Plasmodium* species was changed to 3-day unsupervised dihydroartemisinin + piperaquine.[83 143 144] In 2007, the supply of DHP ran out at RSMM for a brief period during which patients were treated with artesunate+amodiaquine. The treatment of severe malaria was also changed from intravenous quinine to intravenous artesunate in May 2005.[145]

Patients with *P. vivax* or mixed infections are given a 14-day unsupervised course of primaquine for radical cure. Over the last seven years at RSMM, there has been a gradual adoption of the higher 0.5 mg/kg/day dose of primaquine from the 0.25 mg/kg/day dose. Testing for G6PD deficiency is not available and not performed at RSMM before primaquine administration. Recent analyses (as yet unpublished) suggest that adherence to

the 14-day regimen is generally poor and that patients prescribed unsupervised primaquine have similar rates of representation as those not prescribed primaquine.

4.2.2 Data collection

Rumah Sakit Mitra Masyarakat (RSMM) is the largest health care facility in Timika with over 90,000 patient visits every year of which malaria accounts for approximately 15% of out-patient visits.[36] The hospital has a 24-hour emergency department, approximately 110 beds for in-patients and a "high care" facility with equipment for intravenous infusions and monitoring. Facilities for mechanical ventilation are not available. The RSMM provides free treatment for Papuans and at a small cost to non-Papuans. The hospital has a radiological department with X-ray facilities and a diagnostic laboratory, capable of testing haematology and biochemical profile and basic microscopy. Complete blood counts are ordered according to clinical indication and are performed by Coulter Counter (Sysmex™ 3-part Differential Automated Hematology Analyzer).

A unique identification number is assigned to every patient who comes to the RSMM out-patient, in-patient or emergency department and is recorded in a Q-Pro® database by the hospital administration. The details recorded include patient name, age, sex, ethnicity, date of presentation and date of discharge. The clinical diagnoses were made by the attending physician and coded according to the International Classification of Diseases by trained clinical records staff. Details of prescriptions filled at the hospital pharmacy such as drug name, dose prescribed and date of prescription were recorded and linked using the same unique identification number.

The diagnosis of malaria at RSMM is usually based on microscopy; clinical diagnosis without laboratory confirmation is rare and actively discouraged. In all patients presenting with fever or other symptoms likely to be malaria, a blood smear is prepared which is examined by Giemsa staining and light microscopy. Diagnosis of malaria is made on the basis of thick film

smears. The RSMM microscopy laboratory participates in an ongoing quality assurance process. In 2004, the hospital microscopy service was assessed for quality control, and a random sample of 1,200 positive slides was re-read by an independent expert microscopist with more than 10 years of experience blind to the original microscopist. Slides were available in 90% (1,083/1,200) of cases with concordance between the readings of 90% (979/1,083). In 1.7% (18/1,083) of slides, the second reading was negative, and in 4% (38/922) of cases slides reported as mono-infection were in fact found to be mixed infections.[57]

4.2.3 Data merging

Electronic records were available from the hospital between 2004 and 2011. These were derived from three sources: i) The clinical records of all patients examined as outpatients or admitted as inpatients; ii) Laboratory data from all patients with haematological investigations and iii) pharmacy data. These data were merged using the unique hospital identification number and date of admission. The merging process was validated by cross checking against data collected independently from a malaria register. The malaria register consisted of data collected by a trained research nurse who reviewed all patients admitted for more than 12 hours with malaria. Data included duration of stay, demographic details, pregnancy status, outcome, and significant complications including admission to the intensive care and death.[36] Since April 2004, 70,167 patients were admitted to the hospital of whom 21,860 were identified as positive for malaria. These records were cross checked with 15,062 (69%) patients from the malaria register. The concordance was 97.2% (14,634/15,062) for gender and 95.3% (14,361/15,062) for age (within 2 years). The concordance of malaria positivity was 90.6% (13,634/15,062).

The resulting database containing all hospital presentations over a seven year period (n=759,824) was then filtered for patients presenting on their first episode to the hospital

(n=132,586). Individual databases were created for selected infectious and non-infectious diagnoses, with preference given for conditions which could be diagnosed robustly given the limited resources available at RSMM. These included 6 infectious conditions: measles, urinary tract infection, acute upper respiratory tract infection (URTI), respiratory tuberculosis (TB), pneumonia and acute gastroenteritis. The 5 non-infectious conditions which were included were: trauma, anaemia, burns, malnutrition, and malignancy. Further details of the study profile are given in Figure 4.2.

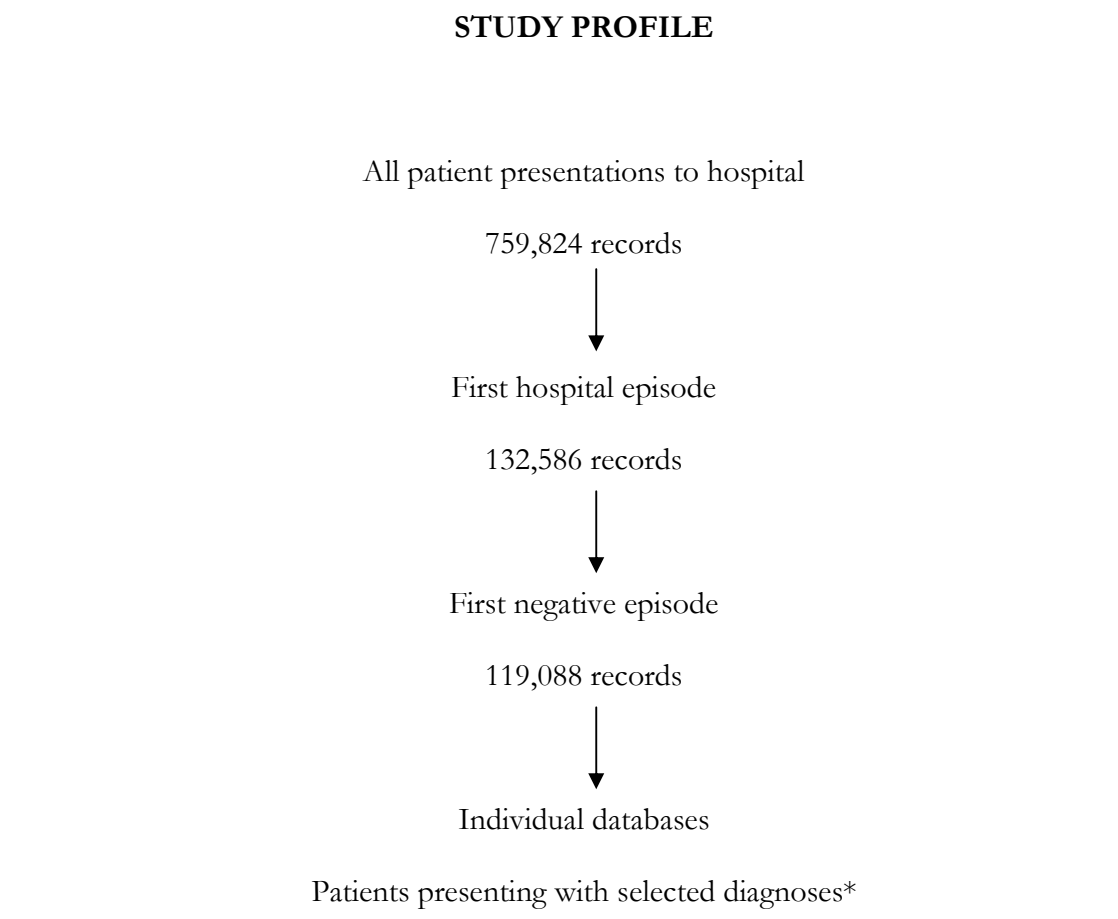

Figure 4.2. **Study Profile**

4.2.4 Statistical Analysis

The analysis of malaria events and their treatments was quantified using the entire dataset of all patients presenting to the hospital and outpatients. The Mann-Whitney U test was used

for nonparametric comparisons, and Student's t-test or one-way analysis of variance for parametric comparisons. Proportions were examined using χ^2 with Yates' correction or by Fisher's exact test.

The risk of representation with *P. vivax* was assessed by survival analysis, in which the cumulative risk of representation was calculated by the Kaplan Meier product limit formula and compared by the Mantel-Haenszel log rank test. A cut off of 63 days was chosen *a priori* to capture early relapses of *P. vivax* malaria which may have been triggered by their initial hospital presentation. Curtailing follow up at 9 weeks minimized the confounding of reinfections. Assuming an incidence of 322 *P. vivax* infections per 1000 patients per year, one would expect 5.6% of patients initially smear negative to represent with *P. vivax* within 9 weeks and this would fall to 3% in patients initially treated with DHA-piperaquine which affords 4 weeks of post treatment prophylaxis. To avoid problems with non-independence of the data, we restricted Kaplan-Meier analyses to the first presentation of the patient to the hospital (n=132,586). Survival analyses were stratified by the species of initial infection, baseline characteristics and initial presentation to the hospital with infectious and non-infectious conditions. The log-rank test for overall difference or trend (as appropriate) was used to compare the hazard of representation for the species of initial infection (Negative, *P. falciparum*, *P. vivax* or mixed infection), age groups (<1 year, 1-5 years, 5-15 years and >15 years), sex, ethnicity (non-Papuan, Highland Papuan and Lowland Papuan), year of initial presentation (2004 to 2011), anaemia (Yes or No), schizontocidal treatment (quinine vs. DHA-piperaquine), radical treatment (Yes or No) and for each of the non-malarial conditions. Cox proportional hazards regression was used for multivariate analyses. Baseline factors identified as significant predictors of representation in univariate analysis in patients initially presenting without malaria were included in the model. We assessed the proportional hazards assumption for each covariable by comparing visually the log (cumulative hazard) by

time of follow-up curves for each covariable category. Year of presentation was found to violate the proportional hazards assumption and therefore the Cox models were stratified for year of presentation.

We investigated the robustness of our results to potential residual confounding by comparing the risk of representation with *P. vivax* malaria at 63 days to the risk of representation with *P. falciparum* malaria at 63 days in all patients and in the 1-5 year age group in the medical conditions showing statistical significance in the multivariate model.

All analyses were done using IBM® SPSS® Statistics for Windows version 20.

4.2.5 Ethical Approval

Ethical approval for this study was obtained from the Oxford Tropical Research Ethics Committee of the University of Oxford and the Health Research Ethics Committees of the University of Gadjah Mada, Indonesia and Menzies School of Health Research, Australia.

4.3 Results

Between 2004 to 2011, 132,586 patients made 759,824 presentations to Rumah Sakit Mitra Masyarakat. The overall median number of visits to hospital was 14 [IQR 5-30]. The age stratified histogram is presented in Figure 4.3. The majority of these presentations were out-patient visits (n=689,657; 91%) with 9% (n=70,167) of presentations being admissions to the hospital wards for at least 6 hours.

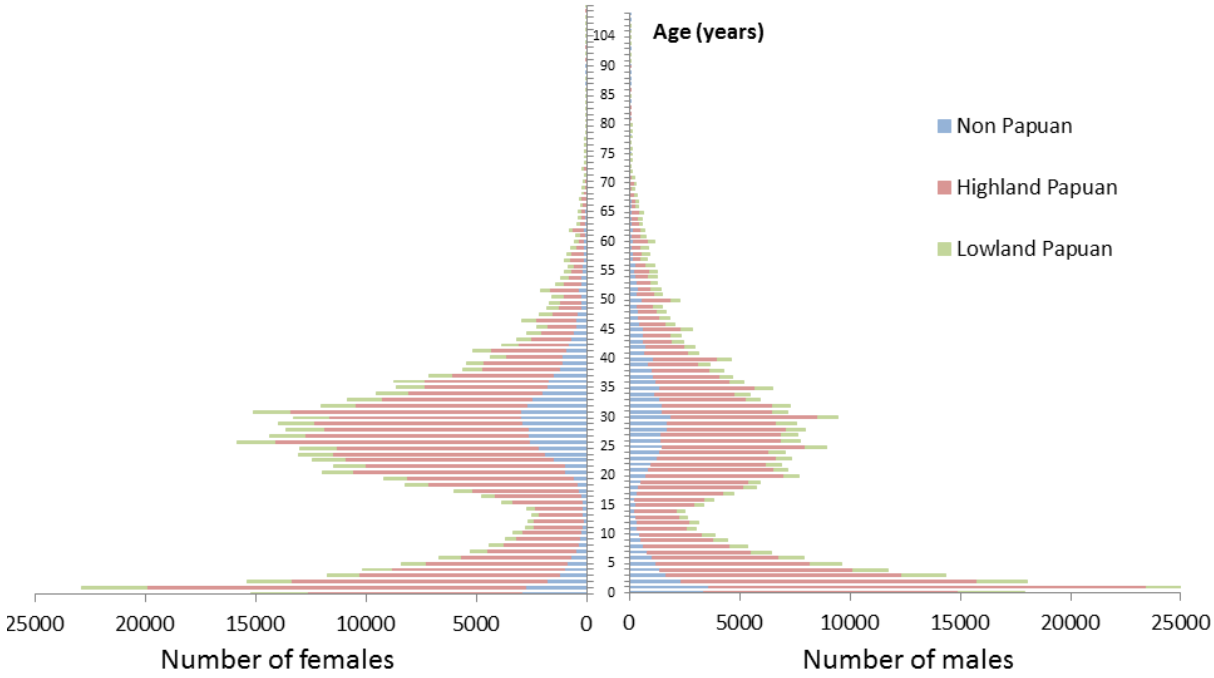

Figure 4.3. **All cause presentations to RSMM from 2004-2011.** This analysis includes all patient presentations to hospital. n=759,824

There were 135,237 (18%) presentations to hospital associated with malaria, *P. falciparum* accounting for 74,967 of these (55%), *P. vivax* for 42,920 (32%), *P. malariae* for 4,005 (3%) and *P. ovale* for 104 (0.8%). Mixed infections were seen in 13,241 (10%) patients. The age distribution of malaria attributable presentations to RSMM is shown in Figure 4.4, with two peaks, one in early childhood (2-5 years) and one in young adults (20-30 years). The median age of patients presenting with vivax malaria was 10 years (IQR 2.6-24), significantly younger than patients with *P. falciparum* (20 years, IQR 6.7-29) or mixed infections (15 years, IQR 3.9-25.5; $p<0.001$). Of the 135,237 presentations with malaria, 21,860 (16.2%) resulted in admission to hospital, the risk being greater in patients with *P. falciparum* (OR=2.81, $p<0.001$) compared to those with pure *P. vivax* infection (OR=1.39, $p<0.001$). Details of malaria attributable presentations are presented in Table 4.1.

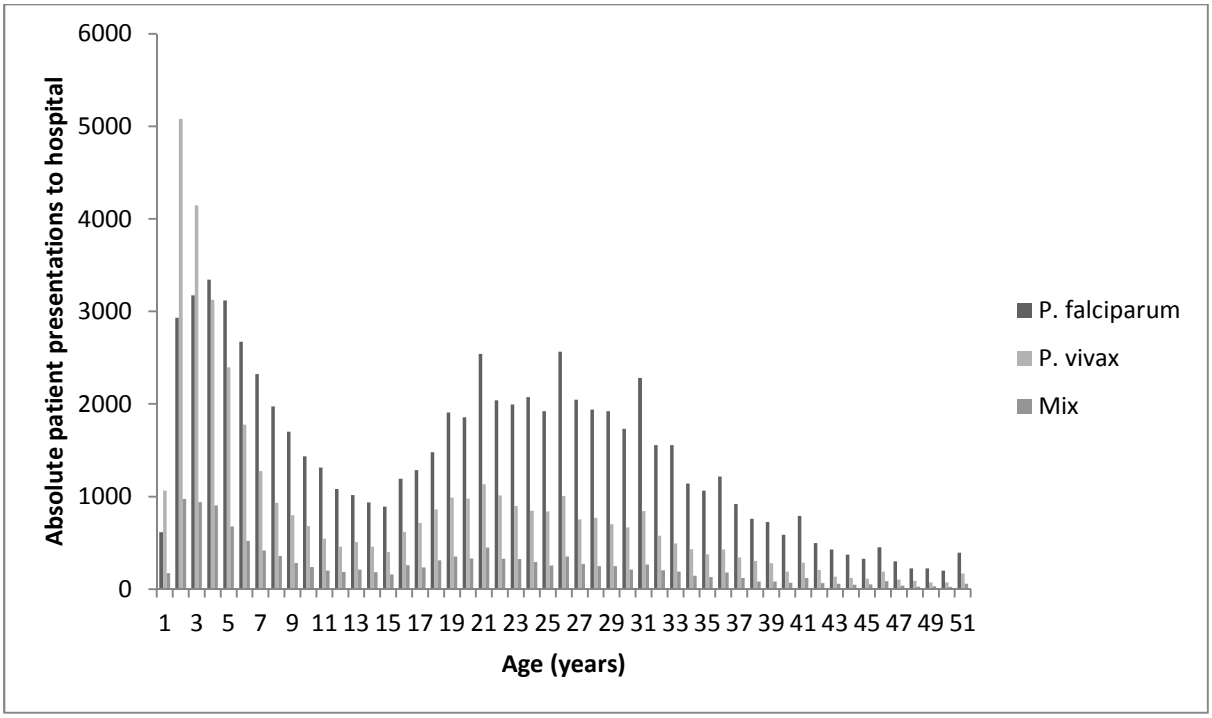

Figure 4.4. **Age distribution of malaria attributable presentations**. This analysis includes all patient presentations to hospital. n=759,824. Patients older than 50 years of age and *P. malariae* and *P. ovale* infections have been omitted from this figure.

