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Clinical trials of new strategies for the prevention and treatment of *Plasmodium falciparum* and *Plasmodium vivax* malaria in north eastern Papua, Indonesia.

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Medicine of the University of London.

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Abstract

Background.
New drug regimens are needed for effective prophylaxis and treatment of drug resistant *Plasmodium falciparum* and *Plasmodium vivax* malaria in northeastern Papua. Mefloquine and doxycycline, two standard prophylactic drugs, had high prophylactic efficacy in northeastern Papua but they have limited application for two vulnerable groups, young children and pregnant women. Azithromycin, an azalide antibiotic, had a prophylactic efficacy of 83% against *P. falciparum* in malaria immune Kenyans. If successful in non immunes, it would be a significant addition to the current prophylactic drugs. Chloroquine, the current first line drug in northeastern Papua, is associated with high rates of treatment failure for falciparum and vivax malaria. Cure rates might be improved by combining with chloroquine with doxycycline, two drugs that are inexpensive and widely available.

Methods.
Two clinical trials were conducted.
(1). The prophylactic efficacy of azithromycin against *P. falciparum* and *P. vivax* was determined in a double blind, placebo-controlled trial in Indonesian adults with limited immunity. After radical cure, three hundred randomised subjects received azithromycin (n=148, 750mg loading dose, 250mg/day), placebo (n=77), or doxycycline (n=75, 100mg/day). The end point was slide proven parasitaemia.
(2). In an open trial chloroquine plus doxycycline (CQD) was compared to chloroquine or doxycycline alone for treating falciparum and vivax malaria. Eight nine falciparum patients were randomised to standard dose chloroquine (n=30), doxycycline [100 mg 12 hourly (7 days), n=20], or chloroquine plus doxycycline (n=39); corresponding numbers for vivax patients were 23, 16, 24. Endpoints were parasite sensitivity (S) or resistance (RI, RII, and RIII) assessed by Day 28.

Findings.
(1). There were 58 *P. falciparum* and 29 *P. vivax* prophylaxis failures over 20 weeks. Based on incidence rates, the prophylactic efficacy of azithromycin relative to placebo was 71.6% (95% CI 50.3-83.8) against *P. falciparum*, and 98.9% (93.1-99.9) against *P. vivax*. Corresponding figures for doxycycline were 96.3% (85.4-99.6) and 98% (88.0-99.9).
(2). Of the 152 recruited patients, 133 reached a parasitological end point. *P. falciparum* cure rates (S) were 26/29 (89.65%) for chloroquine plus doxycycline patients [RIII (n=3)] vs. 5/23 (21.7%) for chloroquine (*P*<0.001), and 12/18 [66.7% (41.0-86.6%)] for doxycycline (CQ vs. D, *P*=0.0037). The corresponding cure rates against *P. vivax* were 15/22 (68.2%) vs. 6/21 (28.6%) vs. 6/14 (42.8%). Only the CQD vs. CQ cure rates were significantly different (*P*=0.01).

**Interpretation.**

Daily azithromycin prophylaxis was highly effective against *P. vivax* but modest against *P. falciparum*. It cannot be recommended as a first line prophylactic agent against *P. falciparum*. Chloroquine plus doxycycline was effective against drug-resistant *P. falciparum* but not chloroquine resistant *P. vivax*. It may offer an alternative to more expensive drugs e.g. mefloquine, atovaquone/proguanil for treating *P. falciparum*. 
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Chapter 1 - Introduction

1.1 Malaria - biology and clinical classification

Human malaria is a parasitic disease caused by one or more of four Plasmodium species; *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*. *P. malariae* has a three-day blood stage life cycle and the other parasites have a two-day cycle. *Plasmodium falciparum* is the most serious form of malaria because it may cause severe disease. Its virulence is attributed to its ability to produce heavy parasite burdens and to cytoadhere to the vascular endothelium leading to sequestration of parasitised red cells in the microvasculature of vital organs. The other three species are often referred to as the benign malarias. They seldom parasitise more than 2% of the erythrocytes and do not cytoadhere. They cause acute febrile illnesses that rarely cause severe morbidity. *P. vivax* and *P. ovale*, the two relapsing malarias, have a dormant liver stage, called the hypnozoite, that gives rise to further bouts of infection after clearance of the blood stage infection.1,2

1.2 Overview of the current global status of malaria

Malaria is currently a serious global health problem that affects predominantly tropical countries (Appendix 1). Although accurate data are lacking, the WHO estimates the global annual incidence of clinical malaria to be 300-500 million cases.3 A more recent review estimates this to be 400 to 900 million African children less than five years of age.4 *Plasmodium falciparum* and *Plasmodium vivax* account for the vast majority of the global malaria burden; *P. vivax* contributes an estimated 70-80 million cases per year.5 *P. falciparum* predominates in sub-Saharan Africa; *P. vivax* predominates in the Middle East, Latin America, and parts of Asia. Within malaria endemic regions, there are many areas of mixed falciparum/vivax malaria where the relative proportions of the species vary. *Plasmodium falciparum* is responsible for virtually all of the estimated 700,000 to 2.7 million deaths per year that occur mostly (75%) in African children.3,4 Sub-Saharan Africa bears most of the global malaria burden, some 90%. Never the less, in absolute terms, malaria represents a significant public health problem in many endemic regions, including Indonesia.6

Compounding the seriousness of the malaria related morbidity and mortality is drug resistance. *P. falciparum* is now resistant to several commonly used antimalarial
drugs. Chloroquine, the mainstay of malaria treatment for the past fifty years, is now ineffective in the vast majority of malaria endemic countries. Chloroquine sensitive *P. falciparum* is confined to Central America, the Dominican Republic and Haiti. Sulfadoxine/pyrimethamine (S/P), an inexpensive second line drug in many countries, has been ineffective in SE Asia and the Amazon basin for several years, and is now losing efficacy in other parts of Asia and Africa. Quinine, the principal drug for treating multidrug resistant malaria and severe malaria in many countries, is still effective despite reports of reduced sensitivity. A recent development has been the emergence of chloroquine resistant *P. vivax* (CRPV) which has been reported in focal areas of India, Burma, Indonesia, Papua New Guinea, Latin America, and the Solomon Islands (Schapira A, personal communication). In Papua, CRPV now constitutes a significant public health problem. CRPV has most often been reported as recurrent parasitaemia occurring by Day 28 but there are also reports of failure to clear parasites, signifying high-grade resistance. Some of these reports have excluded inadequate CQ absorption as a cause of treatment failure by measuring CQ drug levels.

The impact of drug resistant falciparum malaria is considerable. Increased childhood morbidity and mortality in Africa due to chloroquine resistance is now well documented. Malaria related anaemia itself is also an important cause of morbidity and mortality. It affects an estimated 1.5 to 6 million African children and is associated with a case fatality rate (CFR) of 15%. Anaemia is also a significant problem in pregnant African women, affecting a median of 8.6%. The detrimental effects of CRPV have yet to be documented to the same extent as drug resistant falciparum malaria. Clinically, *P. vivax* may cause a debilitating febrile illness and anaemia in pregnant and non-pregnant patients. Rarely, vivax malaria may cause serious pathology in the form of pulmonary oedema and splenic rupture.

For malaria control, recommending optimal treatment and prophylactic regimens is clearly more difficult in the face of drug resistance. From a drug policy perspective, drug resistance is the most critical factor in reducing the useful life span of a drug and undermining drug policy. How to detect and overcome drug resistance, and deploy effective measures to control malaria is the art and science of malaria control itself. From the research perspective, the development of new antimalarial drugs or drug combinations
of either new or currently available drugs that will be effective over the medium to long term is an important way forward.

1.3 Overview of malaria in Indonesia - published reports and unpublished data from the Indonesian Ministry of Health

1.3.1 Epidemiology

Indonesia is an archipelago consisting of 13,767 islands. There are six main islands and 6,000 uninhabited islands (Appendix 2). There are two seasons, dry from June to September, and wet from December to March. The average humidity is 70-90%. The total population is estimated at 180 million; 70% of the population are rural inhabitants. Malaria transmission occurs in all parts of Indonesia but endemicity varies (Figure 1.1). Approximately 50% of the total population live in areas of little or no malaria transmission, chiefly Java and Bali (Appendix 3). The population at risk is located mostly in the outer eastern islands. Malaria surveillance is conducted chiefly through a system of passive case detection. This system covers completely Java and Bali but not all of the outer islands. The number of reported malaria cases, diagnosed either clinically or microscopically, runs currently at about 2 million per year with 100 deaths (F. Laihad, Indonesian MoH, personal communication). The WHO estimate is 6 million and 700, respectively. In 1992, of all the causes of under five mortality, malaria ranked fifth, accounting for 6.3% of the total.

Java, the largest island, has the least intense transmission. In 1997, the overall annual incidence of malaria was 0.12/1,000 population. (F. Laihad, personal communication). Malaria has been eradicated in large areas of eastern and western Java but there remain foci of low transmission, especially in the central southern part. The annual parasite incidence (API) rates for east and west Java are very low, between 0 to 0.1/1000 person-years; the figure for central Java (excluding Yogjakarta) averaged 0.5.6 The focal nature of malaria in central Java has been described by Baird.33 Malaria surveys conducted in the Yogyakarta area found that most of the malaria was confined to three areas of the sub district of Kokap: their APIs were ~ 13, ~ 94 and ~ 170. These areas were hilly with many potential mosquito breeding sites e.g. streams, open wells. Java owes its success in reducing the malaria burden because of a campaign of household DDT spraying that began in the 1950s. However, in 1997 Java saw a resurgence of malaria because of a breakdown in malaria control efforts due to the economic crisis of that time.
The results of a retrospective investigation in Purworejo, a coastal district in central Java, have been reported by Barcus et al.\textsuperscript{34}

This area is geographically diverse with fish ponds and coconut groves in the tidal zone that give way to a coastal plain of rice fields which then rise to form the forested Menoreh hills of some 900 metres. \textit{Anopheles sundaicus} predominates in the tidal zone, \textit{An. aconitus} and \textit{barbirostris} in the costal plan, and \textit{An. maculatus} and \textit{balabacensis} in the hills. Malaria transmission in this area is perennial and peaks in the dry season because suitable breeding sites are not washed away by the rains; dry rocky streambeds are the favoured breeding sites for \textit{An. maculatus} and \textit{balabacensis}, the two species responsible for Menoreh hills epidemic. A review of malaria data between 1986 and 2000 showed that malaria was at a low level in 1995 (mean API < 5/1000) but this rose gradually in 1996 and 1997 with ever larger annual increases in malaria to reach a mean API of 44.5 in 2000. Geographically, the epidemic was confined almost exclusively to the hills which have a higher a population density than the costal and tidal areas. Coincident with this epidemic was a reduction in economic resources for vector control, with reduced spraying activity, and a reduction in the number of malaria control health workers.

This report illustrates well the potential for epidemic malaria to appear in areas where malaria control has been successful. Several factors were responsible. The economic crisis of 1997 reduced drastically malaria control efforts. The epidemic was confined to the hills due to vectors that breed well in small pools of water. The population was poor, living in poorly constructed wooden buildings, and having limited access to health care.

The highest level of malaria transmission occurs in the eastern outer islands and is related to the opening up of new lands for industrial development and resettlement by mostly transmigrants (transmigrasi) from other parts of Indonesia. The cutting down of mangrove forest promotes the breeding of \textit{An. sundaicus} and increased economic activity in the forests of east Kalimantan, exposes workers to \textit{An. balabacensis}, a mosquito that breeds easily in such an environment.\textsuperscript{35} Irian Jaya, renamed Papua in 1998, has the highest cumulative incidence rates of malaria, $\sim 15\%$ and $\sim 17\%$ in 1992 and 1993, respectively; this compares with the overall figure for Java of 0.98\%.\textsuperscript{33}

The two main species transmitted in Indonesia are \textit{P. falciparum} and \textit{P. vivax}. Most studies have reported falciparum:vivax ratios of 2:1 (Figure 1.1). In central,
southern Java, ratios have varied from 2:1 to 0.4:1.\textsuperscript{13} \textit{P. malariae} and \textit{P. ovale} account for very little of the malaria burden; the latter being reported mostly from the eastern islands.\textsuperscript{36,37,38} Several anopheline species transmit malaria in different parts of Indonesia (Table 1.1).\textsuperscript{6}

Figure 1.1 Point prevalent surveys conducted from 1993 to 1997 by the US Navy and the Indonesian Ministry of Health in several parts of Indonesia. The numbers in parentheses refer to the year of study conduct. Data from Lt. Cdr. D. Fryauff, US Navy.
Table 1.1 Malaria vectors and their distribution on the main islands of Indonesia.

<table>
<thead>
<tr>
<th>Islands</th>
<th>Vector species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Java and Bali</td>
<td><em>An. aconitus</em>, <em>An. sundaicus</em>, <em>An. subpictus</em>, <em>An. balabacensis</em>,</td>
</tr>
<tr>
<td></td>
<td><em>An. maculatus</em></td>
</tr>
<tr>
<td>Sumatra</td>
<td><em>An. sundaicus</em>, <em>An. maculatus</em>, <em>An. hyrcanus group</em>, <em>An. letifer</em>,</td>
</tr>
<tr>
<td></td>
<td><em>An. aconitus</em></td>
</tr>
<tr>
<td>Kalimantan</td>
<td><em>An. balabacensis</em>, <em>An. Sundaicus</em>, <em>An. hyrcanus group</em>, <em>An. letifer</em>,</td>
</tr>
<tr>
<td></td>
<td><em>An. umbrosus</em></td>
</tr>
<tr>
<td>Sulawesi</td>
<td><em>An. barbirostris</em>, <em>An. sundaicus</em>, <em>An. subpictus</em>, <em>An. hyrcanus group</em>,</td>
</tr>
<tr>
<td></td>
<td><em>An. flaviostris</em>, <em>An. minimus</em>, <em>An. nigerrimus</em></td>
</tr>
<tr>
<td>Nusa Tenggara</td>
<td><em>An. barbirostris</em>, <em>An. sundaicus</em>, <em>An. subpictus</em>, <em>An. aconitus</em></td>
</tr>
<tr>
<td>Irian Jaya/Maluku</td>
<td><em>An. punctulatus</em>, <em>An. koliensis</em>, <em>An. farauti</em>, <em>An. bancroftii</em>,</td>
</tr>
<tr>
<td></td>
<td><em>An. karwari</em></td>
</tr>
</tbody>
</table>

1.3.2 Malaria control and resistance monitoring

Malaria control activities encompass active and passive case detection by microscopic confirmation, presumptive and radical drug treatment, indoor house spraying, and environmental management (e.g. land drainage). In the Outer islands, the focus is on spraying operations and malarialmetric surveys, passive case detection, treatment of clinical malaria cases, and the control of malaria outbreaks. Malaria drug resistance surveillance is conducted by the Ministry of Health (called Litbangkes) using both the WHO 28 day *in vivo* test and the WHO *in vitro* microtest (Mark II); the latter has been used since 1984 for *P. falciparum* only. The focus has been *P. falciparum* but *in vivo* tests of *P. vivax* have also been conducted in collaboration with the US Navy.