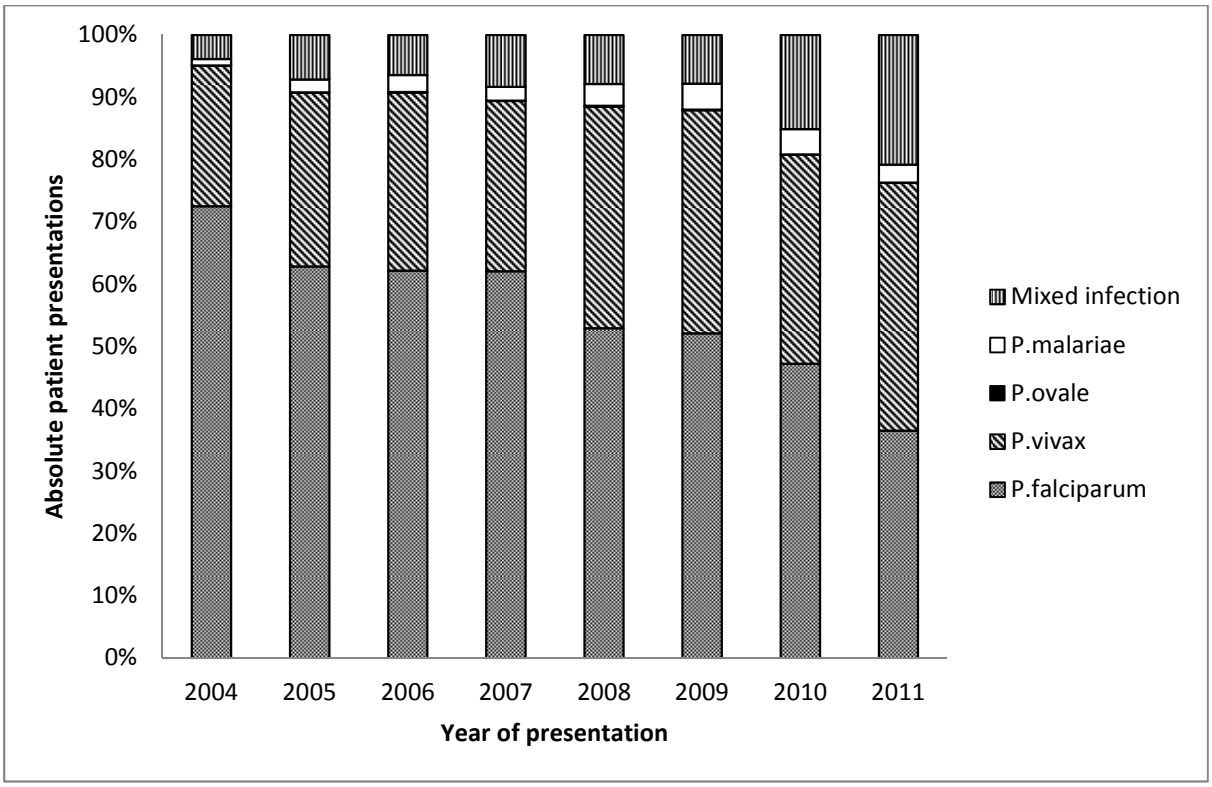

Figure 4.5. Temporal trends in absolute numbers of malaria attributable presentations to RSMM. This analysis includes all patient presentations to hospital. n=759,824

Table 4.1: Distribution of baseline factors according to species of Plasmodium. This analysis includes all patient presentations to hospital. n=759,824

Factors	Negative	*P. falciparum*	*P. vivax*	Mix
Age				
0-1 yr.	51,662 (8.3%)	1,875 (2.5%)	3,511 (8.2%)	604 (4.6%)
1-5 yrs.	85,570 (13.7%)	12,562 (16.8%)	13,153 (30.7%)	3,312 (25.0%)
5-14 yrs.	61,229 (9.8%)	14,442 (19.3%)	7,151 (16.7%)	2,584 (19.5%)
≥15 yrs.	426,052 (68.2%)	46,083 (61.5%)	19,094 (44.5%)	6,738 (50.9%)
	624,513	74,962	42,909	13,268
Gender				
Male	276,290 (44.2%)	37,563 (50.1%)	21,601 (50.3%)	6,837 (51.6%)
Female	348,297 (55.8%)	37,404 (49.9%)	21,319 (49.7%)	6,404 (48.4%)
	624,587	74,967	42,920	13,241
Ethnicity				
Highland Papuan	418,820 (67.2%)	61,241 (81.8%)	34,928 (81.5%)	11,539 (87.2%)
Lowland Papuan	91,194 (14.6%)	7,761 (10.4%)	3,227 (7.5%)	881 (6.7%)
Non Papuan	113,166 (18.2%)	5,878 (7.8%)	4,720 (11.0%)	818 (6.2%)
	623,180	74,880	42,875	13,238
Source				
OP	576,280 (92.3%)	60693 (81.0%)	38,444 (89.6%)	10,510 (79.4%)
IP	48,307 (7.7%)	14,274 (19.0%)	4,476 (10.4%)	2,731 (20.6%)
	624,587	74,967	42,920	13,241
Year				
2004	53,127 (8.5%)	7,149 (9.5%)	2,230 (5.2%)	384 (2.9%)
2005	73,646 (11.8%)	9,279 (12.4%)	4,114 (9.6%)	1,060 (8.0%)
2006	80,632 (12.9%)	9,611 (12.8%)	4,417 (10.3%)	997 (7.5%)
2007	83,255 (13.3%)	14,161 (18.9%)	6,223 (14.5%)	1,905 (14.4%)
2008	80,536 (12.9%)	9,291 (12.4%)	6,240 (14.5%)	1,382 (10.4%)
2009	89,672 (14.4%)	11,011 (14.7%)	7,573 (17.6%)	1,658 (12.5%)
2010	92,235 (14.8%)	9,601 (12.8%)	6,823 (15.9%)	3,080 (23.3%)
2011	71,484 (11.4%)	4,864 (6.5%)	5,300 (12.3%)	2,775 (21.0%)
	624,587	74,967	42,920	13,241

4.3.1 Antimalarial Treatments Administered

Of the 42,920 episodes of *P. vivax* malaria, 25,020 (58%) were treated with dihydroartemisinin-piperaquine. Other blood stage treatments administered were oral quinine (4,752, 11.1%), artesunate-amodiaquine (2,846, 6.6%), IV artesunate (1,838, 4.3%), IV quinine (694, 1.6%), clindamycin (607, 1.4%), doxycycline (554, 1.3%), sulfadoxine-pyrimethamine (177, 0.4%) and chloroquine (27, 0.1%) (Note: some patients received more than one of these treatments). Primaquine was prescribed for radical cure in 26,959 (62.8%) of these patients. Over the seven years there were 74,967 episodes of *P. falciparum* malaria. Dihydroartemisinin-piperaquine was used in 53.6 % (40,191) of these episodes with other treatments being oral quinine (14,618, 19.5%), artesunate-amodiaquine (5,567, 7.4 %), IV

artesunate (7,127, 9.5%), IV quinine (2,891, 3.9%), clindamycin (3,527, 4.7%), doxycycline (6,686, 8.9%), sulfadoxine-pyrimethamine (2,321, 3.1%) and chloroquine (32, 0.04%).

4.3.2 Representation with Malaria

Analysis of the risk of representation with malaria was restricted to the 132,586 patients on their first presentation to hospital. Of these patients 16,521 (12.5%) were reported to have *P. falciparum* infection, 7,789 (5.9%) *P. vivax*, 612 (0.5%) had *P. malariae*, 12 (0.0%) had *P. ovale* and 2,463 (1.9%) had mixed infection. The remaining 79.3% (105,189) were asymptomatic on presentation or had a negative blood film examination and were assumed to be negative. A total of 18.7% (24,764/132,586) patients represented to hospital within 1 year with either *P. falciparum* (11.2%, 14,881/132,586) or *P. vivax* **(**7.5%, 9,883/132,586) infection. The median time to representation with *P. vivax* was 97 days (IQR: 38-201) in patients initially presenting without malaria, 67 days (IQR 33-119) in those initially presenting with *P. vivax*, 77 days (IQR 45-145) in those presenting with *P. falciparum* and 64 (IQR 34-130) in patients with mixed infection. Of the representations of *P. vivax* within 1 year, 3,998 (40.5%) occurred within 63 days. The corresponding times for representation with *P. falciparum* was 99 days (IQR: 36-203.5) in patients initially presenting without malaria, 75 days (IQR 38-150) in those initially presenting with *P. vivax*, 57 days (IQR 21-137) in those presenting with *P. falciparum* and 54.5 (IQR 22-126.3) in patients with mixed infection, with 42.7% (6347/14881) of 1 year representations occurring within 63 days.

Risk factors for representation within 63 days with *P. vivax* were assessed in patients on their first presentation to hospital (n=132,586). The risk of representation with *P. vivax* differed significantly with the diagnosis of initial presentation. The cumulative risks stratified by the initial species of infection are presented in Table 4.2 and Figure 4.6. Patients initially presenting with *P. vivax* were at greatest risk of representation (10.6%, 95%CI: 9.82-11.38), followed by patients with mixed infection (9.4%, 95%CI: 8.22-10.58). Compared to patients

initially presenting without malaria the hazard of representation with *P. vivax* was 2.18 (95% CI: 2.10-2.27; p<0.001) in patients initially with *P. vivax,* 2.04 (95% CI: 1.91-2.19; p<0.001) in those with mixed infection and 1.30 (95% CI 1.24-1.36; p<0.001) in patients with *P. falciparum* (Table 4.2).

Table 4.2: **Risk of representation to hospital with *P. vivax* within 63 days according to initial species of Plasmodium.** This analysis includes only each patient's first recorded presentation to hospital. n=132,586

Species	Number of presentations to hospital	Cumulative risk of representation with *P. vivax* in next 63 days %, 95% CI	Univariate Hazard Ratio (HR), 95% CI	P value
Negative	105,189 (79.3%)	2.3% [2.30,2.30]	1.00	
P. vivax	7,789 (5.9%)	10.6% [9.82-11.38]	2.18[2.10-2.27]	<0.001
P. falciparum	16,521 (12.5%)	3.9% [3.51-4.29]	1.30[1.24-1.36]	<0.001
Mix	2,463 (1.9%)	9.4% [8.22-10.58]	2.04[1.91-2.19]	<0.001

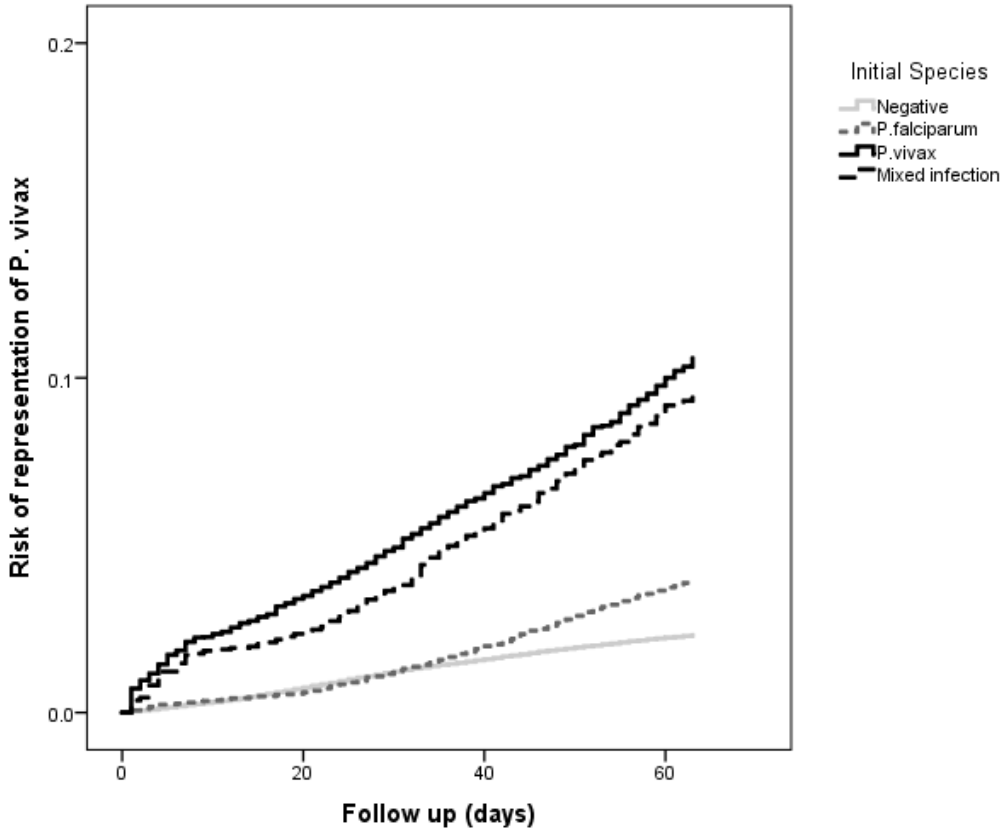

Figure 4.6. **Cumulative risk of representation with *P.vivax* within 63 days**. This analysis includes only each patient's first recorded presentation to hospital. n=132,586

Other baseline factors influencing the risk of representation with *P. vivax* in patients presenting at the initial visit are presented in Table 4.3. Children in the 1-5 years age group had the greatest risk of representation with *P. vivax* (4.8% (95% CI 4.41-5.19) compared to older children and adults; Hazards Ratio (HR) =1.36 (95% CI: 1.28-1.46); p<0.001. The risk of representation was also higher in females compared to males (HR=1.15, 95%CI: 1.08-1.22; p<0.001), Highland Papuans compared to lowlanders (HR=3.02, 95%CI: 2.84-3.20; p<0.001) and outpatients compared to inpatients (HR=1.41, 95% CI: 1.29-1.55; p<0.001). There was a trend for increasing risk of representation with *P. vivax* from 2.1% in 2004 to 4.6% in 2009 (p<0.001).

The multivariate Cox proportional hazards model adjusted for age, gender, ethnicity, source, year, anaemia and stratified by species is presented in Table 4.3. Age less than 5 years, female gender, Highland Papuan ethnicity, out-patient status, presentation to hospital later than 2009 and anaemia remained significant risk factors for representation with *P. vivax* (all p<0.001).

In comparison to quinine, patients treated with dihydroartemisinin-piperaquine had only half the risk of representing with *P. vivax* within 63 days after adjusting for potential confounders (AHR=0.50, 95% CI 0.38-0.65; p =0.00). There was no difference in the recurrence rates of *P. vivax* within 63 days in patients given unsupervised primaquine compared to those given no primaquine (AHR=1.07, 95%CI 0.94-1.22; p=0.307).