1.3.3 Plasmodium falciparum

A comprehensive review of drug resistant malaria has come from Tjitra. Chloroquine resistant *P. falciparum* was first documented in Kutai, in East Kalimantan in 1973. Soon afterwards, two cases of RI CQ resistance were reported in two Japanese adults from East Kalimantan and Irian Jaya. All major provinces now have evidence of *in vivo* (RI/RII/RIII) or *in vitro* chloroquine resistant *P. falciparum*. Recent data from Java (Menoreh hills epidemic) and East Timor (post independence) have shown high rates (50 and 60%, respectively) of chloroquine resistant *P. falciparum*.41 42

The recommended second line drug for chloroquine resistant *P. falciparum* is sulfadoxine/pyrimethamine. Clinical resistance to S/P was first reported in 1979. Data on the distribution of either *in vitro* or *in vivo* S/P resistance are limited. By 1995, *in vivo* or *in vitro* resistance to S/P was reported from 11 of 14 provinces. All three levels of *in
vivo resistance have been reported. RI occurs most commonly but has generally been low, ~2% of patients tested in South Sulawesi, and ~5% in Papua.\textsuperscript{39, 45} However, the rate was 12% (14/119) from central Java, 22% in the Menoreh hills epidemic, and 15% in NE Papua.\textsuperscript{39, 41, 46} High rates of \textit{in vitro} S/P resistance have been documented in Aceh (67%), central Java (71%), East Kalimantan (85-100%), East Timor (27%), Sulawesi (50%), and Irian Jaya (~80%).\textsuperscript{39} Foci of \textit{in vitro} multidrug (chloroquine, S/P, amodiaquine, and mefloquine) resistant \textit{P. falciparum} have been reported from two provinces, East Timor and East Kalimantan.

Several other antimalarial drugs have undergone either \textit{in vivo} or \textit{in vitro} testing. \textit{In vitro} mefloquine resistance has only been reported from East Kalimantan [1/37 (3%)] in 1991. \textit{In vivo} resistance (2/36) to Fansimef (S/P and mefloquine) in Papua.\textsuperscript{45} RI resistance to halofantrine has been documented in East Kalimantan [1/63 (2%)], and North Sulawesi [7/59 (12%)] but resistance was not found in Papua (n=36). Evidence of \textit{in vitro} quinine resistance has been reported from a number of provinces, including Aceh [14/21 (67%)], central Java [27/38 (71%)], and south Sulawesi [11/19 (58%)], north Sulawesi [12/20 (60%)], and east Kalimantan [74/82 (90%)]. Few studies of quinine efficacy have been conducted. Of those with a sample size >30, no resistance was found in Aceh (n=32) but resistance was found in central Java in 14 [RI (n=3), RII (n=11)] of 119 (12%) patients, south Sulawesi [1 (RII)/62 (2%)], and Irian Jaya [1(RII)/41 (2.4%)]. Although amodiaquine is not used in Indonesia, several small \textit{in vitro} studies have documented a high proportion of \textit{P. falciparum} isolates as resistant in east Kalimantan [27/27 (100%)], south Sulawesi [9/9 (100%)], and Irian Jaya [8/8 (100%)]; these areas also have high rates of \textit{in vitro} and \textit{in vivo} chloroquine resistance.

1.3.4 \textbf{Plasmodium vivax}

Chloroquine resistant \textit{P. vivax} (CRPV) was first documented in 1991 in an American visitor to Nias island off the west coast of Sumatra.\textsuperscript{47} He developed symptomatic vivax malaria despite unfailing adherence to CQ prophylaxis. His illness recrudesced despite two therapeutic courses of CQ, the second of which included primaquine 15 mg/day for 14 days. He was eventually cured with a single dose of mefloquine (500mg). Surveys of \textit{in vivo} resistance have found full sensitivity of \textit{P. vivax} to CQ in Lombok, Gag Island, and Java.\textsuperscript{48, 49, 50} However, there was evidence of reduced vivax sensitivity in the Lombok study because some patients had Day 0 chloroquine
levels above the Berliner adapted reference of 100 ng/ml (see 1.7.1.3). *P. vivax* resistant to prophylaxis was suspected in an Australian female traveller who had visited Bali and Lombok on the basis of fever whilst on chloroquine and Malaprim® prophylaxis. However, no malaria slide was done at the time. Seven weeks after stopping her prophylaxis, she developed acute vivax malaria that was cured with chloroquine and primaquine. Resistance of *P. vivax* to chloroquine treatment has been documented in Papua, north Sulawesi, and West Kalimantan. Evidence of vivax resistance has been the failure to clear parasites or the recurrence of vivax parasitaemia despite total, whole blood CQ concentrations ≥ 100ng/ml. Papua has the most serious problem with CRPV where high-grade vivax resistance has been documented.

S/P resistant vivax has now been documented in Java and Irian Jaya. These studies have involved small numbers of treated patients, so failure rates are broad e.g. 4/6 [66% (22-95)] in Java (S/P), and 2 of 6 [33% (4-78)] in Irian Jaya (CQ & S/P). Treatment failures have been persistent parasitaemia during the first seven days of follow up, or recurrence of parasitaemia. Preliminary studies have identified mutations in the vivax dihydrofolate reductase gene at positions 57, 58, 61, and 117 that were associated with poor parasitological responses to vivax.

1.3.5 *Plasmodium malariae* and *ovale*

There are no recent data on the drug sensitivity of *P. ovale*. Indeed, as mentioned earlier, this species is now quite rare in Indonesia. Similarly, little is known about the epidemiology of *P. malariae* in Indonesia but foci were described in Papua in the 1950s (see 1.4 and 1.5 below). Recently, chloroquine resistant *P. malariae* has been reported from Legundi island off the southern tip of Sumatra. Using standard dose chloroquine and a 28 day follow up, two of 28 patients failed to clear their parasites by Day 8. The total, whole blood concentrations of chloroquine were 205 and 680 µg/L on Day 2 and 100 and 170 µg/L on Day 8. These levels confirm CQ absorption but the level of 205 is somewhat low. Although the therapeutic range of chloroquine against *P. malariae* is unknown (see 1.7.6), these data are consistent with CQ resistance.

1.4 Synopsis of the malaria situation in Papua

1.4.1 Malaria epidemiology

Papua is Indonesia's most eastern province and has diverse physical features that include central highlands, and areas of forestation and swamps (Appendix 4). Gunawan
has provided an excellent overview of the malaria situation in Irian Jaya. Historical texts dating from the early 1800s describe a swamp fever that affected the young indigenous population as well as the Dutch colonial settlers. Fort de Bus, a Dutch military post, had to be abandoned eight years after it was established because of the deleterious effects of swamp fever. Systematic data from the 1950's come from Dutch researchers. Using spleen rates as the epidemiological marker of transmission, they found malaria ranged in endemicity from hypoendemic (spleen rate 10%) to holoendemic (> 75%; see Table 2.1). The highest rates of malaria were found on the south coast where transmission was hyperendemic with foci of holoendemic malaria. Malaria transmission mirrored the rainfall pattern and decreased with increasing altitude. Unstable transmission with the risk of epidemics occurred at altitudes between 350 to 1,600 m.

In 1979, malaria was the diagnosis in 83,171 outpatient and clinic consultations throughout the province, representing 20% of all diagnoses. From 1975-79, malaria was responsible for 16% of all hospital admissions and contributed 14% of all hospital deaths. The case fatality rate of malaria at the Rumah Sakit Umum (public hospital) in Jayapura was 3.5%.  

1.4.1.1 Highland malaria

Two epidemics were described by Metselaar in 1959 in the Kemboe and Baliem valleys in the central highlands. In Kemboe, a dry weather spell, and visits of highlanders to a salt well were important factors in this epidemic which was first signalled by an increase in fever deaths. Malarriometric surveys conducted at different altitudes of the valley showed that villagers from high altitude villages had lower parasite prevalence rates (7-15%) than those living lower down (38%) with the exception of Boegelo (1,900 m) which had a prevalence rate of 48%. Inhabitants from Boegelo would visit frequently a salt well, situated lower down at 1,400 m, and spend the night there; thus, increasing their risk of malaria. In addition, dry climatic conditions favoured the preservation of mosquito breeding sites which would normally be washed away by the rains. In the Baliem valley (1,600 m) near Wamena, evidence of low levels of local malaria transmission was found; spleen (0.2%) and parasite rates (1%) were low. An increase in malaria was thought to be due to contact with traders from outside the valley who had parasite rates of 12%.
Another survey of malaria in the central highlands was conducted in the Oksibil valley (1,200-1,500 m), close to Papua New Guinea (Appendix 4). Five malariometric surveys were conducted in four villages in 1990-91. The overall malaria prevalence was 25% (844 of 3,380 slides examined); prevalence by age groups were: 10% (infants), 50% (children aged 1-4y), 35% (5-9y), 28% (10-14y), and 16% for adults. The spleen rate was 96% in children < 5 years of age. Transmission of all four malaria species was documented. *P. falciparum* accounted for 55%, *P. vivax* 31%, *P. malariae* 13%, and *P. ovale* 0.5%. In the village of Kutdol (1,500 m), *P. malariae* accounted for 43%; rates for *falciparum* and *vivax* malaria were 32 and 23%, respectively. Asymptomatic falciparum parasitaemia was common in children and adults. Increased transmission was associated with an increase in mosquito breeding sites brought about by replacing traditional huts with more modern housing. This required the construction of drainage ditches to drain off rainwater from the tin roofs. The mean mosquito biting rate was 10 bites/person/night (p/n) in the newly constructed houses compared to 1 bite/p/n in the traditional hut that served as a separate outbuilding. *Anopheles punctulatus* was the predominant vector, accounting for 97% of mosquitoes examined; *An. koliensis* accounted for the remaining 3%. Based on the high spleen rates, and rates of asymptomatic parasitaemia, it was concluded that this area had had stable malaria transmission for many years. The construction of new housing favoured the breeding of *An. punctulatus* with a consequential increase in transmission.

In 1997, a series of localised malaria epidemics were reported from the Jayawijaya district, a large area of the central highlands, at elevations of 1,000 and 2,200 metres (Appendix 4). These epidemics affected isolated and marginalised communities scattered over the highlands. Although data in the paper are based on clinical diagnoses, it is estimated that during a period of 10 weeks, there were more than 550 deaths. Several factors played a role in this epidemic. There was a drought in the highlands that resulted in increased breeding of vectors of the *An. punctulatus* complex which in turn enhanced local transmission of *P. falciparum*. Food shortages forced communities to search for food increasing their exposure to malaria, especially in the endemic lowlands. Poor health infrastructure and limited access to effective treatment also contributed to the high mortality.
1.4.1.2 Lowland malaria

In 1995, malaria surveys were conducted in three villages 30 km south-east of Nabire, a coastal town 120 km west of Jayapura (Appendix 4). Irianese lived in two villages, Topo and Urusuma, and transmigrants, who had arrived five years earlier, lived in Margajaya. The spleen rates in children aged 2-9 were, respectively, 27%, 79%, and 10%; the corresponding malaria prevalence rates were 9% (n=199), 18% (n=157), and 9% (n=573). The falciparum:vivax ratios were 2.5:1, 1.1:1, and 1.4:1, respectively. More recent work conducted on new transmigrasi villages in Armopa area on the north coast west of Jayapura have shown a higher prevalence of vivax over falciparum malaria: this was 2:1 in adults after 6 months of settlement; corresponding incidence rates were 1.8 and 1.5 infections per person-year.

In the Timika area of southern Papua, Pribadi et al. conducted malarialometric surveys in six villages. Four were long established, where local villagers had life long malaria exposure, and two were newly established. One housed Irianese from the highlands, and the other transmigrants from other parts of Indonesia. The spleen rates in children aged 2-9 years varied from 5-92% and correlated with the parasite rates that ranged from 14-60% (Figure 2). The lowest and highest rates were in Mapurujaya and Mwapi, respectively, and represent the spectrum of malaria endemicity in this area. Mapurujaya was hypo- and Mwapi holoendemic. The overall falciparum to vivax ratio was 2.5 to 1.

Figure 1.2 Spleen and malaria parasite rates conducted in the Timika area of southern Irian Jaya.
1.4.2 Vector studies

Of the Anopheles vectors studied in Papua, the important species are *Anopheles punctulatus*, *An. koliensis*, *An. farauti*, *An. longirostris*, *An. karwari*, and *An. bancrofti*. *An. punctulatus* needs little sunshine and breeds indiscriminately in all types of water e.g. water near pig sties, ditches, drums of stagnant water, irrigation ditches associated with the growing of *kangkung*, a popular spinach like plant. *An. punctulatus* numbers vary with the season, peaking during November to April (rains) and declining significantly during May to October (dry season). Measured biting rates were 64 and 20 bites/person-night, respectively. The prevalence of *An. punctulatus* dropped during dry spells within the rainy season, indicating environmental vulnerability. *An. punctulatus* is endophilic (rests indoors) and endophagous (bites indoors).

1.5 Malaria control in Irian Jaya

In 1954, hut spraying with 5% DDT at six monthly intervals was started in northern and southern parts of Irian Jaya. This was later supplemented by mass drug administration of weekly chloroquine (e.g. 450 mg base for adults), or chloroquine combined with pyrimethamine (e.g. 50 mg for adults) administered at least at monthly intervals. Malaria transmission was reduced but not eradicated by these measures. In the Demta district along the north coast west of Jayapura, DDT spraying reduced substantially the parasite rate of all three species (11.2%) compared to baseline (41%). This reduction was augmented following the administration of weekly CQ for eight weeks resulting in a parasite rate of 0.1%. The effect was greatest for *P. vivax* and *P. malariae*. However, in two villages that were close to those where no malaria control measures were in place, the asexual and gametocyte parasite rates actually increased because of a combination of poor quality housing and frequent contact with the untreated villages. In the Sentani area, there was also a decline in the three main species (falciparum, vivax, and malariae) but this was subsequently followed by a relative increase in the proportion of falciparum cases due to the high sensitivity of *P. vivax* and *P. malariae* to the drugs used and the greater increase in gametocyte carriage by those infected with *P. falciparum*.