Table 4.3. **Effect of baseline factors on risk of representation to hospital with *P. vivax* within 63 days.** This analysis includes only each patient's first recorded presentation to hospital. n=132,586. Adjusted Hazards Ratio (AHR) derived from multivariable Cox regression model after adjusting for age, gender, ethnicity, source, year and anaemia and stratifying by species

Factors	Number of presentations to hospital	Cumulative Risk of representation with *P. vivax* within 63 days %, 95% CI	Univariate HR, 95%CI	p value	Multivariate AHR, 95%CI	p value
Age						
0-1 yr.	14,869 (11.2%)	3.2% [3.00-3.40]	**0.88 [0.81-0.95]**	0.001	**1.18[1.09-1.27]**	<0.001
1-5 yrs.	14,630 (11.0%)	4.8% [4.41-5.19]	**1.36 [1.28-1.46]**	<0.001	**1.34[1.25-1.43]**	<0.001
5-14 yrs.	14,804 (11.2%)	4.0% [3.61-4.39]	**1.12 [1.05-1.20]**	0.001	**0.88[0.82-0.95]**	<0.001
≥15 yrs.	88,258 (66.6%)	2.7% [2.50-2.90]	1.00		1.00	
	132,561					
Gender						
Male	70,620 (53.3%)	2.9% [2.70-3.10]	1.00		1.00	
Female	61,966 (46.7%)	3.3% [3.10-3.50]	**1.15 [1.08-1.22]**	<0.001	**1.20[1.12-1.27]**	<0.001
	132,586					
Ethnicity						
Highland Papuan	71,662 (54.0%)	5.0% [4.80-5.20]	**3.02 [2.84-3.20]**	<0.001	**2.84[2.67-3.02]**	<0.001
Lowland Papuan	22,229(16.8%)	1.0% [0.80-1.20]	**0.58 [0.53-0.64]**	<0.001	**0.61[0.55-0.67]**	<0.001
Non Papuan	38,116 (28.7%)	1.0% [0.80-1.20]	1.00		1.00	
	132,007					
Source						
OP	109,140 (82.3%)	3.3% [3.10-3.50]	**1.41 [1.29-1.55]**	<0.001	**1.94[1.76-2.14]**	<0.001
IP	23,446 (17.7%)	2.4% [2.20-2.60]	1.00		1.00	
	132,586					
Year						
2004	25,864 (19.5%)	2.1% [1.90-2.30]	1.00		1.00	
2005	19,826 (15.0%)	2.8% [2.60-3.00]	**0.86 [0.79-0.93]**	<0.001	**0.90[0.83-0.98]**	0.011
2006	18,977 (14.3%)	2.6% [2.40-2.80]	**0.81 [0.75-0.88]**	<0.001	**0.84[0.77-0.91]**	<0.001
2007	18,210 (13.7%)	3.4% [3.20-3.60]	1.04 [0.97-1.13]	0.265	**1.08[1.00-1.17]**	0.045
2008	14,864 (11.2%)	3.0% [2.80-3.20]	0.94 [0.86-1.02]	0.137	1.04[0.95-1.13]	0.420
2009	13,008 (9.8%)	4.6% [4.21-4.99]	**1.43 [1.32-1.54]**	<0.001	**1.31[1.21-1.42]**	<0.001
2010	12,307 (9.3%)	4.1% [3.71-4.49]	**1.27 [1.17-1.38]**	<0.001	**1.20[1.10-1.30]**	<0.001
2011	9,530 (7.2%)	4.1% [3.71-4.49]	**1.26 [1.15-1.39]**	<0.001	**1.23[1.11-1.35]**	<0.001
	132,586					

Anaemia						
No	125,593 (94.7%)	3.0% [3.00]	1.00		1.00	
Yes	6,993 (5.3%)	5.4% [4.81-5.99]	**1.84[1.66-2.05]**	**<0.001**	**1.37[1.22-1.54]**	**<0.001**
	132,586					
Treatment						
Quinine	8,196	5.7%[5.11-6.29]	1.00		1.00	
DHP	13,255	5.8%[5.41-6.19]	0.994[0.88-1.12]	0.926	**0.50[0.38-0.65]**	**0.000**
Primaquine						
No	9,954 (40%)	5.4%[5.01-5.79]	1.00		1.00	
Yes	14,956 (60%)	5.8%[5.41-6.19]	1.07	0.225	1.07[0.94-1.22]	0.307
	24,910					

4.3.3 Effect of anaemia on the risk of representation with *P. vivax*

Among patients presenting to the hospital on their first visit, 40,299 (30.4%) had their haemoglobin levels measured. Among these patients, 12,613 patients (31.3%) had no anaemia (Hb ≥ 12.0 mg/dl, both sexes) whereas 6,993 (17.4%) had severe anaemia (Hb ≤ 7.0 mg/dl). Patients presenting with mixed infections were at greatest risk of developing anaemia compared to malaria negative individuals (OR 7.16, 95% CI 6.43 - 7.97, p<0.001), followed by patients with *P. falciparum* (OR 5.21, 95% CI 4.93-5.52, p<0.001) and *P. vivax* (OR 4.34, 95% CI 4.02-4.68). Children were also at a higher risk of presenting with anaemia compared to adults (1-5 year age group OR 1.42, 95% CI 1.35 - 1.50, p<0.001 and 5-14 years age group OR 1.32, 95% CI1.25 - 1.39, P<0.001).There was also significant variation among the sexes, with females having a higher risk compared to males (OR 1.38, 95% CI 1.31 - 1.45, p<0.001). Highland Papuans had a higher risk of presenting with anaemia compared to lowland Papuans (OR 1.28, 95% CI 1.20-1.63, p<0.001) whereas non Papuans had a lower risk (OR 0.37, 95% CI 0.34 - 0.41, p<0.001).

Overall, the risk of representation with *P. vivax* within 63 days was 5.4% (95%CI: 4.81-5.99) in anaemic patients presenting on their first visit to the hospital compared to 3% (95% CI: 3.00, 3.00) in non-anaemic patients (HR=1.37, 95% CI: 1.22-1.54; p<0.001). The risk of representation in anaemia varied according to the initial species; see Table 4.4. The risk of

representation with *P. vivax* associated with anaemia was greatest in those initially infected with *P. vivax* (HR 1.50, 95%CI: 1.25-1.81; p<0.001) or *P. falciparum* (HR =1.41, 95%CI: 1.15-1.73; p=0.001), but was not apparent in patients without malaria or those with mixed infections. The risk of anaemia in patients with *P. vivax* or *P. falciparum* remained significant even after controlling for other confounding factors determined in table 4.3; p<0.001.

Table 4.4. **Effect of anaemia on risk of representation to hospital with *P. vivax* within 63 days according to species of initial infection.** This analysis includes only each patient's first recorded presentation to hospital. n=132,586

Species	Number of presentations to hospital	Anaemia (Hb<7.0g/dl)	No. of patients	Cumulative risk (%), 95%CI	Univariate HR, 95%CI	P value
Negative	105,189 (79.3%)	Yes	3211 (3.1%)	2.4% [1.81-2.99]	1.05[0.84-1.32]	0.668
		No	101978 (96.9%)	2.3% [2.3]	1	
P. vivax	7,789 (5.9%)	Yes	936 (12.0%)	14.6% [12.25-16.95]	**1.50 [1.25-1.81]**	**<0.001**
		No	6853 (88.0%)	10.0% [9.22-10.78]	1	
P. falciparum	16,521 (12.5%)	Yes	2330 (14.1%)	5.2% [4.22-6.18]	**1.41 [1.15-1.73]**	**0.001**
		No	14191 (85.9%)	3.7% [3.31-4.09]	1	
Mix	2,463 (1.9%)	Yes	453 (18.4%)	10.4% [7.46-13.34]	1.15 [0.83-1.60]	0.402
		No	2010 (81.6%)	9.2% [7.83-10.57]	1	

4.3.4 Risk factors for representation with *P. vivax* in non-malarial patients

To assess the risk associated with other comorbidities on subsequent recurrence with *P. vivax,* the analysis was focused on the 105,189 (79%) of patients presenting with their first episode without malaria. Six infectious and five non-infectious diagnoses were assessed to address the *a priori* hypothesis that febrile illnesses would be associated with a greater risk of representation. Patients presenting with trauma and thus unlikely to have fever were chosen as the control group. There were significant differences in baseline characteristics between disease categories; these are presented in Table 4.5. Trauma patients were more likely to be

older with 75% (16,658/22,300) adult patients compared to measles and malnutrition where only 7% (n=40) and 4% (n=43) of patients were aged greater than 15 years respectively. Patients with urinary tract infections, anaemia and malignancy were more likely to be females (68%, 60% and 58% respectively) whereas patients with trauma, burns, TB and pneumonia were more likely to be males (66%, 57%, 56% and 57% respectively). More than 80% of patients selected were out-patients except in anaemia, malnutrition and pneumonia where the number of in-patients was higher (table 4.5). The number of measles cases showed peaks in 2005, 2009 and 2011 which were anecdotally associated with outbreaks of measles in the community.

Table 4.5. **Distribution of baseline characteristics in patients presenting with non-malarial illnesses.** These patient groups exclude those known to have co-infection with malaria

Infectious conditions

Factors	Measles	UTI	Gastroenteritis	Respiratory TB	Pneumonia	Acute URTI
Source						
OP	511 (87.7%)	7899 (89.7%)	15,047 (84.1%)	7,599 (86.3%)	5,803 (68.6%)	41,449 (99.4%)
IP	72 (12.3%)	907 (10.3%)	2,836 (15.9%)	1,204 (13.7%)	2,655 (31.4%)	266 (0.6%)
Total	583	8,806	17,883	8,803	8,458	41,715
Age						
0-1 yr.	125 (21.4%)	99 (1.1%)	3,289 (18.4%)	336 (3.8%)	2,222 (26.3%)	7,109 (17.0%)
1-5 yrs.	342 (58.7%)	360 (4.1%)	4,679 (26.2%)	1,223 (13.9%)	2,010 (23.8%)	6,679 (16.0%)
5-14 yrs.	76 (13.0%)	535 (6.1%)	2,000 (11.2%)	1,045 (11.9%)	743 (8.8%)	5,542 (13.3%)
≥15 yrs.	40 (6.9%)	7,812 (88.7%)	7,906 (44.2%)	6,199 (70.4%)	3,476 (41.1%)	22,377 (53.7%)
Total	583	8,806	17,874	8,803	8,451	41,707
Gender						
Male	289 (49.6%)	2,809 (31.9%)	9,177 (51.3%)	4,893 (55.6%)	4,794 (56.7%)	21,177 (50.8%)
Female	294 (50.4%)	5,997 (68.1%)	8,706 (48.7%)	3,910 (44.4%)	3,664 (43.3%)	20,538 (49.2%)
Total	583	8,806	17,883	8,803	8,458	41,715
Ethnicity						
Highland Papuan	432 (74.2%)	5,497 (62.6%)	13,176 (73.8%)	6,192 (70.4%)	6,035 (71.4%)	28,728 (69.1%)
Lowland Papuan	74 (12.7%)	1,429 (16.3%)	2,334 (13.1%)	1,756 (20.0%)	1,394 (16.5%)	6,385 (15.4%)
Non Papuan	76 (13.1%)	1,855 (21.1%)	2,336 (13.1%)	842 (9.6%)	1,020 (12.1%)	6,479 (15.6%)
Total	582	8,781	17,846	8,790	8,449	41,592
Year						
2004	29 (5.0%)	505 (5.7%)	2,254 (12.6%)	1,239 (14.1%)	1,263 (14.9%)	5,626 (13.5%)
2005	108 (18.5%)	995 (11.3%)	2,487 (13.9%)	1,678 (19.1%)	1,424 (16.8%)	5,517 (13.2%)
2006	49 (8.4%)	1,153 (13.1%)	3,049 (17.0%)	1,170 (13.3%)	1,106 (13.1%)	5,858 (14.0%)
2007	8 (1.4%)	1,441 (16.4%)	2,439 (13.6%)	1,202 (13.7%)	1,023 (12.1%)	5,599 (13.4%)
2008	10 (1.7%)	1,377 (15.6%)	2,123 (11.9%)	1,062 (12.1%)	769 (9.1%)	5,069 (12.2%)
2009	167 (28.6%)	1,427 (16.2%)	2,163 (12.1%)	1,010 (11.5%)	936 (11.1%)	5,482 (13.1%)
2010	11 (1.9%)	1,118 (12.7%)	1,995 (11.2%)	895 (10.2%)	1,121 (13.3%)	4,945 (11.9%)
2011	201 (34.5%)	790 (9.0%)	1,373 (7.7%)	547 (6.2%)	816 (9.6%)	3,619 (8.7%)
Total	583	8,806	17,883	8,803	8,458	41,715

Non-infectious conditions

Factors	Trauma	Malnutrition	Malignancy	Burns	Anaemia
Source					
OP	20,501 (91.9%)	416 (39.4%)	1,361 (81.7%)	920 (88.3%)	6,486 (65.2%)
IP	1,803 (8.1%)	640 (60.6%)	305 (18.3%)	122 (11.7%)	3,459 (34.8%)
Total	22,304	1,056	1,666	1,042	9,945
Age					
0-1 yr.	276 (1.2%)	243 (1.2%)	36 (2.2%)	57 (5.5%)	862 (8.7%)
1-5 yrs.	2,057 (9.2%)	593 (56.2%)	73 (4.4%)	339 (32.5%)	1,530 (15.4%)
5-14 yrs.	3,309 (14.8%)	176 (16.7%)	84 (5.0%)	142 (13.6%)	1,212 (12.2%)
≥15 yrs.	16,658 (74.7%)	43 (4.1%)	1,473 (88.4%)	504 (48.4%)	6,338 (63.7%)
Total	22,300	1,055	1,666	1,042	9,942
Gender					
Male	14,633 (65.6%)	550 (52.1%)	698 (41.9%)	593 (56.9%)	3,954 (39.8%)
Female	7,671 (34.4%)	506 (47.9%)	968 (58.1%)	449 (43.1%)	5,991 (60.2%)
Total	22,304	1,056		1,042	9,945
Ethnicity					
Highland Papuan	12,386 (55.7%)	739 (70.0%)	830 (50.0%)	728 (69.9%)	7,723 (77.7%)
Lowland Papuan	3,775 (17.0%)	255 (24.2%)	336 (20.2%)	140 (13.4%)	1,554 (15.6%)
Non Papuan	6,086 (27.4%)	61 (5.8%)	495 (29.8%)	173 (16.6%)	663 (6.7%)
Total	22,247	1,055	1,661	1,041	9,940
Year					
2004	1,889 (8.5%)	62 (5.9%)	157 (9.4%)	49 (4.7%)	1,027 (10.3%)
2005	2,869 (12.9%)	88 (8.3%)	200 (12.0%)	101 (9.7%)	1,375 (13.8%)
2006	3,796 (17.0%)	100 (9.5%)	194 (11.6%)	138 (13.2%)	1,155 (11.6%)
2007	3,420 (15.3%)	161 (15.2%)	246 (14.8%)	147 (14.1%)	1,399 (14.1%)
2008	3,154 (14.1%)	177 (16.8%)	212 (12.7%)	151 (14.5%)	1,222 (12.3%)
2009	2,522 (11.3%)	225 (21.3%)	223 (13.4%)	156 (15.0%)	1,758 (17.7%)
2010	2,560 (11.5%)	140 (13.3%)	240 (14.4%)	169 (16.2%)	1,192 (12.0%
2011	2,094 (9.4%)	103 (9.8%)	194 (11.6%)	131 (12.6%)	817 (8.2%)
Total	22,304	1,056	1,666	1,042	9,945

Overall, 2.3% (2,374/105,189) of patients with non-malarial diagnoses represented with *P. vivax* malaria within 63 days and 6% (n=6,260) within 1 year. Baseline characteristics which could potentially confound the relationship between the non-malarial diagnosis and the risk of representing with *P. vivax* are presented in Table 4.6. In general these were similar to the risks derived from the whole dataset (Table 4.3), with the exception of anaemia which was not a risk factor in non-malarial patients.

Table 4.6. **Effect of baseline factors on risk of representation to hospital with *P. vivax* within 63 days in non-malarial presentations**. This analysis includes only each patient's first recorded presentation to hospital, excluding those presenting on their first visit with malaria. n=105,189

Factors	Number of presentations to hospital	Cumulative risk of representation with *P. vivax* in next 63 days %, 95% CI	Univariate HR, 95%CI	p value
Age				
0-1 yr.	13,776 (13.1%)	2.2%[2.00-2.40]	**0.88 [0.80-0.97]**	**0.007**
1-5 yrs.	11,259 (10.7%)	3.1%[2.71-3.50]	**1.24 [1.14-1.36]**	**<0.001**
5-14 yrs.	10,065 (9.6%)	2.8%[2.41-3.19]	1.09 [0.99-1.20]	0.088
≥15 yrs.	70,066 (66.6%)	2.1%[1.90-2.30]	1.00	
Gender				
Male	54,929 (52.2%)	2.1%[1.90-2.30]	1.00	
Female	50,260 (47.8%)	2.5%[2.30-2.70]	**1.18[1.09-1.28]**	<0.001
Ethnicity				
Highland Papuan	52,448 (50.1%)	4.0%[3.80-4.20]	**3.13[2.90-3.38]**	<0.001
Lowland Papuan	19,209 (18.4%)	0.7%[0.50-0.90]	**0.58[0.51-0.65]**	<0.001
Non Papuan	33,016 (31.5%)	0.7%[0.70]	1.00	
Source				
OP	88,131 (83.8%)	2.5%[2.30-2.70]	**1.84[1.61-2.11]**	<0.001
IP	17,058 (16.2%)	1.4%[1.20-1.60]	1.00	
Year				
2004	21,201 (20.2%)	1.4%[1.20-1.60]	1.00	
2005	15,907 (15.1%)	1.7%[1.50-1.90]	**0.693 [0.62-0.78]**	**<0.001**
2006	15,200 (14.5%)	2.0%[1.80-2.20]	**0.811 [0.73-0.90]**	**<0.001**
2007	13,629 (13.0%)	2.4%[2.20-2.60]	0.994 [0.90-1.12]	0.909
2008	11,774 (11.2%)	2.4%[2.20-2.60]	1.005 [0.90-1.12]	0.935
2009	9,923 (9.4%)	4.0%[3.80-4.20]	**1.65 [1.50-1.82]**	**<0.001**
2010	9,738 (9.3%)	3.2%[2.81-3.59]	**1.31 [1.18-1.46]**	**<0.001**
2011	7,817 (7.4%)	3.4%[3.01-3.79]	**1.41 [1.25-1.58]**	**<0.001**
Anaemia				
No	101,978 (96.9%)	2.3%[2.30]	1.00	
Yes	3,211 (3.1%)	2.4%[1.81-2.99]	1.05[0.84-1.32]	0.668

The risks of representation to the hospital with *P. vivax* in the selected infectious and non-infectious conditions are presented in Table 4.7. The cumulative risk of representing to the hospital with *P. vivax* at 63 days was 2.6%, (95%CI: 2.40-2.80) in the non-febrile control group of patients with trauma. Among the febrile conditions, the risk was highest in patients with measles (10.4%, 95%CI: 7.85-12.95), whereas in the non-febrile group, the risk of

representation with *P. vivax* was greatest in patients with burns (7.6%, 95%CI: 6.03-6.17) and malnutrition (7.5%, 95%CI: 5.93-9.07). All patients with febrile diseases had statistically significantly higher hazards for representation compared to trauma, the greatest risk being in patients with measles (HR=2.02, 95%CI: 1.77-2.32; p <0.001) (Table 4.7). In the non-febrile group, patients with malnutrition were at greatest risk (HR= 2.95, 95%CI: 2.32-3.74; p<0.001) with burns patients also at significantly higher risk (HR 1.25, 95%CI: 1.19-1.31; p<0.001).