In 1959, a medicated salt campaign was set up in villages along the north coast of Papua extending westwards towards Sarmi, the Tor river basin (some 230 km west of Jayapura), Arso, and Waris (south of Arso). There were three phases. Pyrimethamine

25
[PYR (⇔ 25 mg/week)] was used initially, followed by a mixture of PYR (⇔ 25 mg/w) and CQ (⇔ 140 mg/w), and finished with CQ (⇔ 140 mg/w) only containing salt. Overall, the salt programme gave disappointing results even in areas of DDT spraying and despite initial falls in parasite prevalence rates. The CQ only phase gave the best results in terms of a reduction in the prevalence of malaria. The main negative effects were an increase in *P. falciparum* prevalence rates six months into the pyrimethamine only programme, relatively less effect on younger children for all salts used, and the failure to interrupt malaria transmission. Pyrimethamine and proguanil resistant *P. falciparum* was reported three and half months into the programme from Arso. The salt programme was discontinued in 1961. Other malaria control activities included the use of larvicides in breeding sites in urban areas, parasite and entomological surveys, *in vivo* tests, staff training, and centralised drug purchase.

### 1.5.1 The effect of DDT spraying

DDT spraying has been conducted in different areas of Irian Jaya. Acceptance by the population was generally high but there was a tendency for urban dwellers to refuse house spraying; consequently, coverage was only 10% in Jayapura, Biak, and Sorong. Parasite rates declined in areas where spraying was done but little effect was seen in Genyem (60 km south west of Jayapura), Jayapura, and Abepura (part of the Jayapura conurbation). This was due to a combination of poor quality spraying and the emergence of DDT and chloroquine resistance. In these areas *P. falciparum* continued to be the predominant species with profound public health consequences.

### 1.5.2 Drug resistance studies in north-eastern Papua

Drug resistant *P. falciparum* was first recorded to pyrimethamine and proguanil by Meuwissen in Arso in 1959. He also documented the first clinical indication of reduced chloroquine sensitivity by recording prolonged parasite clearance times of 144 hours in 12 (41%) of 29 local residents following the administration of standard dose chloroquine. By the mid 1970s, chloroquine resistant falciparum malaria was confirmed clinically using the WHO *in vivo* test in residents from Jayapura and Hamadi (part of the Jayapura conurbation). Seven of 35 patients had recurrent parasitaemia, one on Day 11 and six on Day 21; these cases were probably RI resistant infections. At about the same time, RI chloroquine resistant *P. falciparum* was reported in two Japanese males who had been working in Irian Jaya, one in the ‘bird's peak’ area of western Irian (Manokwari...
Clyde characterised a strain of *P. falciparum* from an English patient who had travelled to several areas of Irian Jaya. By experimentally inducing malaria in non-immune American volunteers, he found resistance to several antimalarials. This strain was RII resistant to chloroquine and amodiaquine, RIII resistant to pyrimethamine (50mg/d x 3d), and had reduced sensitivity to varying doses of quinine but was fully sensitive to mefloquine. Daily proguanil as prophylaxis was also unsuccessful with breakthrough parasitaemia occurring on days 19 and 20.

In 1980, more *in vivo* and *in vitro* data of chloroquine resistant falciparum malaria were obtained in 21 patients from Nimboran district (~100 km west of Jayapura), an area of hyperendemic malaria by spleen rate. Two (10%) had a RII response to standard dose chloroquine and six isolates were resistant using the WHO microtest. In Nabire, an *in vivo* test was conducted of *P. falciparum* (n=26), *P. vivax* (n=22), and *P. malariae* (n=3), mixed falciparum/vivax (n=11), and mixed vivax/malariae (n=1); falciparum cases were counted as 37 and vivax as 34. Patients were transmigrants of five years residence and local Irianese. Chloroquine was well absorbed; Day 2 levels ranged from 691-3,119 ng/ml. Cumulative treatment failure rates were high: 30%, 58%, and 89% in the falciparum arm, and 15%, 45%, and 64% in the vivax arm on Days 7, 14 and 28, respectively. In Timika, seven day *in vivo* tests conducted in 78 patients (ages not stated) found a high proportion (49%) of RII (44%) and RIII (5%) resistance. *In vitro* testing (WHO microtest) also found evidence of chloroquine resistance but no resistance to S/P, quinine, or mefloquine. Clinical resistance (RI) to S/P was first reported in 1979 in a resident of Jayapura and a non-immune visitor to Timika.

*In vivo* tests combined with the molecular markers of chloroquine and S/P resistance were conducted between 1996 and 1999 in Armopa. All grades of chloroquine resistance were associated highly with the presence of mutant codons at position 86 (tyrosine-86) or 1042 (asparagine-1042) of the *pfmdr1* gene. Similarly, gene mutations that encode for dihydrofolate reductase (dhfr) and dihydropteroate synthetase (dhps) enzymes were associated with S/P resistance. The dhfr changes, Asn-108 and Arg-59, in combination with the dhps change of Gly-437 were associated with RI and RII/RIII resistance. The presence of the dhps mutation Gly-540, either alone, in combination with the dhfr mutations and/or the dhps Gly-437 mutation, was associated only with RII/RIII resistance.
S/P resistance. In another study conducted in Arso, the pfmdr1 genetic changes were consistent: the K1 (86-tyr) and 7G8 (Asp-1246) mutations were associated with \textit{in vivo} chloroquine resistance. An assessment of the T76 mutation of the pfcr gene has been carried out in Armopa in 107 transmigrasi adults and children. Using the 28 day \textit{in vivo} test end points (S or any degree of resistance), the T76 mutation had a sensitivity of 96% (79/82) for detecting CQ resistant falciparum malaria with a specificity of 52% (12/25). Using molecular techniques to reclassify recurrent parasitaemias (merozoite surface protein 2) as new or RI infections, and to detect subpatent parasitaemia (small subunit ribosomal RNA analysis) in microscopically diagnosed sensitive infections as sensitive or RI resistant infections, the sensitivity and specificity were 93% and 82%, respectively.

\subsection*{1.6 Towards an understanding of drug resistant malaria}

Most of our knowledge of drug resistance has been gained by studying \textit{P. falciparum}. Clinicians tend to assume drug resistance exists if sick malaria patients fail treatment despite taking properly prescribed, effective treatment or subjects develop malaria despite unfailing compliance with chemoprophylaxis. The clinical pharmacologist will observe a declining pharmacodynamic response of an antimalarial drug over time, indicating reduced parasite sensitivity. The laboratory scientist will observe an increase in the concentration of drug required to kill malaria parasites in culture, \textit{in vitro} resistance, and the molecular biologist will detect changes in certain malaria parasite genes that are associated with \textit{in vivo} and \textit{in vitro} resistance. Deciding which indicator/s of resistance is/are reliable is of particular importance for malaria control programmes for the formulation of drug policy.