Table 4.7. **Risk of representation with *P. vivax* within 63 days following different medical diagnoses in patients presenting with a non-malarial illness.** These patient groups exclude those known to have co-infection with malaria

Diagnosis	Number of presentations to hospital	Cumulative Risk of representation with *P. vivax* within 63 days %, 95% CI	Hazard Ratio (HR) (unadjusted)	P value	Adjusted Hazard Ratio(AHR) (adjusted for age, gender, ethnicity, year, source)	P value
Trauma (control)	22,304	2.6%[2.40-2.80]	1.00		1.00	
Measles	583	10.4%[7.85-12.95]	2.02[1.77-2.32]	<0.001	1.07 [0.91-1.24]	0.432
UTI	8,806	4.2%[3.81-4.59]	1.17 [1.12-1.23]	<0.001	**1.14 [1.09-1.19]**	**<0.001**
Gastroenteritis	17,883	5.8%[5.41-6.19]	1.23[1.20-1.26]	<0.001	**1.08 [1.05-1.11]**	**<0.001**
Pneumonia	8,458	5.4%[5.01-5.79]	1.16 [1.13-1.19]	<0.001	1.025 [0.99-1.06]	0.111
Respiratory TB	8,803	3.8%[3.41-4.19]	1.05 [1.03-1.07]	<0.001	**1.02[1.00-1.04]**	**0.041**
Acute URTI	41,715	5.2%[5.00-5.40]	1.08 [1.07-1.09]	<0.001	**1.05 [1.04-1.06]**	**<0.001**
Malnutrition	1,056	7.5%[5.93-9.07]	2.95 [2.32-3.74]	<0.001	1.07 [0.79-1.45]	0.663
Malignancy	1,666	2.9%[2.12-3.68]	1.04 [0.94-1.15]	0.489	1.08 [0.98-1.20]	0.143
Burns	1,042	7.6%[6.03-6.17]	1.25 [1.19-1.31]	<0.001	**1.09 [1.03-1.14]**	**0.001**
Anaemia	9,945	5.5%[5.11-5.89]	1.09 [1.08-1.11]	<0.001	**1.03 [1.02-1.05]**	**<0.001**

In view of the significant differences in baseline characteristics between diagnostic categories, the risk of representation with *P. vivax* was determined after controlling for other known factors in a multivariate model. Age, gender, ethnicity, source and year of presentation were

included in the model. After accounting for these factors, only urinary tract infection (AHR 1.14, 95% CI: 1.09-1.19; p <0.001), gastroenteritis (AHR 1.08, 95%CI: 1.05-1.11; p<0.001), acute upper respiratory tract infection (AHR 1.05, 95%CI:1.04-1.06; p<0.001**)** and respiratory TB (AHR 1.02, 95% CI: 1.00-1.04; p=0.041) showed statistically significant increased risk compared to the control group of trauma patients. Among non-febrile conditions, the risk of representation was significantly, greater in patients with burns (AHR 1.09, 95% CI: 1.03-1.14; p=0.001) and anaemia (AHR 1.03, 95%CI: 1.02-1.05; p<0.001).

4.3.5 Risk of representation with *P. vivax* compared with *P. falciparum*

Our *a priori* hypothesis was that fever would increase the risk of activation of hypnozoites. If true, one would expect that fever would result in higher rates of *P. vivax,* but not *P. falciparum* reinfection and that the risk would be greatest in those infectious conditions where the grade of fever was highest. However the risk of representation with *P. vivax* was similar to that with *P. falciparum* across the medical diagnoses (see table 4.8 and 4.9). The AHR was similar in both groups at 63 days. A sub group analysis was conducted in children aged 1 to 5 years (table 4.9), a group known to be at highest risk of representation. Young children presenting with burns were at significantly greater risk of *P. vivax* (AHR 1.09 (95%CI 1.02-1.17; p=0.016) compared to children in the same group presenting with trauma; although this risk was similar for *P. falciparum* representation (AHR=1.02) this did not reach statistical significance. Anaemia in the 1-5 year age group was associated with significantly greater risk of representing within 63 days with either *P. vivax* (AHR 1.04, 95%CI: 1.01-1.07; p=0.003) or *P. falciparum* (AHR 1.05, 95%CI: 1.02-1.08; p=0.001) compared to children in the same age group presenting with trauma.

Table 4.8. **Risk of representation with parasitaemia within 63 days. All patients.** These patient groups exclude those known to have co-infection with malaria. The AHR represent the risk of representation at 63 days compared to the control group of patients presenting with trauma after controlling for age, gender, ethnicity, source and stratifying for year of presentation.

	Cumulative risk of representation with *P. vivax* within 63 days	**AHR for representation with *P. vivax* at 63 days**		**Cumulative risk of representation with *P. falciparum* within 63 days**	**AHR for representation with *P. falciparum* at 63 days**	
	All patients	**All patients**		**All patients**	**All patients**	
Diagnosis		**AHR**	**P value**		**AHR**	**P value**
Trauma	2.6%[2.40-2.80]	1		3.9%[4.10-3.70]	1	
Measles	10.4%[7.85-12.95]	1.07[0.91-1.24]	0.432	6.2%[4.24-8.16]	1.02[0.84-1.24]	0.842
UTI	4.2%[3.81-4.59]	**1.14[1.09-1.19]**	**<0.001**	6.20%[5.61-6.79]	**1.12[1.07-1.16]**	**<0.001**
Gastroenteritis	5.8%[5.41-6.19]	**1.08[1.05-1.11]**	**<0.001**	6.2%[5.81-6.59]	**1.08[1.05-1.11]**	**<0.001**
Acute URTI	5.2%[5.00-5.40]	**1.05 [1.04-1.06]**	**<0.001**	6.40%[6.20-6.60]	**1.05[1.04-1.05]**	**<0.001**
Respiratory TB	3.8%[3.41-4.19]	**1.02[1.00-1.04]**	**0.041**	5.20%[4.81-5.59]	1.01[1.00-1.03]	0.131
Burns	7.6%[6.03-6.17]	**1.09[1.03-1.14]**	**0.001**	6.80%[5.23-8.37]	**1.06[1.01-1.12]**	**0.023**
Anaemia	5.5%[5.11-5.89]	**1.03[1.02-1.05]**	**<0.001**	6.90%[6.31-7.49]	**1.04[1.02-1.05]**	**<0.001**

Table 4.9. **Risk of representation with parasitaemia within 63 days. Age group 1-5 years.** These patient groups exclude those known to have co-infection with malaria. The AHR represent the risk of representation at 63 days compared to the control group of patients presenting with trauma after controlling for age, gender, ethnicity, source and stratifying for year of presentation.

	Cumulative risk of representation with *P. vivax* within 63 days	**AHR for representation with *P. vivax* at 63 days**		**Cumulative risk of representation with *P. falciparum* within 63 days**	**AHR for representation with *P. falciparum* at 63 days**	
	1-5 years	**1-5 years**		**1-5 years**	**1-5 years**	
Diagnosis		**AHR**	**P value**		**AHR**	**P value**
Trauma	7.10%[5.92-8.28]	1		5.2%[4.22-6.18]	1	
Measles	13.70%[9.98-17.42]	1.14[0.94-1.38]	0.186	6.7[3.96-9.44]	1.05[0.81-1.36]	0.727
UTI	10.50%[7.17-13.83]	1.10[0.97-1.24]	0.148	5.30%[2.95-7.65]	0.99[0.84-1.18]	0.940
Gastroenteritis	8.50%[7.72-9.28]	1.03[0.98-1.08]	0.239	6.3%[5.52-7.08]	1.00[0.94-1.06]	0.984
Acute URTI	6.70%[6.11-7.29]	0.99[0.97-1.02]	0.578	6.30%[5.71-7.29]	1.01[0.99-1.04]	0.417
Respiratory TB	7.40%[5.83-8.97]	0.99[0.95-1.02]	0.465	6.80%[5.43-8.17]	1.00[0.96-1.04]	0.981
Burns	**13.1%[9.86-16.82]**	**1.09[1.02-1.17]**	**0.016**	7.40%[4.46-10.34]	1.02[0.93-1.12]	0.619
Anaemia	**12.5%[10.74-14.26]**	**1.04[1.01-1.07]**	**0.003**	10.50%[8.93-12.07]	**1.05[1.02-1.08]**	**0.001**

4.4 Discussion

The municipality of Timika presents a unique setting for the study of malaria. The presence of a multi-ethnic population with varying degrees of anti-malarial immunity and the existence of all malarial species except *P. knowlesi* coupled with abundance of Anopheles mosquito vectors have resulted in a significant burden of disease.[57] The analyses of hospital surveillance data from Timika highlight several important findings. Firstly, as expected, patients presenting to the hospital with *P. vivax* infection (either alone or mixed) were at two fold greater risk of representing within 63 days than patients presenting initially without malaria. Interestingly, patients infected with P. *falciparum* were also at higher risk (HR 1.30, 95%CI: 1.24-1.36) of representation with *P. vivax* within 63 days. In addition to the species of infection, patients with severe anaemia (Hb<7.0g/dl) were at a higher risk of early representation with *P. vivax* malaria and this was apparent in patients with either *P. vivax* or *P. falciparum* infections. In areas with a high malaria burden, it is likely that severe anaemia is a proxy indicator of repeated malarial infections, thus, predisposing towards a higher *P. vivax* hypnozoite load compared to other patients thereby increasing their risk of future relapses.

Patients with *P. vivax* parasitaemia have clearly had prior exposure to sporozoite infected mosquitos and are thus most likely to harbour hypnozoites. However, individuals presenting to the hospital with malaria are likely to be at greater risk of transmission of *Plasmodium*, either due to the geographical location where they reside or work, or due to their socioeconomic circumstances. Hence patients with known infection with *P. falciparum* are more likely to have had prior infection with *P. vivax*, and thus harbour dormant hypnozoites. Alternatively these patients might have acquired *P. vivax* around the same time as the falciparum infection, but it might have been present at low levels and therefore missed by microscopic blood film examination.

Acquired immunity against *P. vivax* malaria develops quickly in regions with high vivax endemicity.[146 147] In our study, children were at 1.3 fold higher risk, in keeping with a greater risk of symptomatic disease. It is possible that some patients with homologous infection will have developed sufficient immunity to remain asymptomatic, and they would either not present to a clinic or if they did present with another illness may not have had a blood film examination. This would account for why adult patients were only three fourth as likely as children to have a symptomatic recurrence of *P. vivax*. Patients from the Papuan highlands were also at greater risk possibly reflecting lower acquired immunity from decreased lifelong exposure. Gender was a significant factor influencing early representation with *P. vivax* in this study, with females having higher risk compared to men. This difference has been previously described in studies which demonstrate a higher risk of infection and severity of *P. vivax* in females, even in early childhood, but pronounced in adulthood.[36 148] Several theories have been proposed for this difference including hormonal differences, lower starting haemoglobin in females and difference in treatment seeking behaviour but none of these theories are an adequate explanation for the gender differences observed in early childhood.[36]

One of the primary aims of this analysis was to test the hypothesis that high grade fever increases the risk of *P. vivax* relapse. Febrile illnesses in our analysis did indeed increase the risk of representation in our study population, but after controlling for potential confounders, the risk was modest and similar for recurrence in *P. vivax* and *P. falciparum*.

It is possible that these correlates could be due to residual confounding by factors such as place of residence and or socioeconomic factors which could not be controlled for in our multivariate analysis. If febrile illnesses increased the risk of relapse then the proportion of patients representing would be expected to be much higher in *P. vivax* than *P. falciparum*; which was not the case in our analysis. Hence although our findings identify certain disease

entities at slightly greater risk of recurrence they do not support the hypothesis of an increased risk of relapse due to non-malarial fevers.

This study of patient presentations to RSMM over seven years has some significant strengths. The large sample size used in this study lends strong statistical validity to the analyses and it is unlikely that the estimates of risk for representation are due to chance variation. The use of high quality microscopy services at RSMM and strict hospital policy actively discouraging clinical diagnosis of malaria have increased the accuracy of parasitological diagnosis in this study. Moreover, the use of routine hospital surveillance data allows the estimation of risk in a real world setting compared to the controlled setting of a clinical trial.

However, the use of routine surveillance from one hospital as in this study also has several limitations. It is possible that a significant amount of attrition may have occurred and that patients with recurring symptoms sought cure from other health providers, especially in the private setting. It is also likely that some degree of non-differential misclassification may occur during routine surveillance which would dilute any associations towards the null. In particular, the diagnoses of non-malarial illnesses were predominantly made clinically with few corroborating laboratory findings. The categories chosen for the study were therefore chosen to represent illnesses with clear clinical scenarios and thus more likely to have robust diagnosis. The surveillance data does not include several important variables such as place of residence which may influence recurrence of *P. vivax* malaria and body weight which may determine adequacy of treatment. In addition, the use of trauma patients as a control group for patients presenting with non-malarial illnesses may have been incorrect considering that it may be an inappropriate control for conditions like measles and malnutrition which were predominantly diagnosed in children. Moreover, major trauma can lead to a stress response which could theoretically increase the risk of relapse by reactivating hypnozoites. This study

only identified patients coming back to the hospital with symptomatic recurrences but it is likely that a substantial proportion of recurrences in this population are asymptomatic. Another potential confounding factor in patients presenting with infection due to different species of malaria was the variation in the prescription of antimalarial treatment which varied widely between treatment groups. However, the differences in risk associated with species of infection remain after controlling for antimalarial treatment. Furthermore, this analysis and previous unpublished studies have shown no difference in the risk for *P. vivax* recurrence in patients receiving primaquine compared to patients receiving no primaquine.

In summary, the results of this study provide evidence to support the focused use of primaquine for radical cure in patients presenting with *P. vivax,* but suggest that patients presenting with *P. falciparum,* particularly those who are anaemic, also constitute an important population at increased risk who should be screened and offered radical cure if no clear contraindications exist. However, as discussed in chapter 3 the benefits of radical cure need to be weighed against the risks of adverse effects, most importantly that of haemolysis which is especially high in G6PD deficient patients. Presently, in the absence of a cheap and effective bedside test for G6PD deficiency, the best option may be to adopt the use of effective blood schizontocidal drugs with slow elimination such as dihydroartemisinin-piperaquine which will prevent or delay initial relapses of the Chesson strain of *P. vivax* prevalent in the region as shown in this study. This will result in a substantial reduction of morbidity, especially in young children who are most at risk of symptomatic *P. vivax* infection.

Chapter 5

5 General Discussion & Conclusion

Malaria due to *P. vivax* has often been neglected in malaria control programs across the world due to the perception that the disease course is benign in comparison to *P. falciparum* malaria. However, as discussed in previous chapters, control of *P. vivax* malaria is key to the elimination of malaria. In particular, the ability of *P. vivax* to relapse and the high prevalence of asymptomatic parasitaemia in high endemicity settings makes control of *P. vivax* a difficult challenge.