The WHO definition of resistance is a good working definition because it deals with the most important consequence of drug resistance, namely, clinical resistance in the sick patient with malaria. Resistance is defined as the “ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject.” This definition was later modified to specify that the antimalarial drug “gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action.” This definition does not apply to prophylaxis failure (the failure to prevent the emergence of parasitaemia). Never the less, the latter is a useful indicator of early drug resistance. A distinction should be made between drug resistance and treatment failure.
because the latter might be interpreted erroneously as resistance by clinicians. Drug resistance is only one cause of treatment failure. Other causes of treatment failure include incorrect dosing, fake drugs, poor patient compliance, poor drug absorption (e.g. kwashiorkor, certain beverages), and diarrhoea. Acute malaria may also affect the absorption of drugs because of reduced food intake (empty stomach), vomiting, and reduced visceral perfusion. One important determinant of treatment failure is the acquisition of malaria immunity. A patient with naturally acquired immunity is more likely to clear resistant parasites than non-immune patients.

1.6.1 The development and spread of drug resistance

Drug resistance develops as a natural phenomenon and arises primarily through an increase in the prevalence of resistance-conferring gene mutations in an environment of selective drug pressure. Parasite replication results in random errors of replication that produce genetic mutations. Most of these mutations result in parasite death but others confer a survival advantage to the parasite when a given drug is present. There is inherent variation in the susceptibility of parasites to antimalarial drugs; highly susceptible parasites are killed by low drug concentrations and vice versa. The greater the biomass (parasitaemia), the more likely the diverse the parasite population regarding drug sensitivity. Failure to kill resistant asexual parasites will result in the passing on of mutant gene/s to the next generation via gametocytes. These sexual forms are more likely to be produced by resistant infections. Other factors associated with resistance include the drug concentrations that the parasites are exposed to, the pharmacological characteristics of the antimalarial drugs used, the local malaria epidemiology, the genetic mechanisms underlying resistance, the level of individual immunity, and the way drugs are used.

1.6.2 Drug pharmacokinetics and pharmacodynamics and resistance

The drug pharmacokinetic (PK), pharmacodynamic (PD) characteristics, and the molecular mechanism of drug resistance are key factors that define the intrinsic susceptibility of drugs to resistance. Drugs more prone to resistance generally have the following properties: (i) intrinsically weak antimalarial activity manifest as a slow reduction of the parasite biomass [a low parasite reduction ratio (PRR)], (ii) a long elimination half life, (iii) a 'flat' dose response or concentration effect relationship, (iv) cross resistance between drugs of a similar class, and (iv) a mechanism of action that can
be overcome easily through single point mutations e.g. atovaquone, rather than through several point mutations, so called polygenic resistance e.g. chloroquine. Selection for resistance occurs in two ways. The first occurs when a primary infection is not cleared by drug treatment. If this infection contains sensitive and mutant parasites, then, as the parasites multiply, many asexual cycles will be exposed to progressively lower blood concentrations, exposing an increasing number of parasites to the drug. This offers a greater opportunity for resistance conferring genetic mutations to occur and for these resistant parasites to survive the drug concentrations. This is more likely to occur if the initial drug concentration is low e.g. because of a sub therapeutic dose, and if the drug has a longer half life, resulting in greater drug exposure. With short half-life drugs e.g. artesunate (T1/2p ~ 1 hour), quinine (T1/2p ~ 12 hours), dividing malaria parasites will encounter maximum therapeutic drug concentrations followed by sub therapeutic concentrations for only a short time. At the next dose, the parasites will again encounter maximum therapeutic drug concentrations. The probability of selecting mutant parasites is, therefore, considerably reduced.

The second mechanism is when a newly acquired infection develops in the presence of residual sub-maximal blood concentrations of an antimalarial drug from an antecedent treatment. These drug concentrations may be sufficient to eradicate a sensitive infection but not a resistant one. In this way, the drug filters out (selects out) the sensitive parasites, leaving the resistant ones to emerge. This is important in enhancing the spread of drug resistance. Again, the longer the terminal elimination half-life, the more likely these parasites will be exposed to low drug concentrations. An interesting example is the case of the antifolate drugs sulfadoxine/pyrimethamine (S/P) and dapsone and chlorproguanil (LapDap). The half lives of the S/P components (S=10days, P=4days) are longer than those of dapsone (20-30h) and cycloproguanil (12-20h). Use of S/P was associated with the loss of wild type parasites and an increase in the prevalence of mutant parasites (mutant dhfr and dhps genes). By contrast, LapDap was not associated with a change in the proportions of wild type dhfr and dhps alleles in the pretreatment and recurrent parasitaemias.

Antimalarial drugs differ in their inhibitory effects on asexual malaria parasite development which and can be measured using the PRR. The PRR=1-parasite count/parasite count0 (48=48 hours). The PRR is related to the drug's stage specificity
(stage/s in the life cycle). Antimalarial drugs reduce the parasite numbers with each asexual cycle by between 10-10,000 (Appendix 5).\(^{88}\) The artemisinin compounds have the highest PRR and the broadest stage specificity, killing young rings, trophozoites, and schizonts.\(^{92}\) The antimalarial antibiotics e.g. doxycycline, azithromycin, have the lowest PRR. In between are quinine, mefloquine, chloroquine, and amodiaquine whose greatest activity is against the mid-late trophozoite, and the antifolate drugs (e.g. sulfadoxine, pyrimethamine, dapsone, proguanil) which act against mature trophozoites and early schizonts. Very young rings and mature schizonts are relatively resistant to all antimalarial drugs. The clinical and parasitological responses are faster for drugs that act against earlier parasite stages.

Parasite killing as a function of drug concentration has a sigmoid relationship (Appendix 6). The \(E_{\text{max}}\) represents the top of the dose-response or concentration-effect relationship and differs widely between the different antimalarial drugs; the more active drugs with a higher PRR have a lower \(E_{\text{max}}\). The lowest drug concentration that results in the \(E_{\text{max}}\) is the minimum parasiticidal concentration (MPC). Parasite reduction appears to be a first order (linear) process. Therefore, a fixed fraction of the population is removed at each successive asexual cycle if the MPC is exceeded. As resistance develops, parasite susceptibility and PRR decrease, and there is a shift to the right of the sigmoid curve i.e. a higher drug concentration is required to achieve the same level of parasite killing but the \(E_{\text{max}}\) is unchanged. The standard \textit{in vitro} drug measurements (IC\(_{50}\), IC\(_{90}\), and IC\(_{99}\)) will increase (see 1.7.4 for definitions). With further parasite resistance, the IC\(_{50}\) increases and the \(E_{\text{max}}\) falls. \textit{In vivo}, parasite clearance still occurs, albeit more slowly, provided the free drug, plasma concentration exceeds the concentration required to maintain the parasite multiplication rate below one, the minimum inhibitory concentration (MIC).

The slope of the sigmoid curve is also relevant to drug resistance. A steep slope indicates large parasite inhibition for a small change in drug concentration whereas a flatter slope indicates that a higher drug concentration is necessary to achieve the same antiparasitic effect. Selection of resistance in bacteria is greatest at 'intermediate' levels of drug activity, generally between 20% to 80% of the maximum effect; this may also apply to malaria parasites.\(^{86}\) Drugs with long elimination half lives are more vulnerable because of the persistence of sub therapeutically drug concentrations. In practice, the drug's pharmacodynamic action may be improved by increasing its dose. In Thailand,
mefloquine was increased from 15 mg/kg to 25 mg/kg with a resulting increase in efficacy but not to pre-resistant levels. However, over time the efficacy of the higher dose also fell.

1.6.3 Antimalarial drug cross resistance

Cross-resistance is the occurrence of resistance in one drug as a result of resistance in another drug. These drugs usually have chemical similarities and share similar molecular mechanisms of resistance. Several examples exist e.g. chloroquine and amodiaquine, halofantrine and mefloquine, quinine and mefloquine. There is one report of an inverse relationship between chloroquine and mefloquine resistance.

Cross resistance may have explained the high IC$_{50}$ values for mefloquine in several west African countries that was interpreted as innate mefloquine resistance because of the lack of mefloquine drug pressure. Supporting evidence for this phenomenon also came from mefloquine prophylaxis failures in non immune travellers. However, as treatment, mefloquine has been highly efficacious (>90%) in west Africa. By contrast, in Cameroon an interesting geographical difference in mefloquine sensitivity has been documented. Using the radiolabelled $^3$H-hypoxanthine method and a cut off limit of 30 nmol/L to define in vitro mefloquine resistance, 26 (20%) of 133 isolates from northern Cameroon were mefloquine resistant compared to one of 73 (1.4%) isolates from southern Cameroon. There was also a close correlation ($r=0.67$) between the in vitro response to quinine and mefloquine. Seven day in vivo tests were also performed using MQ 25 mg/kg in asymptomatic children aged 1-10 years. RII-RIII responses were found in six of 46 (13%) northern children compared to no resistance in 40 southern children. Mefloquine was not in use at the time of the study. Indeed, MQ has never been registered in Cameroon (P. Ringwald, personal communication, 2002). However, quinine was widely used in north Cameroon and may have led to secondary mefloquine resistance.

1.6.4 Epidemiological factors and resistance

Drug resistance will spread and increase if the malaria life cycle is completed and resistant genes are transmitted to new hosts. The rate at which resistance spreads depends on the relative reproductive rates of the drug sensitive versus the drug resistant parasites. Several factors are involved: (i) the probability of drug resistant parasites transmitting viable gametocytes rather than sensitive parasites; this is a function of the duration of
patent gametocytaemia in man, and their infectivity to mosquitoes; (ii) the intensity of transmission, (iii) the immunity of the human population that affects both asexual and sexual forms, (iv) the extent of antimalarial drug use, and (v) the ability of the drug to reduce gametocyte carriage.\textsuperscript{107, 108, 109, 110} The intensity of transmission is also important but different factors favour the selection and spread of drug resistance in low and high transmission areas.

Chloroquine resistance was first documented in the low transmission areas of Venezuela and Colombia in the late 1950's, and the early 1960's in Thailand.\textsuperscript{111, 112, 113} Drug pressure was the main reason why resistance developed in these areas. In low transmission areas, high biomass infections are more likely, there is less naturally acquired immunity against asexual (less eradication) and sexual forms (effect on transmissibility), drug pressure is high because most patients are symptomatic and seek treatment, and male and female gametocytes are usually derived from the same infection.\textsuperscript{86} As transmission increases, the rate at which resistance spreads increases until premunition (anti parasitic immunity) is acquired and is a major factor in retarding the rate of spread of resistance. Thereafter, an increasing proportion of transmission derives from older children and adults who harbour asymptomatic infections that are subject to less selective drug pressure. The combination of less selective pressure and high background immunity are the main reasons for a slower rate of transmission of drug resistance.

1.6.5 Operational and behavioural factors and resistance

Drug use by communities is an important determinant of drug resistance. Practices that lead to sub therapeutic drug levels promote drug resistance by failing to kill all parasites. In endemic areas, antimalarial drugs should, ideally, only be used to treat confirmed malaria cases and at therapeutic doses. However, limited access to the formal health sector often leads to the purchase of drugs from local vendors or pharmacies. The high cost of drugs may preclude the purchase of a therapeutic course. Inappropriate antimalarial dosages and inappropriate drugs may also be dispensed. Fever is often regarded as malaria and leads to over use of antimalarial drugs.\textsuperscript{114, 115} Compliance is another key issue. Mothers commonly give their children a partial dose, saving the rest of the drug for siblings or the next bout of fever.\textsuperscript{116} Many patients also stop taking drugs when they feel better before the full course has been taken; this is more likely after
rapidly acting drugs like artesunate, or chloroquine and amodiaquine because of their anti-inflammatory properties. Long or complex drug regimens, and poor tolerability also compromise compliance.\textsuperscript{117} The formulation and proper implementation of drug policy by malaria control programmes is crucial to ensure that all parts of the health system are properly informed and understand the basics of prescribing appropriate drug regimens.

### 1.7 Measuring and detecting drug resistance

*In vivo* and *in vitro* tests, and the detection of the genetic markers of resistance are used for measuring and detecting resistance. For clinicians, the *in vivo* test is the most important. *In vitro* testing and the genetic markers of resistance are more useful in epidemiological studies.\textsuperscript{118} The roles of these methods in malaria control programmes is a constantly changing field. Although the *in vivo* test provides the best data, it is costly and requires long follow up which can be difficult to achieve in the field outside of the research setting. *In vitro* testing has its technical problems, several methodologies exist, and *in vitro in vivo* correlations vary.\textsuperscript{119} Molecular markers are potentially good public health tools for resistance surveillance because of the ease of obtaining filter paper blood spots. Correlation of these markers with the *in vivo* tests varies and is the subject of much research.

#### 1.7.1 *In vivo* tests of resistance

##### 1.7.1.1 WHO test of parasitological response of *Plasmodium falciparum*

This test was designed originally to assess the *P. falciparum* parasitological response to chloroquine in asymptomatic individuals but has since become used for all antimalarial drugs in symptomatic patients.\textsuperscript{75} \textsuperscript{120} This *in vivo* test was used for the clinical trials in this thesis for both vivax and falciparum malaria because at the time there were no published WHO recommendations for vivax or falciparum malaria testing in areas of low transmission.

The parasitological responses for *P. falciparum* are divided into four categories: parasite sensitivity (S) or resistance at three levels: RI, RII, or RIII. The parasitological definitions are:

- S or S/R1. In the extended test, the parasites are sensitive if no asexual parasites are found by Day 7 and parasites do not reappear by Day 28. In the 7-day field test, the
infection may be either S or resistant at RI (S/RI) level if no asexual parasites are present on Day 7.

- **RI.** In the extended test, parasites are resistant at the RI level if asexual parasites disappear by Day 7 but return within 28 days, if reinfection has been excluded. In the 7-day field test, parasites are resistant at the RI level if asexual parasites disappear for at least 2 consecutive days but return and are present on Day 7.

- **RII.** The parasites are resistant at RII level if asexual parasite does not clear by Day 7 but is reduced to 25% or less of the original pre-test level during the first 48 hours of treatment.

- **RIII.** The parasites are resistant at RIII level if asexual parasitaemia is reduced by less than 75% during the first 48 hours, or if it continues to rise or remain stable.