5.1 Variation in relapse interval in *P. vivax*

One of the major challenges in *P. vivax* malaria is that relapse occurs at varying intervals after primary infection in different parts of the world (Figure 5.1). This has been attributed to several factors, one of which is the existence of various strains of *P. vivax,* some relapsing earlier than others. Classically, the strains of *P. vivax* have been classified according to the geographical region of origin and intervals to primary infection and relapse. The temperate strains, best characterized by the St. Elizabeth strain in USA, had a short prepatency period of 9-16 days and early primary infections followed by late relapses several months after the original infection.[59] In contrast, tropical strains like the Chesson strain of New Guinea and the Madagascar strain led not only to early primary infections but also to early relapses, usually within a few weeks of the primary infection.[59] This classification held true for most strains until the discovery of several South American strains which, despite being in the tropical region geographically, demonstrated characteristics of the temperate group.[149] In USSR, Nikolaev in 1949 proposed a new subspecies of *P. vivax*, *P. vivax* hibernans, which had

a long prepatency period and relapsed after very long latent periods.[150] This was similar to the characteristics seen in several Northern European strains prevalent in the area which have been reported in literature since the early 1900s. In 1969, in an attempt at classification of the different strains, a WHO report described strains of *P. vivax* as belonging to three types.[150] Type I was characterized by short incubation period of 12-20 days, frequent relapses and no prolonged latent period. Type II strains also had a short incubation period similar to Type I strains but a prolonged period of latency to first relapse or had series of relapses at short intervals. Type III stains were characterized by a prolonged incubation period of 6 months or more prior to the primary attack followed by a series of relapses at short intervals and then by a second period of latency and subsequent relapses.

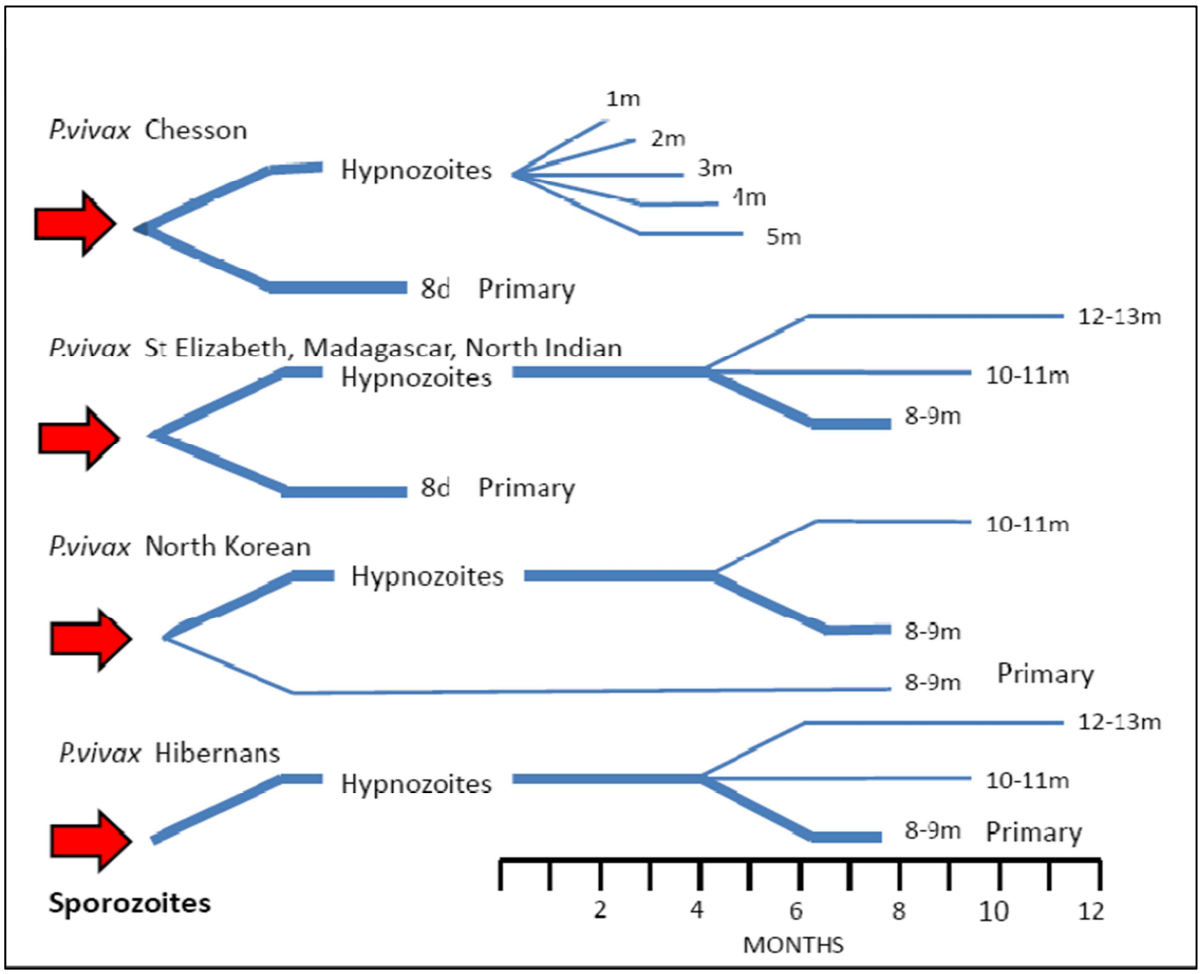

Figure 5.1. **Relapse intervals in different strains of *P. vivax*.** From White, N. (2011). "Determinants of relapse periodicity in *Plasmodium vivax* malaria." Malaria Journal 10(1): 297. Reproduced with permission.

Another important factor in the variation of relapse interval is the difference in sporozoite load. In the first few decades of the 1900s, the St. Elizabeth strain of *P. vivax* was used for artificial inoculation studies to test the ability of different drugs to treat malaria in the United States. Another strain popular in Europe was the Madagascar strain, first isolated in an Indian merchant who had previously stopped in Madagascar. Both these strains were used for malaria therapy, the practice of artificially inoculating malarial parasites in patients suffering from syphilis. Malaria caused by these two strains caused early infection and relapsed 8-12 months after the primary infection. It was during these malaria therapy experiments that the importance of sporozoite load in determining timing of relapses was demonstrated. The heavier the sporozoite inoculum, the earlier relapses occurred, irrespective of the malaria strain used.[151]

Despite evidence of varying periods before relapse in different geographical regions, there is still uncertainity if the true reason of this variation is attributable to genetic differences between *P. vivax* from different locations, or simply increased sporozoite load leading to increased burden of hypnozoites and thus more frequent relapses. In the absence of non-invasive methods to confirm and quantify the existence of hypnzoites in the liver of patients, it is unlikely that this matter will be conclusively resolved in the near future. Other factors have also been found to influence the interval to relapse, these include acquired immunity against the infecting genotype and the post prophylaxis effect of anti-malarial drugs used in treatment.[59]

5.2 Trigger factors for *P. vivax* relapses

The exact mechanism behind the biology of relapse remains elusive and it is likely that re-awakening of hypnozoites is governed by multiple influences. The factors triggering *P. vivax* relapse are poorly understood. Schmidt proposed that after invasion of host hepatocytes,

some sporozoites are pre-programmed to re-awaken at certain periods in time.[152] [153] These periods coincide with seasons of increased breeding of the vector, the *Anopheles* mosquito, and therefore results in increased chances of survival and transmission of the parasite.[154] [155] A related hypothesis put forward by Hulden *et al* proposed that certain protein factors in the saliva of the feeding *Anopheles* mosquito, when injected into the host trigger signalling pathways which activate dormant hypnozoites.[154] This is supported by malaria data collected in Finland which showed a correlation between seasonal malaria cases and hatching of uninfected *Anopheles* females.[156] An *Anopheles* bite triggered relapse, the authors say would "ensure the survival of the parasite through a vector free season, allow effective dispersal to new areas and provide better possibilities for the parasite to reach a vector".[154]

Current evidence from genotyping studies suggest that the parasite populations responsible for relapses following primary infection are different from the *P. vivax* populations causing the primary infection. The 'activation of latent hypnozoite' hypothesis proposes that sporozoites from previous *P. vivax* infections remain dormant in the liver and are re-activated as a result of a new infection. The high risk of *P. vivax* recurrence demonstrated following pure *P. falciparum* infection and treatment with effective blood schizontocidals as demonstrated in Chapter 3 lends credence to the possibility that in areas of moderately high *P. vivax* endemicity (such as Southeast Asia, Oceania and India), hypnozites from previous asymptomatic infections lie dormant in the livers of a large proportion of the population, primed to re-activate through a yet unknown mechanism. One of the mechanisms proposed for this activation has been the systemic illness associated with the primary *P. falciparum* infection.[59] If this is true, it is possible that significant systemic derangement due to other high grade febrile illness could also lead to activation of hypnozoites. This hypothesis was the focus of Chapter 4 of this thesis but our analysis of hospital surveillance data from Timika did not demonstrate an increased risk of relapse after non-malarial illnesses. It is

likely that the results may have been subject to confounding by factors outside the surveillance dataset which could not be controlled. This hypothesis needs to be tested using a prospective cohort in controlled settings wherein factors like age group, body weight, treatment, place of residence and adherence to treatment are uniform and residual confounding be minimized to as little as possible.

5.3 Strategies for improving radical cure in *P. vivax*

There are very few anti-hypnozoitocidal agents in the pipeline. Bulaquine and Tafenoquine are the only candidates, both belonging to the same class of 8-aminoquinolines as primaquine.[76] Bulaquine is a rapidly cleaved enamine prodrug of primaquine which is currently available only in India, with an efficacy and safety profile similar to primaquine.[157] Tafenoquine is another 8-aminoquinoline agent in development with a longer half-life effective against hypnozoites, but at best is unlikely to become available for at least 5-10 years.[158] The main concern with 8-aminoquinolines is their propensity to cause haemolysis, with G6PD deficient individuals being at greater risk of life threatening haemolysis. An alternative regimen of 8 weekly doses of 45mg primaquine (0.75mg/kg) has been recommended by the WHO for individuals with mild to moderate G6PD deficiency while primaquine is contraindicated in severe G6PD deficiency. The effects of low dose primaquine (15mg/day for 14 days) in G6PD deficient patients range from no adverse effects in individuals with mild deficiency[159] such as the G6PD A^- variant prevalent in Africans to life threatening intravascular haemolysis, acute cardiac failure, renal failure and death in patients with severe G6PD deficiency such as the Mediterranean variant B^- or Asian variants.[160] The potential to cause haemolysis limits the use of higher doses in patients and prevents the use of primaquine in mass drug administrations as a public health tool to reduce the burden of *P. vivax* malaria in the population. The development of cheaper and highly

sensitive kits to identify G6PD deficiency in the field will enable the wider use of primaquine. Future efforts should be directed at identifying pharmaceutical agents outside the 8-aminoquinoline class effective against hypnozoites.

The literature review on primaquine highlights the variation in hypnozoitocidal treatment and its effectiveness across the world. Presently, there are only a handful of studies using high dose primaquine with long term follow up to support the recommendation for high dose primaquine. Even when a higher dose is used, adherence to treatment ultimately decides drug effectiveness. Unpublished reports from Papua demonstrate no benefit of unsupervised primaquine at a dose of 0.25 mg/kg/day or 0.5 mg/kg/day in reducing representation with vivax malaria at 365 days. Shorter high dose primaquine regimens delivering same total dosage as the standard 14 day regimen could ensure higher rates of completion of treatment and thereby, efficacy of radical treatment.[161] Strategies such as directly observed treatment and community based monitoring of treatment also have the potential to improve adherence as demonstrated in Chapter 2 and need to be explored individually for each region since local factors will play an important role in its successful implementation. Efforts to increase adherence through increased awareness, patient education and indirect supervision should be undertaken alongside attempts to develop newer drugs with slower elimination to replace primaquine.

One of the primary objectives of this thesis was to identify particular patient groups at greatest risk of recurrent *P. vivax* so as to target the deployment of primaquine radical cure. Three groups could be identified – children aged 1 to 5 years old, those with *P. vivax* or *P. falciparum* and those with severe anaemia. The number of people in these groups in the current study and the recurrences hypothetically preventable assuming a fully effective radical cure is presented in table 5.1. Targeting radical cure in all children in the 1-5 year age group and patients presenting with *P. falciparum* malaria would prevent 17% and 15% of all

recurrences within 63 days respectively in patients who otherwise would not have been treated but would come at the expense of treating an additional 13-15,000 in each case who would not have recurrent *P. vivax* infection within 63 days. Narrowing the targeted group to patients presenting with *P. falciparum* and severe anaemia in the 1-5 age group would decrease the yield of recurrences prevented but would also narrow down the number of unnecessary treatments significantly.

Table 5.1. **Targeting high risk groups for radical cure**

Targeted Group	Number to treat	No. with *P. vivax* within 63 days	% with *P. vivax* within 63 days	If all treated, % of all recurrence prevented	Number of people without recurrence needed to be tested
All	132,586	3,998	3.0%	100%	128,588
All Age 1-5	14,630	685	4.7%	17.0%	13,945
***P. vivax* , >1yr old**	7,259	688	9.5%	17.2%	6,571
P. falciparum	16,521	599	3.6%	15.0%	15,922
***P. falciparum* + Age 1-5**	1801	101	5.6%	2.5%	1,700
***P. falciparum* + Severe anaemia**	2330	113	4.9%	2.8%	2,217
***P. falciparum* + Severe anaemia + Age 1-5**	500	28	5.6%	0.7%	472
***P. falciparum* + Severe anaemia + Age 1-15**	968	50	5.2%	1.3%	918

In conclusion, there is a need to improve current strategies for radical cure of *P. vivax*. Shortening radical cure regimens to ensure greater adherence and widening the target of radical cure to include high risk groups as identified in this study would be some initial steps which would work towards achieving this goal. The development of an accurate and cost effective G6PD screening test would enable the use of higher primaquine dosages to be administered in shorter regimens for radical cure. It would also enable the use of other 8-aminoquinoline drugs like tafenoquine which also have the propensity to cause life threatening haemolysis in G6PD deficient patients. Ultimately, newer drugs outside the 8-aminoquinoline class will need to be discovered for radical cure. The pharmaceutical, medical and political communities will need to work together to ensure that the control of *P. vivax*, a

disease which puts greater than 40% of the world's population at risk remains a priority for the present and future.

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7 Appendix

7.1 Table T1 Complete list of all articles included in the analysis in Chapter 2

7.2 Table T2 Study design of articles included in the analysis in Chapter 2

7.3 Table T3 Recurrence rates reported for all primaquine treatment arms in Chapter 2

7.4 Table T4 Severe adverse events reported in Chapter 2

Supplementary Table 1: Study References

Authors	Year	Title	Journal	Volume	Issue	Pages	Reason for Exclusion
Edgcomb et. al.	1950	Primaquine, SN 13272, a new curative agent in vivax malaria; a preliminary report	Journal National Malaria Society	9	4	285-92	Full text not available
Alving et. al.	1953	Korean vivax malaria. II. Curative treatment with pamaquine and primaquine	The American journal of tropical medicine and hygiene	2	6	970-6	
Coatney et. al.	1953	Korean vivax malaria. V. Cure of the infection by primaquine administered during long-term latency	The American journal of tropical medicine and hygiene	2	6	985-8	
Cooper et. al.	1953	Studies in human malaria. XXXI. Comparison of primaquine, isopentaquine, SN-3883, and pamaquine as curative agents against Chesson strain vivax malaria	The American journal of tropical medicine and hygiene	2	6	949-57	
Di Lorenzo et. al.	1953	Korean vivax malaria. IV. Curative effect of 15 milligrams of primaquine daily for 7 days	The American journal of tropical medicine and hygiene	2	6	983-4	
Singh et. al.	1953	Preliminary studies on 8-aminoquinolines	Indian journal of malariology	7		289-94	Full text not available
Thaeler et. al.	1953	A clinical study of primaquine (S. N. 13,272) in the treatment of malaria among the Miskito Indians of Nicaragua	The American journal of tropical medicine and hygiene	2	6	989-99	
Singh et. al.	1954	Antirelapse treatment with primaquine and pyrimethamine	Indian journal of malariology	8	2	127-36	Full text not available
Alving et. al.	1955	Potentiation of the curative action of primaquine in vivax malaria by quinine and chloroquine	The Journal of laboratory and clinical medicine	46	2	301-6	
Alving et. al.	1960	Mitigation of the haemolytic effect of primaquine and enhancement of its action against exoerythrocytic forms of the Chesson strain of Piasmodium vivax by intermittent	Bulletin of the World Health Organization	22		621-31	
Basavaraj et. al.	1960	Observations on the treatment of 678 malaria cases with primaquine in an area free from maiaria transmission in Mysore State, India	Indian journal of malariology	14		269-81	
Diwan et. al.	1962	Primaquin Trials as Antirelapse (Radical) Treatment for Malaria conducted in National Malaria Eradication Programme Units (East) Meerut and Mathura (Uttar Pradesh)	Bulletin of the National Society of India for Malaria and Other Mosquito Borne Diseases	10	2	91-102	Full text not available
Mendoza et. al.	1963	Comparative study of two regimens of radical treatment of vivax malaria in Mexico	WHO	527.65			
Martelo et. al.	1969	Malaria in American soldiers	Archives of internal medicine	123	4	383-7	
Fisher et. al.	1970	Malaria in soldiers returning from Vietnam. Epidemiologic, therapeutic, and clinical studies	The American journal of tropical medicine and hygiene	19	1	27-39	
Charoenlarp et. al.	1973	Relapses of vivax malaria after a conventional course of primaquine and chloroquine: report of 2 cases	The Southeast Asian journal of tropical medicine and public health	4	1	135-7	Full text not available
Contacos et. al.	1973	Five day primaquine therapy--an evaluation of radical curative activity against vivax malaria infection	The American journal of tropical medicine and hygiene	22	6	693-5	
Sharma et. al.	1973	Effectiveness of drug schedule being followed under the National Malaria Eradication Programme, India, for radical cure of vivax malaria cases.	Journal of Communicable Diseases	5		167-74	
Contacos et. al.	1974	Combined chloroquine-primaquine therapy against vivax malaria	The American journal of tropical medicine and hygiene	23	2	310-2	
Kaplan et. al.	1974	Improved therapy for Vietnam acquired vivax malaria	Military medicine	141	6	444-8	
Miller et. al.	1974	Sensitivity of four Central American strains of Plasmodium vivax to primaquine	The American journal of tropical medicine and hygiene	23	2	309-10	
Clyde et. al.	1977	Radical cure of Chesson strain vivax malaria in man by 7, not 14, days of treatment with primaquine	The American journal of tropical medicine and hygiene	26	3	562-3	
Roy et. al.	1977	Efficacy of 5-day radical treatment of P. vivax infection in Tamil Nadu	The Indian journal of medical research	65	5	652-6	

Supplementary Table 1: Study References

Authors	Year	Title	Journal	Volume	Issue	Pages	Reason for Exclusion
Saint-Yves et. al.	1977	Comparison of treatment schedules for Plasmodium vivax infections in the Solomon Islands	Papua and New Guinea medical journal	20	2	62-5	
Cedillos et. al.	1978	Field evaluation of primaquine in the control of Plasmodium vivax	The American journal of tropical medicine and hygiene	27	3	466-72	
Roy et. al.	1979	Results of 5-day course of radical treatment of Plasmodium vivax in six districts of Tamil Nadu	The Indian journal of medical research	69		939-43	
Kondrashin et. al.	1981	Comparative studies on responses to 5-day treatment with primaquine of indigenous and imported cases of P. vivax in Nepal in 1974-76	Nepal Medical Association Journal	19		06-015	Full text not available
Appavoo et. al.	1984	Results of 3-day radical treatment of Plasmodium vivax in North Arcot and South Arcot Districts of Tamil Nadu	Indian journal of malariology	21	1	21-Apr	
Lapierre et. al.	1984	Drug resistance of Plasmodium falciparum and Plasmodium vivax strains in Cambodia (Cardamone Massif). Morphological characteristics of Plasmodium vivax	Medecine tropicale : revue du Corps de sante colonial	44	4	339-49	No translation available
Dixon et. al.	1985	A clinical trial of mefloquine in the treatment of Plasmodium vivax malaria	The American journal of tropical medicine and hygiene	34	3	435-7	
Harinasuta et. al.	1985	Trials of mefloquine in vivax and of mefloquine plus 'fansidar' in falciparum malaria	Lancet	1	8434	885-8	Insufficient information
Ohtomo et. al.	1987	Clinical evaluation of antimalarial regimens in Japan	Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene. Series A Medical microbiology infectious diseases virology	264	3-Apr	513-20	No translation available
Rombo et. al.	1987	Seven patients with relapses of Plasmodium vivax or P. ovale despite primaquine treatment	Tropical medicine and parasitology : official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft	38	1	49-50	No translation available
Arias et. al.	1989	Low response of Colombian strains of Plasmodium vivax to classical antimalarial therapy	Tropical medicine and parasitology : official organ of Deutsche Tropenmedizinische Gesellschaft and of Deutsche Gesellschaft	40	1	21-Mar	No translation available
Sinha et. al.	1989	Efficacy of 5 day radical treatment of primaquine in Plasmodium vivax cases at the BHEL industrial complex, Hardwar (U.P.)	Indian journal of malariology	26	2	83-6	
Singh et. al.	1990	Radical treatment of vivax malaria in Madhya Pradesh, India	Indian journal of malariology	27	1	55-6	
Tanabe et. al.	1990	Clinical evaluation of antimalarial drugs	Kansenshogaku zasshi. The Journal of the Japanese Association for Infectious Diseases	64	6	668-73	No translation available
Boulos et. al.	1991	Frequency of malaria relapse due to Plasmodium vivax in a non-endemic region (Sao Paulo, Brazil)	Revista do Instituto de Medicina Tropical de Sao Paulo	33	2	143-6	No translation available
Prasad et. al.	1991	Relapse/reinfection patterns of Plasmodium vivax infection: a four year study	The Southeast Asian journal of tropical medicine and public health	22	4	499-503	
Ronn et. al.	1993	Recurrence problems with preventive primaquine treatment in patients with malaria	Ugeskrift for laeger	155	48	3901-4	No translation available
Bunnag et. al.	1994	High dose of primaquine in primaquine resistant vivax malaria	Transactions of the Royal Society of Tropical Medicine and Hygiene	88	2	218-9	
Pukrittayakamee et. al.	1994a	Antimalarial effects of rifampin in Plasmodium vivax malaria	Antimicrobial agents and chemotherapy	38	3	511-4	
Pukrittayakamee et. al.	1994b	Blood stage antimalarial efficacy of primaquine in Plasmodium vivax malaria	The Journal of infectious diseases	169	4	932-5	
Baird et. al.	1995	Treatment of chloroquine-resistant Plasmodium vivax with chloroquine and primaquine or halofantrine	The Journal of infectious diseases	171	6	1678-82	
Jelinek et. al.	1995	Long-term efficacy of primaquine in the treatment of vivax malaria in nonimmune travelers	The American journal of tropical medicine and hygiene	52	4	322-4	
Tan-ariya et. al.	1995	Clinical response and susceptibility in vitro of Plasmodium vivax to the standard regimen of chloroquine in Thailand	Transactions of the Royal Society of Tropical Medicine and Hygiene	89	4	426-9	

Supplementary Table 1: Study References

Authors	Year	Title	Journal	Volume	Issue	Pages	Reason for Exclusion
Phillips et. al.	1996	Failure of combined chloroquine and high-dose primaquine therapy for Plasmodium vivax malaria acquired in Guyana, South America	Clinical infectious diseases : an official publication of the Infectious Diseases Society of America	23	5	1171-3	Case report
Srivastava et. al.	1996	Studies on Plasmodium vivax relapse pattern in Kheda district, Gujarat	Indian journal of malariology	33	4	173-9	
Fryauff et. al.	1997	Halofantrine and primaquine for radical cure of malaria in Irian Jaya, Indonesia	Annals of tropical medicine and parasitology	91	1	Jul-16	
Smoak et. al.	1997	Plasmodium vivax infections in U.S. Army troops: failure of primaquine to prevent relapse in studies from Somalia	The American journal of tropical medicine and hygiene	56	2	231-4	
Gogtay et. al.	1998	A 5 days primaquine regimen as anti-relapse therapy for Plasmodium vivax	Transactions of the Royal Society of Tropical Medicine and Hygiene	92	3	341	
Pinto et. al.	1998	Clinical efficacy of four schemes for vivax malaria treatment in children	Jornal de pediatria	74	3	222-7	No translation available
Soto et. al.	1998	Primaquine prophylaxis against malaria in nonimmune Colombian soldiers: efficacy and toxicity. A randomized, double-blind, placebo-controlled trial	Annals of internal medicine	129	3	241-4	
Fang et. al.	1999	Imported malaria: successful treatment of 31 patients in the era of chloroquine resistance	Journal of the Formosan Medical Association = Taiwan yi zhi	98	10	683-7	
Gogtay et. al.	1999	Efficacies of 5- and 14-day primaquine regimens in the prevention of relapses in Plasmodium vivax infections	Annals of tropical medicine and parasitology	93	8	809-12	
Li et. al.	1999	Observation on efficacy of artemether compound against vivax malaria	Zhongguo ji sheng chong xue yu ji sheng chong bing za zhi = Chinese journal of parasitology & parasitic diseases	17	3	175-7	
Looareesuwan et. al.	1999a	Chloroquine sensitivity of Plasmodium vivax in Thailand	Annals of tropical medicine and parasitology	93	3	225-30	
Looareesuwan et. al.	1999b	Atovaquone and proguanil hydrochloride followed by primaquine for treatment of Plasmodium vivax malaria in Thailand	Transactions of the Royal Society of Tropical Medicine and Hygiene	93	6	637-40	
Luxemburger et. al.	1999	Treatment of vivax malaria on the western border of Thailand	Transactions of the Royal Society of Tropical Medicine and Hygiene	93	4	433-8	
Rowland et. al.	1999	Randomized controlled trials of 5- and 14-days primaquine therapy against relapses of vivax malaria in an Afghan refugee settlement in Pakistan	Transactions of the Royal Society of Tropical Medicine and Hygiene	93	6	641-3	
Soto et. al.	1999	Double-blind, randomized, placebo-controlled assessment of chloroquine/primaquine prophylaxis for malaria in nonimmune Colombian soldiers	Clinical infectious diseases : an official publication of the Infectious Diseases Society of America	29	1	199-201	Prophylaxis study
Wilairatana et. al.	1999	Efficacy of primaquine regimens for primaquine-resistant Plasmodium vivax malaria in Thailand	The American journal of tropical medicine and hygiene	61	6	973-7	
Bergonzoli et. al.	2000	Therapeutic efficacy of different antimalarial regimens in the Costa Rica-Nicaragua border region	Revista panamericana de salud publica = Pan American journal of public health	7	6	366-70	
Kitchener et. al.	2000	Malaria in the Australian Defence Force during and after participation in the International Force in East Timor (INTERFET)	The Medical journal of Australia	173	11-Dec	583-5	Prophylaxis study
Pukrittayakamee et. al.	2000	Therapeutic responses to different antimalarial drugs in vivax malaria	Antimicrobial agents and chemotherapy	44	6	1680-5	
Schwartz et. al.	2000	Short report: a consideration of primaquine dose adjustment for radical cure of Plasmodium vivax malaria	The American journal of tropical medicine and hygiene	62	3	393-5	
Singh et. al.	2000	Emergence of chloroquine-resistant vivax malaria in south Bihar (India)	Transactions of the Royal Society of Tropical Medicine and Hygiene	94	3	327	
Villalobos-Salcedo et. al.	2000	In-vivo sensitivity of Plasmodium vivax isolates from Rond nia (western Amazon region, Brazil) to regimens including chloroquine and primaquine	Annals of tropical medicine and parasitology	94	8	749-58	
Abdon et. al.	2001	Assessment of the response to reduced treatment schemes for vivax malaria	Revista da Sociedade Brasileira de Medicina Tropical	34	4	343-8	

Supplementary Table 1: Study References

Authors	Year	Title	Journal	Volume	Issue	Pages	Reason for Exclusion
Adak et. al.	2001	Plasmodium vivax polymorphism in a clinical drug trial	Clinical and diagnostic laboratory immunology	8	5	891-4	
Buchachart et. al.	2001	Effect of primaquine standard dose (15 mg/day for 14 days) in the treatment of vivax malaria patients in Thailand	The Southeast Asian journal of tropical medicine and public health	32	4	720-6	
Dua et. al.	2001	Plasmodium vivax relapses after 5 days of primaquine treatment, in some industrial complexes of India	Annals of tropical medicine and parasitology	95	7	655-9	
Duarte et. al.	2001	Association of subtherapeutic dosages of a standard drug regimen with failures in preventing relapses of vivax malaria	The American journal of tropical medicine and hygiene	65	5	471-6	
Soto et. al.	2001	Plasmodium vivax clinically resistant to chloroquine in Colombia	The American journal of tropical medicine and hygiene	65	2	90-3	Primaquine given at end of follow up
Taylor et. al.	2001	Chloroquine/doxycycline combination versus chloroquine alone, and doxycycline alone for the treatment of Plasmodium falciparum and Plasmodium vivax malaria in	The American journal of tropical medicine and hygiene	64	5-Jun	223-8	Primaquine given at end of follow up
Valecha et. al.	2001	Comparative antirelapse efficacy of CDRI compound 80/53 (Bulaquine) vs primaquine in double blind clinical trial	Current Science	80		561-563	Details same as Adak-2001
Lacy et. al.	2002	Atovaquone/proguanil therapy for Plasmodium falciparum and Plasmodium vivax malaria in Indonesians who lack clinical immunity	Clinical infectious diseases : an official publication of the Infectious Diseases Society of America	35	9	e92-5	
Congpuong et. al.	2002	Sensitivity of Plasmodium vivax to chloroquine in Sa Kaeo Province, Thailand	Acta tropica	83	2	117-21	
Hamedi et. al.	2002	Plasmodium vivax malaria in Southeast Iran in 1999-2001: establishing the response to chloroquine in vitro and in vivo	The Southeast Asian journal of tropical medicine and public health	33	3	512-8	
Yadav et. al.	2002	Radical curative efficacy of five-day regimen of primaquine for treatment of Plasmodium vivax malaria in India	The Journal of parasitology	88	5	1042-4	
Da Silva et. al.	2003	Short course schemes for vivax malaria treatment	Revista da Sociedade Brasileira de Medicina Tropical	36	2	235-9	
Fernandopulle et. al.	2003	Efficacy of a five-day course of primaquine in preventing relapses in Plasmodium vivax malaria--a pilot study	The Ceylon medical journal	48	1	32	
Machado et. al.	2003	Correlation between Plasmodium vivax variants in Belem, Para State, Brazil and symptoms and clearance of parasitaemia	The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases	7	3	175-7	
Rajgor et. al.	2003	Efficacy of a 14-day primaquine regimen in preventing relapses in patients with Plasmodium vivax malaria in Mumbai, India	Transactions of the Royal Society of Tropical Medicine and Hygiene	97	4	438-40	
Silachamroon et. al.	2003	Clinical trial of oral artesunate with or without high-dose primaquine for the treatment of vivax malaria in Thailand	The American journal of tropical medicine and hygiene	69	1	14-Aug	
Valibayov et. al.	2003	Clinical efficacy of chloroquine followed by primaquine for Plasmodium vivax treatment in Azerbaijan	Acta tropica	88	1	99-102	
Walsh et. al.	2004	Randomized trial of 3-dose regimens of tafenoquine (WR238605) versus low-dose primaquine for preventing Plasmodium vivax malaria relapse	Clinical infectious diseases : an official publication of the Infectious Diseases Society of America	39	8	1095-103	
Hamedi et. al.	2004	Therapeutic efficacy of artesunate in Plasmodium vivax malaria in Thailand	The Southeast Asian journal of tropical medicine and public health	35	3	570-4	
Leslie et. al.	2004	Compliance with 14-day primaquine therapy for radical cure of vivax malaria--a randomized placebo-controlled trial comparing unsupervised with supervised treatment	Transactions of the Royal Society of Tropical Medicine and Hygiene	98	3	168-73	
Vijaykadga et. al.	2004	Assessment of therapeutic efficacy of chloroquine for vivax malaria in Thailand	The Southeast Asian journal of tropical medicine and public health	35	3	566-9	Primaquine given at end of follow up
Dunne et. al.	2005	A double-blind, randomized study of azithromycin compared to chloroquine for the treatment of Plasmodium vivax malaria in India	The American journal of tropical medicine and hygiene	73	6	1108-11	
Yeramian et. al.	2005	Efficacy of DB289 in Thai patients with Plasmodium vivax or acute, uncomplicated Plasmodium falciparum infections	The Journal of infectious diseases	192	2	319-22	

Supplementary Table 1: Study References

Authors	Year	Title	Journal	Volume	Issue	Pages	Reason for Exclusion
Alvarez et. al.	2006	Efficacy of three chloroquine-primaquine regimens for treatment of Plasmodium vivax malaria in Colombia	The American journal of tropical medicine and hygiene	75	4	605-9	
Carmona-Fonseca et. al.	2006	Plasmodium vivax malaria: treatment of primary attacks with primaquine, in three different doses, and a fixed dose of chloroquine, Antioquia, Colombia, 2003-2004	Biomedica : revista del Instituto Nacional de Salud	26	3	353-65	Details same as Ref No. 93
Haghdoost et. al.	2006	Estimating the relapse risk of Plasmodium vivax in Iran under national chemotherapy scheme using a novel method	Journal of vector borne diseases	43	4	168-72	
Krudsood et. al.	2006	Safety and tolerability of elubaquine (bulaquine, CDRI 80/53) for treatment of Plasmidium vivax malaria in Thailand	The Korean journal of parasitology	44	3	221-8	
Maguire et. al.	2006	Mefloquine is highly efficacious against chloroquine-resistant Plasmodium vivax malaria and Plasmodium falciparum malaria in Papua, Indonesia	Clinical infectious diseases : an official publication of the Infectious Diseases Society of America	42	8	1067-72	
Tasanor et. al.	2006	Clinical-parasitological response and in-vitro sensitivity of Plasmodium vivax to chloroquine and quinine on the western border of Thailand	Transactions of the Royal Society of Tropical Medicine and Hygiene	100	5	410-8	
Dao et. al.	2007	Vivax malaria: preliminary observations following a shorter course of treatment with artesunate plus primaquine	Transactions of the Royal Society of Tropical Medicine and Hygiene	101	6	534-9	
Hasugian et. al.	2007	Dihydroartemisinin-piperaquine versus artesunate-amodiaquine: superior efficacy and posttreatment prophylaxis against multidrug-resistant Plasmodium falciparum and	Clinical infectious diseases : an official publication of the Infectious Diseases Society of America	44	8	1067-74	Primaquine given at end of follow up
Krudsood et. al.	2007	Clinical efficacy of chloroquine versus artemether-lumefantrine for Plasmodium vivax treatment in Thailand	The Korean journal of parasitology	45	2	111-4	
Elmes et. al.	2008	The efficacy and tolerability of three different regimens of tafenoquine versus primaquine for post-exposure prophylaxis of Plasmodium vivax malaria in the	Transactions of the Royal Society of Tropical Medicine and Hygiene	102	11	1095-101	Post Exposure Prophylaxis
Krudsood et. al.	2008	High-dose primaquine regimens against relapse of Plasmodium vivax malaria	The American journal of tropical medicine and hygiene	78	5	736-40	
Leslie et. al.	2008	A randomised trial of an eight-week, once weekly primaquine regimen to prevent relapse of plasmodium vivax in Northwest Frontier Province, Pakistan	PloS one	3	8	e2861	
Carmona-Fonseca et. al.	2009	Prevention of Plasmodium vivax malaria recurrence: efficacy of the standard total dose of primaquine administered over 3 days	Acta tropica	112	2	188-92	
Lee et. al.	2009	Biological resistance of hydroxychloroquine for Plasmodium vivax malaria in the Republic of Korea	The American journal of tropical medicine and hygiene	81	4	600-4	
Moon et. al.	2009	Recurrence rate of vivax malaria in the Republic of Korea	Transactions of the Royal Society of Tropical Medicine and Hygiene	103	12	1245-9	
Orjuela-Sanchez et. al.	2009	Recurrent parasitemias and population dynamics of Plasmodium vivax polymorphisms in rural Amazonia	The American journal of tropical medicine and hygiene	81	6	961-8	
Carmona-Fonseca et. al.	2010	Vivax malaria in children: Recurrences with standard total dose of primaquine administered in 3 vs. 7 days	IATREIA	23	1	10--20	No translation available
Pukrittayakamee et. al.	2010	A comparison of two short-course primaquine regimens for the treatment and radical cure of Plasmodium vivax malaria in Thailand	The American journal of tropical medicine and hygiene	82	4	542-7	
Takeuchi et. al.	2010	Directly-observed therapy (DOT) for the radical 14-day primaquine treatment of Plasmodium vivax malaria on the Thai-Myanmar border	Malaria journal	9		308	
Yeshiwondim et. al.	2010	Therapeutic efficacy of chloroquine and chloroquine plus primaquine for the treatment of Plasmodium vivax in Ethiopia	Acta tropica	113	2	105-13	
Maneeboonyang et. al.	2011	Directly observed therapy with primaquine to reduce the recurrence rate of plasmodium vivax infection along the Thai-Myanmar border	The Southeast Asian journal of tropical medicine and public health	42	1	Sep-18	
Muhamed et. al.	2011	Monitoring of clinical efficacy and in vitro sensitivity of Plasmodium vivax to chloroquine in area along Thai Myanmar border during 2009-2010	Malar J	10		44	
Poravuth et. al.	2011	Pyronaridine-artesunate versus chloroquine in patients with acute Plasmodium vivax malaria: a randomized, double-blind, non-inferiority trial	PloS one	6	1	e14501	Primaquine given at end of follow up

Supplemetary Table 2: Design of Studies Included in the Analysis

First Author	Year of Publication	Country	No.of trial arms	Prospective Study	Patient Enrolment	Parasitemia Criteria	Randomization	Control Arm	Age Lower Limit (yrs)	Age Upper Limit (yrs)	Duration of Follow Up (days)
Alving	1953	USA	3	Yes	Soldiers (Korean)	No	No	Yes			120
Coatney	1953	USA	2	Yes	Soldiers (Korean)	No	No	No			180
Cooper	1953	USA	9	Yes	Volunteers (Chesson)	No	No	No	21	45	350
Di Lorenzo	1953	USA	2	Yes	Soldiers (Korean)	No	No	Yes			90
Thaeler	1953	Nicaragua	3	Yes	In hospital	No	No	No			360
Alving	1955	USA	3	Yes	Volunteers (Chesson)	No	No	No			365
Alving	1960	USA	8	Yes	Volunteers (Chesson)	No	No	Yes			120
Basavaraj	1960	India	1	No	Out patient	No	No	No			480
Mendoza	1963	Mexico	2	Yes	Community	No	No	No	1		270
Martelo	1969	USA	2	Yes	Soldiers (Vietnam)	No	No	No			180
Fisher	1970	USA	2	No	Soldiers (Vietnam)	No	No	No			180
Contacos	1973	USA	1	Yes	Volunteers (Chesson)	No	No	No			200
Sharma	1973	India	1	Yes	Community	No	No	No	1		365
Contacos	1974	USA	2	Yes	Volunteers (W Pakistan)	No	No	No			133
Kaplan	1974	USA	1	Yes	Soldiers (Somalia)	No	No	No			895
Miller	1974	USA	1	Yes	Volunteers (Chesson)	No	No	No			270
Clyde	1977	USA	1	Yes	Volunteers (Chesson)	No	No	No	23	41	293
Roy	1977	India	1	Yes	Community	No	No	No			365
Saint-Yves	1977	PNG	3	Yes	Out patient	No	Yes	No	10	40	180
Cedillos	1978	El Salvador	4	Yes	Community	No	No	No			270
Roy	1979	India	1	Yes	Community	No	No	No			365
Appavoo	1984	India	1	Yes	Out patient	No	No	No			365
Dixon	1985	Thailand	3	Yes	In hospital	<100,000/µL	No	Yes	18		28
Sinha	1989	India	1	Yes	Out patient	No	No	No	1		395
Singh	1990	India	2	Yes	Out patient	No	No	No			240

Supplemetary Table 2: Design of Studies Included in the Analysis

First Author	Year of Publication	Country	No.of trial arms	Prospective Study	Patient Enrolment	Parasitemia Criteria	Randomization	Control Arm	Age Lower Limit (yrs)	Age Upper Limit (yrs)	Duration of Follow Up (days)
Prasad	1991	India	4	Yes	Community	No	No	No			1440
Bunnag	1994	Thailand	2	Yes	In hospital	No	Yes	No	15	60	540
Pukrittayakamee	1994a	Thailand	3	Yes	In hospital	No	Yes	No	15	50	30
Pukrittayakamee	1994b	Thailand	3	Yes	In hospital	No	Yes	Yes	15	50	28
Baird	1995	Indonesia	6	Yes	Community	>40/µL	Yes	Yes	6	54	28
Jelinek	1995	Germany	1	Yes	Travelers	No	No	No	6	74	540
Tan-ariya	1995	Thailand	1	Yes	Out patient	No	No	No	17	35	100
Srivastava	1996	India	3	Yes	Community	No	No	No	2		540
Fryauff	1997	Indonesia	2	Yes	Community	0.001%-1%	No	No	15		28
Smoak	1997	USA	2	Yes	Soldiers (Somalia)	No	No	No	18	39	300
Gogtay	1998	India	1	Yes	Out patient	No	No	No			180
Fang	1999	Taiwan	1	No	Travelers	No	No	No	6	64	540
Gogtay	1999	India	3	Yes	In hospital	No	Yes	Yes	16	63	180
Li	1999	China	3	Yes	Out patient	Unknown	No	No	4	53	270
Looareesuwan	1999a	Thailand	2	Yes	In hospital	No	Yes	Yes	12	63	28
Looareesuwan	1999b	Thailand	1	Yes	In hospital	>40/µL	No	No	15	52	84
Luxemburger	1999	Thailand	2	Yes	Out patient	No	No	Yes	2	55	63
Rowland	1999	Pakistan	2	Yes	Out patient	No	Yes	Yes	3		300
Wilairatana	1999	Thailand	4	Yes	In hospital	No	No	No	15	65	28
Bergonzoli	2000	Nicaragua, Costa Rica	4	Yes	Community	No	Yes	No	15		180
Pukrittayakamee	2000	Thailand	9	Yes	In hospital	No	Yes	Yes	15	64	28
Schwartz	2000	Israel	1	No	Travelers (Ethiopia)	No	No	No			365
Singh	2000	India	1	Yes	In hospital	No	No	No	14	70	28
Villalobos-Salcedo	2000	Brazil	2	Yes	Community	>100/µL	Yes	No	12	40	90
Abdon	2001	Brazil	3	Yes	Out patient	No	Yes	No	12	67	180

Supplemetary Table 2: Design of Studies Included in the Analysis

First Author	Year of Publication	Country	No.of trial arms	Prospective Study	Patient Enrolment	Parasitemia Criteria	Randomization	Control Arm	Age Lower Limit (yrs)	Age Upper Limit (yrs)	Duration of Follow Up (days)
Adak	2001	India	3	Yes	Out patient	No	Yes	Yes	15	65	365
Buchachart	2001	Thailand	1	Yes	In hospital	No	No	No	12	60	28
Dua	2001	India	1	Yes	Out patient	No	No	No			540
Duarte	2001	Brazil	1	Yes	Community	No	No	No	14	77	180
Congpuong	2002	Thailand	1	Yes	In hospital	No	No	No	14		28
Hamedi	2002	Iran	1	Yes	In hospital	2000-35,000/µl	No	No	15	59	28
Lacy	2002	Indonesia	1	Yes	Community	No	No	No	15	48	28
Yadav	2002	India	2	Yes	Community	No	No	Yes	1		365
Da Silva	2003	Brazil	8	Yes	Out patient	No	Yes	No	14		180
Fernandopulle	2003	Sri Lanka	1	Yes	In hospital	No	No	No			180
Machado	2003	Brazil	1	Yes	Community	No	No	No			30
Rajgor	2003	India	2	Yes	Out patient	No	Yes	Yes	16	88	180
Silachamroon	2003	Thailand	4	Yes	In hospital	No	Yes	No	15		28
Valibayov	2003	Azerbaijan	1	Yes	Out patient	>250/µL	No	No	6	85	28
Hamedi	2004	Thailand	1	Yes	In hospital	No	No	No	12	56	28
Leslie	2004	Pakistan	3	Yes	Out patient	No	Yes	Yes	3		270
Walsh	2004	Thailand	5	Yes	In hospital	No	Yes	Yes	18	55	168
Dunne	2005	India	2	Yes	In hospital	<100,000/µL	Yes	No	18	65	28
Yeramian	2005	Thailand	1	Yes	In hospital	<25,000/µL	No	No	18	30	28
Alvarez	2006	Columbia	3	Yes	Out patient	No	Yes	No	15		180
Haghdoost	2006	Iran	1	No	Out patient	No	No	No			2555
Krudsood	2006	Thailand	2	Yes	In hospital	No	Yes	No	16	51	28
Maguire	2006	Indonesia	2	Yes	Community	No	Yes	No	6	58	28
Tasanor	2006	Thailand	2	Yes	Out patient	1000-22,000/µl	Yes	No	15	60	28
Dao	2007	Vietnam	1	Yes	In hospital	500-50,000/µL	No	No			28

Supplemetary Table 2: Design of Studies Included in the Analysis

First Author	Year of Publication	Country	No.of trial arms	Prospective Study	Patient Enrolment	Parasitemia Criteria	Randomization	Control Arm	Age Lower Limit (yrs)	Age Upper Limit (yrs)	Duration of Follow Up (days)
Krudsood	2007	Thailand	2	Yes	In hospital	No	Yes	No	15	65	28
Krudsood	2008	Thailand	6	Yes	In hospital	No	Yes	No	12	60	28
Leslie	2008	Pakistan	3	Yes	Out patient	No	Yes	Yes	4	80	330
Carmona-Fonseca	2009	Columbia	4	Yes	Out patient	>1000/µL	Yes	No	2	50	120
Lee	2009	Republic of Korea	1	Yes	In hospital	No	No	No	19	50	28
Moon	2009	Republic of Korea	1	No	In hospital	No	No	No			660
Orjuela-Sanchez	2009	Brazil	2	Yes	Community	No	No	No	1	75	336
Pukrittayakamee	2010	Thailand	2	Yes	In hospital	No	Yes	No	14	61	28
Takeuchi	2010	Thailand	2	Yes	Community	No	Yes	No			90
Yeshiwondim	2010	Ethiopia	3	Yes	Out patient	No	Yes	Yes	4	65	157
Maneeboonyang	2011	Thailand	2	Yes	Community	No	No	No	1	80	90
Muhamed	2011	Thailand	1	Yes	Out patient	1000-100,000/µ	No	No	15	60	42

Supplementary Table 3: Recurrence Rates

First Author	Year of Publication	Country	Partner drug	Duration of Treatment (days)	Total primaquine dose (mg/kg)	Primaquine Supervision	Duration of Follow Up (days)	Sample Size	Recurrence Rate (%) of P. vivax at end of study
Very Low Dose Primaquine (≤ 2.5 mg/kg Total Dose)									
Baird	1995	Indonesia	Chloroquine	3	2.50	Not stated	28	19	15.8
Dixon	1985	Thailand	Chloroquine	5	1.25	Not stated	28	40	0.0
Krudsood	2008	Thailand	Artesunate	5	2.50	Not stated	28	60	15.0
Di Lorenzo	1953	USA (Korea)	Chloroquine	7	1.75	Not stated	90	31	3.0
Villalobos-Salcedo	2000	Brazil	Chloroquine	5	2.50	All doses	90	30	20.0
Alving	1960	USA (Chesson)	Chloroquine	28	2.00	Not stated	120	41	90.0
Carmona-Fonseca	2009	Columbia	Chloroquine	3	1.75	All doses	120	26	50.0
Carmona-Fonseca	2009	Columbia	Chloroquine	3	2.50	All doses	120	26	57.7
Abdon	2001	Brazil	Chloroquine	5	2.50	All doses	180	40	20.0
Alvarez	2006	Columbia	Chloroquine	7	1.75	All doses	180	62	41.9
Alvarez	2006	Columbia	Chloroquine	3	0.75	All doses	180	65	49.2
Bergonzoli	2000	Nicaragua, Costa Rica	Chloroquine	5	2.00	All doses	180	30	0.0
Bergonzoli	2000	Nicaragua, Costa Rica	Chloroquine	1	0.75	All doses	180	25	4.0
Da Silva	2003	Brazil	Chloroquine	5	2.50	Not stated	180	26	15.4
Da Silva	2003	Brazil	Artesunate (100mg)	5	2.50	Not stated	180	25	16.0
Da Silva	2003	Brazil	Artesunate (200mg)	5	2.50	Not stated	180	20	25.0
Da Silva	2003	Brazil	Artesunate (150mg)	5	2.50	Not stated	180	20	25.0
Fernandopulle	2003	Sri Lanka	Not stated	5	1.25	All doses	180	6	83.3
Gogtay	1998	India	Chloroquine	5	1.25	All doses	180	100	15.0
Gogtay	1999	India	Chloroquine	5	1.25	All doses	180	62	25.8
Saint-Yves	1977	PNG	Chloroquine	1	0.75	All doses	180	9	67.0
Contacos	1973	USA (W Pakistan)	Chloroquine	5	1.25	Not stated	200	5	100.0
Singh	1990	India	Chloroquine	5	1.25	Not stated	240	995	13.2
Cedillos	1978	El Salvador	Amodiaquine	5	1.25	Part supervised	270	90	21.1
Cedillos	1978	El Salvador	Amodiaquine	1	0.75	Part supervised	270	67	23.9
Mendoza	1963	Mexico	Chloroquine	5	1.25	All doses	270	389	20.6
Rowland	1999	Pakistan	Chloroquine	5	1.25	All doses	300	250	51.2
Cooper	1953	USA (Chesson)	Quinine	14	2.33	Not stated	350	34	65.0

Supplementary Table 3: Recurrence Rates

First Author	Year of Publication	Country	Partner drug	Duration of Treatment (days)	Total primaquine dose (mg/kg)	Primaquine Supervision	Duration of Follow Up (days)	Sample Size	Recurrence Rate (%) of P. vivax at end of study
Cooper	1953	USA (Chesson)	Chloroquine	7	2.33	Not stated	350	10	80.0
Thaeler	1953	Nicaragua	Chloroquine	14	2.33	Not stated	360	121	0.0
Adak	2001	India	Chloroquine	5	1.25	All doses	365	220	26.8
Appavoo	1984	India	Chloroquine	3	1.25	Not stated	365	425	3.8
Prasad	1991	India	Chloroquine	5	1.25	All doses	365	883	2.0
Roy	1977	India	Chloroquine	5	1.25	Not stated	365	6393	1.3
Roy	1979	India	Chloroquine	5	1.25	All doses	365	1389	0.7
Sharma	1973	India	Chloroquine	5	1.25	All doses	365	140	9.3
Yadav	2002	India	Chloroquine	5	1.25	Not stated	365	759	6.5
Sinha	1989	India	Chloroquine	5	1.25	All doses	395	725	6.9
Basavaraj	1960	India	Chloroquine	5	1.25	Not stated	480	563	6.0
Dua	2001	India	Chloroquine	5	1.25	Not stated	540	5541	9.2
Srivastava	1996	India	Chloroquine	5	1.25	Not stated	540	173	5.8
Prasad	1991	India	Chloroquine	5	1.25	All doses	720	1439	4.0
Prasad	1991	India	Chloroquine	5	1.25	All doses	1080	2484	5.7
Prasad	1991	India	Chloroquine	5	1.25	All doses	1440	8914	23.2
Low Dose Primaquine (>2.5 to <5.0 mg/kg Total Dose)									
Looareesuwan	1999a	Thailand	Chloroquine	14	3.5	All doses	28	441	0.5
Singh	2000	India	Chloroquine	14	3.5	Part supervised	28	75	16.0
Buchachart	2001	Thailand	Chloroquine	14	3.5	Not stated	28	364	0.0
Congpuong	2002	Thailand	Chloroquine	14	3.5	Not stated	28	26	0.0
Hamedi	2002	Iran	Chloroquine	14	3.5	All doses	28	39	0.0
Valibayov	2003	Azerbaijan	Chloroquine	14	3.5	Not stated	28	143	0.0
Hamedi	2004	Thailand	Artesunate	14	3.5	All doses	28	42	4.8
Yeramian	2005	Thailand	DB289	14	3.5	Not stated	28	9	11.1
Krudsood	2006	Thailand	Chloroquine	7	3.5	All doses	28	68	1.5
Maguire	2006	Indonesia	Mefloquine	14	3.5	All doses	28	310	1.3
Maguire	2006	Indonesia	Chloroquine	14	3.5	All doses	28	249	21.2

Supplementary Table 3: Recurrence Rates

First Author	Year of Publication	Country	Partner drug	Duration of Treatment (days)	Total primaquine dose (mg/kg)	Primaquine Supervision	Duration of Follow Up (days)	Sample Size	Recurrence Rate (%) of P. vivax at end of study
Tasanor	2006	Thailand	Chloroquine	14	3.5	All doses	28	24	0.0
Tasanor	2006	Thailand	Quinine	14	3.5	All doses	28	23	0.0
Krudsood	2007	Thailand	Chloroquine	14	3.5	Not stated	28	42	0.0
Krudsood	2007	Thailand	Artemether-Lumefantri	14	3.5	Not stated	28	38	2.6
Krudsood	2008	Thailand	Artesunate	9	4.5	Not stated	28	56	4.0
Krudsood	2008	Thailand	Artesunate	7	3.5	Not stated	28	57	11.0
Lee	2009	Republic of Korea	Chloroquine	14	3.5	Part supervised	28	108	0.0
Pukrittayakamee	2010	Thailand	None	7	3.5	Not stated	28	31	30.0
Pukrittayakamee	1994b	Thailand	Chloroquine	14	3.5	Not stated	28	25	0.0
Pukrittayakamee	1994b	Thailand	None	14	3.5	Not stated	28	30	10.0
Pukrittayakamee	2000	Thailand	Chloroquine	14	3.5	Not stated	28	22	0.0
Pukrittayakamee	2000	Thailand	None	14	3.5	Not stated	28	26	11.5
Pukrittayakamee	1994a	Thailand	Chloroquine	14	3.5	Not stated	30	20	0.0
Pukrittayakamee	1994a	Thailand	Rifampicin	14	3.5	Not stated	30	20	5.0
Machado	2003	Brazil	Chloroquine	14	3.5	Not stated	30	30	0.0
Muhamed	2011	Thailand	Chloroquine	14	3.5	Part supervised	42	130	0.0
Luxemburger	1999	Thailand	Chloroquine	14	3.5	All doses	63	43	7.0
Villalobos-Salcedo	2000	Brazil	Chloroquine	14	3.5	All doses	90	31	6.5
Takeuchi	2010	Thailand	Chloroquine	14	3.5	All doses	90	90	3.3
Takeuchi	2010	Thailand	Chloroquine	14	3.5	Unsupervised	90	97	12.4
Maneeboonyang	2011	Thailand	Chloroquine	14	3.5	All doses	90	43	0.0
Maneeboonyang	2011	Thailand	Chloroquine	14	3.5	Unsupervised	90	33	15.1
Tan-ariya	1995	Thailand	Chloroquine	14	3.5	Part supervised	100	50	12.0
Alving	1953	USA (Korea)	Chloroquine	14	3.5	Not stated	120	348	0.0
Alving	1960	USA (Chesson)	Chloroquine	14	3.5	Not stated	120	60	27.0
Alving	1960	USA (Chesson)	Chloroquine	28	4.0	Not stated	120	20	30.0
Alving	1960	USA (Chesson)	Chloroquine	28	3.0	Not stated	120	15	40.0
Alving	1960	USA (Chesson)	Chloroquine	56	4.0	Not stated	120	61	55.7
Carmona-Fonseca	2009	Columbia	Chloroquine	14	3.5	All doses	120	66	15.2

Supplementary Table 3: Recurrence Rates

First Author	Year of Publication	Country	Partner drug	Duration of Treatment (days)	Total primaquine dose (mg/kg)	Primaquine Supervision	Duration of Follow Up (days)	Sample Size	Recurrence Rate (%) of P. vivax at end of study
Carmona-Fonseca	2009	Columbia	Chloroquine	3	3.5	All doses	120	63	58.7
Yeshiwondim	2010	Ethiopia	Chloroquine	14	3.5	All doses	157	132	3.0
Walsh	2004	Thailand	Chloroquine	14	3.5	All doses	168	12	25.0
Coatney	1953	USA (Korea)	None	14	3.5	Not stated	180	294	0.0
Martelo	1969	USA (Vietnam)	Chloroquine	14	3.5	Not stated	180	21	14.3
Fisher	1970	USA (Vietnam)	Chloroquine	14	3.5	All doses	180	133	7.5
Gogtay	1999	India	Chloroquine	14	3.5	All doses	180	63	0.0
Bergonzoli	2000	Nicaragua, Costa Rica	Chloroquine	14	3.5	All doses	180	26	0.0
Bergonzoli	2000	Nicaragua, Costa Rica	Chloroquine	9	2.8	All doses	180	28	3.6
Abdon	2001	Brazil	Chloroquine	7	3.5	All doses	180	39	0.0
Abdon	2001	Brazil	Chloroquine	14	3.5	All doses	180	40	5.0
Duarte	2001	Brazil	Chloroquine	14	3.5	Part supervised	180	50	14.0
Da Silva	2003	Brazil	Artesunate (100mg)	7	3.5	Not stated	180	22	0.0
Da Silva	2003	Brazil	Chloroquine	7	3.5	Not stated	180	25	4.0
Da Silva	2003	Brazil	Artesunate (200mg)	7	3.5	Not stated	180	20	5.0
Da Silva	2003	Brazil	Artesunate (150mg)	7	3.5	Not stated	180	24	20.8
Rajgor	2003	India	Chloroquine	14	3.5	All doses	180	103	5.8
Alvarez	2006	Columbia	Chloroquine	15	3.5	All doses	180	64	18.8
Mendoza	1963	Mexico	Chloroquine	14	3.5	All doses	270	363	10.2
Miller	1974	USA (Chesson)	Chloroquine	14	3.5	Not stated	270	57	3.5
Li	1999	China	Chloroquine	8	3.0	All doses	270	35	22.9
Leslie	2004	Pakistan	Chloroquine	14	3.5	All doses	270	210	19.0
Leslie	2004	Pakistan	Chloroquine	14	3.5	All doses	270	173	19.7
Smoak	1997	USA (Somalia)	Chloroquine / Quinine	14	3.5	Part supervised	300	32	25.0
Smoak	1997	USA (Somalia)	Chloroquine / Quinine	14	3.5	Part supervised	300	28	64.2
Rowland	1999	Pakistan	Chloroquine	14	3.5	All doses	300	100	32.0
Orjuela-Sanchez	2009	Brazil	Chloroquine	7	3.5	Part supervised	336	87	71.5
Cooper	1953	USA (Chesson)	Quinine	14	4.7	Not stated	350	34	15.0
Cooper	1953	USA (Chesson)	Chloroquine	7	3.5	Not stated	350	10	90.0

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First Author	Year of Publication	Country	Partner drug	Duration of Treatment (days)	Total primaquine dose (mg/kg)	Primaquine Supervision	Duration of Follow Up (days)	Sample Size	Recurrence Rate (%) of P. vivax at end of study
Orjuela-Sanchez	2009	Brazil	Chloroquine	7	3.5	Part supervised	355	77	36.6
Thaeler	1953	Nicaragua	Chloroquine	14	3.5	Not stated	360	151	0.0
Thaeler	1953	Nicaragua	Chloroquine	14	4.7	Not stated	360	49	0.0
Alving	1955	USA (Chesson)	Quinine	14	3.5	Not stated	365	19	5.3
Alving	1955	USA (Chesson)	Chloroquine	14	3.5	Not stated	365	19	26.3
Alving	1955	USA (Chesson)	None	14	3.5	Not stated	365	19	78.9
Saint-Yves	1977	PNG	Chloroquine	14	3.50	All doses	365	10	0.0
Schwartz	2000	Israel	Chloroquine	14	3.5	Not stated	365	15	33.3
Bunnag	1994	Thailand	Chloroquine	14	3.5	Not stated	540	81	8.6
Jelinek	1995	Germany	Chloroquine	14	3.5	Not stated	540	56	12.5
Fang	1999	Taiwan	Chloroquine	14	3.5	Not stated	540	12	8.3
Moon	2009	Republic of Korea	Chloroquine	14	3.5	Not stated	660	3881	1.6
Haghdoost	2006	Iran	Chloroquine	14	3.5	Not stated	2555	12337	7.4
High Dose Primaquine (≥ 5.0 mg/kg Total Dose)									
Baird	1995	Indonesia	Chloroquine	27	10.0	Not stated	28	26	15.4
Baird	1995	Indonesia	Chloroquine (10mg/kg)	27	10.0	Not stated	28	23	13.0
Fryauff	1997	Indonesia	Halofantrine	28	10.0	All doses	28	26	0.0
Fryauff	1997	Indonesia	Chloroquine	28	10.0	All doses	28	27	11.1
Wilairatana	1999	Thailand	Artesunate	14	7.0	Not stated	28	15	0.0
Wilairatana	1999	Thailand	Sulfadoxine-Pyrimethar	14	7.0	Not stated	28	19	0.0
Wilairatana	1999	Thailand	None	14	7.0	Not stated	28	17	0.0
Lacy	2002	Indonesia	Atovaquone/Proguanil	14	7.0	Not stated	28	16	0.0
Silachamroon	2003	Thailand	Artesunate (5d)	14	7.0	Not stated	28	142	0.0
Silachamroon	2003	Thailand	Artesunate (7d)	14	7.0	Not stated	28	157	0.0
Dao	2007	Vietnam	Artesunate	7	5.3	All doses	28	28	3.6
Krudsood	2008	Thailand	Artesunate	11	5.5	Not stated	28	48	0.0
Krudsood	2008	Thailand	Artesunate	14	7.0	Not stated	28	52	0.0
Krudsood	2008	Thailand	Artesunate	7	7.0	Not stated	28	49	4.0

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First Author	Year of Publication	Country	Partner drug	Duration of Treatment (days)	Total primaquine dose (mg/kg)	Primaquine Supervision	Duration of Follow Up (days)	Sample Size	Recurrence Rate (%) of P. vivax at end of study
Pukrittayakamee	2010	Thailand	None	7	7.0	Not stated	28	33	6.0
Looareesuwan	1999b	Thailand	Atovaquone/Proguanil	14	7.0	Not stated	84	35	5.7
Alving	1960	USA (Chesson)	Chloroquine	56	8.0	Not stated	120	51	5.9
Alving	1960	USA (Chesson)	Chloroquine	56	6.0	Not stated	120	40	10.0
Alving	1960	USA (Chesson)	Chloroquine	98	7.0	Not stated	120	30	20.0
Contacos	1974	USA (Chesson)	Chloroquine	56	6.0	Not stated	133	10	0.0
Martelo	1969	USA (Vietnam)	Chloroquine	56	6.0	Not stated	180	21	28.6
Fisher	1970	USA (Vietnam)	Chloroquine	56	6.0	Unsupervised	180	94	22.3
Clyde	1977	USA (Chesson)	Chloroquine	7	7.0	All doses	293	11	0.0
Leslie	2008	Pakistan	Chloroquine	14	7.0	All doses	330	54	1.9
Leslie	2008	Pakistan	Chloroquine	56	6.0	All doses	330	68	5.9
Saint-Yves	1977	PNG	Chloroquine	14	5.3	All doses	365	10	0.0
Bunnag	1994	Thailand	Chloroquine	14	5.3	Not stated	540	86	1.2
Kaplan	1974	USA (Somalia)	Chloroquine	21	5.3	All doses	895	207	4.3
Primaquine Dose Not Stated									
Dunne	2005	India	Azithromycin	14	Not Stated	Not stated	28	100	0.0
Dunne	2005	India	Chloroquine	14	Not Stated	Not stated	28	97	0.9
Control Arms (No Primaquine)									
Dixon	1985	Thailand	Chloroquine		No Primaquine		28	11	0.0
Pukrittayakamee	1994b	Thailand	Chloroquine		No Primaquine		28	30	0.0
Baird	1995	Indonesia	Chloroquine		No Primaquine		28	23	87.0
Baird	1995	Indonesia	Chloroquine		No Primaquine		28	22	68.2
Looareesuwan	1999a	Thailand	Chloroquine		No Primaquine		28	445	0.5
Pukrittayakamee	2000	Thailand	Chloroquine		No Primaquine		28	21	0.0
Walsh	2004	Thailand	Chloroquine		No Primaquine		56	10	80.0
Luxemburger	1999	Thailand	Chloroquine		No Primaquine		63	69	55.1
Di Lorenzo	1953	USA (Korea)	Chloroquine		No Primaquine		90	46	50.0

Supplementary Table 3: Recurrence Rates

First Author	Year of Publication	Country	Partner drug	Duration of Treatment (days)	Total primaquine dose (mg/kg)	Primaquine Supervision	Duration of Follow Up (days)	Sample Size	Recurrence Rate (%) of P. vivax at end of study
Alving	1953	USA (Korea)	Chloroquine		No Primaquine		120	355	38.6
Alving	1960	USA (Chesson)	Chloroquine		No Primaquine		120	74	95.9
Yeshiwondim	2010	Ethiopia	Chloroquine		No Primaquine		157	108	8.3
Gogtay	1999	India	Chloroquine		No Primaquine		180	60	11.7
Rajgor	2003	India	Chloroquine		No Primaquine		180	101	12.9
Leslie	2004	Pakistan	Chloroquine		No Primaquine		270	212	40.6
Rowland	1999	Pakistan	Chloroquine		No Primaquine		300	250	51.6
Rowland	1999	Pakistan	Chloroquine		No Primaquine		300	100	49.0
Leslie	2008	Pakistan	Chloroquine		No Primaquine		330	68	32.4
Adak	2001	India	Chloroquine		No Primaquine		365	224	40.2
Yadav	2002	India	Chloroquine		No Primaquine		365	723	8.6

Supplementary Table 4: Adverse Events

First Author	Year of Publication	Sample Size	Co-administered Drug	Primaquine Supervision	Duration of Treatment (days)	Total Primaquine Dose (mg/kg)	Day of Adverse Event	Adverse Event Reported	No. of Patients	Action Taken
Cooper	1953	34	Quinine	Not stated	14	4.67	Not Stated	Cyanosis	2	None
Fisher	1970	133	Chloroquine	All doses	14	3.5	Not Stated	Haemolysis (G6PDd)	1	None
Clyde	1977	11	Chloroquine	All doses	7	7	4	Severe abdominal cramps, vomiting and cyanosis	1	Treatment stopped
Silachamroon	2003	157	Artesunate	Not stated	14	7	4	13% reduction in haematocrit (G6PDd)	1	Treatment stopped
Silachamroon	2003	157	Artesunate	Not stated	14	7	6	14% and 8% reduction in haematocrit (G6PDd)	2	Treatment stopped
Silachamroon	2003	157	Artesunate	Not stated	14	7	7	16% reduction in haematocrit (G6PDd)	1	Treatment stopped
Dunne	2005	97	Chloroquine	Not stated	14	Not Stated	Not Stated	Maculopapular rash	1	Treatment stopped
Dunne	2005	97	Chloroquine	Not stated	14	Not Stated	Not Stated	Severe pruritis	1	Treatment stopped