Clearly, the duration of follow-up affects the interpretation of the test e.g. the 7-day *in vivo* test only detects high grade resistance. Ideally, the duration of *in vivo* tests should be extended e.g. to 42 or 63 days for drugs with long half lives such as CQ, MQ, S/P because drug concentrations persist that may be pharmacologically active against sub patent parasites. The longer the follow up, the greater the probability of detecting a late resistant infection but this also increases the chance of a new infection. With a mean incubation period for *P. falciparum* of 12 days, a recurrent parasitaemia within 14 days is more likely to be a resistant (RI) infection.

**1.7.1.2 The WHO assessment of therapeutic efficacy for *P. falciparum***

The WHO introduced a new *in vivo* test in 1996 (Appendix 7) that was designed primarily for use in high transmission areas as a public health tool for drug policy makers. This protocol has since been revised and published following a consultation in 2002. The 2003 edition uses both clinical and parasitological criteria and has four end points: (i) adequate clinical and parasitological response (ACPR), (ii) early treatment failure, and (iii) late clinical failure, and (iv) late parasitological failure (Appendix 8). Follow up extends to Day 14 or Day 28, if adequate resources are available, including the use of the polymerase chain reaction (PCR) to differentiate cases of recurrent parasitaemia. This protocol removes asymptomatic recurrent parasitaemia from the definition of ACR, a weakness of the 1996 protocol. Another improvement is a minimum level of parasitaemia for entry into the test to reflect the pyrogenic density (the level of
parasitaemia at which symptoms and fever occur) for different areas of transmission. This allows for comparisons between studies.

1.7.1.3 An in vivo test of *P. vivax*

An in vivo test for *P. vivax* has only recently been developed by the WHO and is detailed in the 2003 document mentioned above. Hitherto, defining vivax resistance could be done by either applying the Berliner adapted system of Baird or to use the WHO parasitological in vivo test for falciparum malaria, as was done for this thesis (see Materials & Methods). Certainly, failure to clear vivax parasitaemia after supervised, standard dose chloroquine is good evidence of resistance, even allowing for the wide interindividual absorption. Differentiating a recurrent vivax parasitaemia cannot be done on clinical grounds. PCR and the timing of the recurrence offer some help but neither can exclude definitively a recrudescence from a new infection or a relapse of liver hypnozoites. A recurrence within 14 days of therapy is more likely to be a resistant infection; after this time, it could be a resistant, or new, or relapse infection. A fully sensitive *P. vivax* infection does not usually reappear within 28 days of follow up; indeed in the early work on chloroquine treatment of *P. vivax*, relapses began to occur after five weeks following standard dose CQ. Using the CQ cut off of ≥ 100 ng/ml at the time of recurrent parasitaemia, the cause of the recurrent parasitaemia is less important (See 1.7.6). Although this is evidence of resistance, this finding does not in itself predict success or failure if standard dose CQ is given to treat this recurrent parasitaemia.

This protocol also sets a lower limit of parasitaemia of 250 parasites per μL. The end points / definitions of treatment failure are defined below:

- Clinical deterioration due to *P. vivax* illness requiring hospitalisation in presence of vivax parasitaemia.
- Presence of parasitaemia and fever (axillary temperature ≥ 37.5 °C) any time between Day 3 and Day 28.
- Presence of parasitaemia on any day between Day 7 and Day 28, irrespective of the clinical condition of the patient.

If a patient has a recurrent parasitaemia due to *P. falciparum* infection after clearance of *P. vivax* parasites, this is not classified as a *P. vivax* treatment failure.
1.7.1.4 Problems with in vivo drug assessments

The WHO parasitological in vivo test only measures the parasitological response. When first used, there was no distinction made between patients with symptomatic and asymptomatic malaria, and there was no lower limit of parasitaemia. If a patient deteriorated clinically within the first 48 hours e.g. due to drug induced vomiting, progression to severe malaria, he/she was excluded from the analysis because of the failure to meet the strict parasitological definition of resistance. The 1996 therapeutic efficacy test for areas of high transmission classified asymptomatic recurrent parasitaemia as ACR and, therefore, underestimated resistance. Slow acting drugs may overestimate the rate of ETF especially in children with high parasitaemias who still have a high proportion of parasites present on Day 3. In a study in two areas of Mali, Bandiagara (lower CQ resistance) and Mopti, the 1996 in vivo test overestimated ETF at both sites. This was more marked in Bandiagara where 10 [71% (95% CI 42-91)] of 14 ETFs were either cured (n=8) or had recurrent parasitaemia (n=2) \(^\text{125}\).

During follow up, recurrence of parasitaemia may occur that may be due to a new infection, a relapse (P. vivax or P. ovale), or a resistant infection. The longer the follow up, the more likely recurrent parasitaemia will occur. A recurrent parasitaemia cannot be distinguished on clinical grounds. A recurrent parasitaemia may also arise if (i) an incubating infection is present at the time of the test, and (ii) there is early reinoculation with the same infecting mosquito. PCR is a useful technology to assist with the interpretation of the in vivo test by genetically characterising and comparing the recurrent parasitaemia with the Day 0 parasitaemia.

1.7.1.5 Polymerase chain reaction and in vivo assessments

PCR is a method of examining the DNA (deoxyribonucleic acid) of malaria parasites and exploits the fact that malaria parasites of the same species have different genetic compositions (parasite diversity). PCR detects the different malaria species and genetic differences within a species \(^\text{126}\). For differentiating recrudescent falciparum infections from new infections, three genes that encode for three highly polymorphic (the occurrence together in the same population of two or more genetically determined phenotypes) antigens are commonly used: glutamate rich protein (GLURP) and the merozoite surface proteins one and two (MSP-1, MSP-2). The principle of differentiating recurrent parasitaemias depends on genetically identifying the Day 0 and recurrent
parasitaemias. For *P. vivax*, the MSP1 and the circumsporozoite protein genes have been used to compare the paired isolates.\textsuperscript{127} A matching pair is consistent with a recrudescent infection and a non-matching pair with a new infection. In addition, for *P. vivax*, a matching pair is also consistent with a relapse parasitaemia from liver hypnozoites of the original infection, and a non matching pair with a relapse from an antecedent infection.

There are certain technical issues with PCR that effect its sensitivity and specificity. These include: (i) the sensitivity of detecting the Day 0 parasitaemia e.g. low density parasites may be missed, (ii) the resolution limits for detecting differences in alleles, (iii) the occurrence of genetically different multiple infections on Day 0 and/or during follow up, (iv) the local transmission patterns that may result in repeat inoculations within a short time span or inoculation with a different malaria species. Therefore, a matching pattern cannot be interpreted with a 100% certainty as a recrudescent infection. However, using a more refined technique e.g. nested PCR with single-stranded conformational polymorphism techniques to analyse GLURP, MSP-1 and MSP-2, the probability of differentiating a recurrent falciparum parasitaemia is ≥ 95%.\textsuperscript{126} There is less experience with the PCR genotyping of *P. vivax* but a matching genotype indicates a >80% probability of a recrudescent or relapse (K. Kain, personal communication).\textsuperscript{127}

1.7.2 The clinical assessment of drug responses

Clinicians assess patients and their responses to treatment using clinical or laboratory measures that include: (i) the fever clearance time, (ii) the parasite clearance time, (iii) cure rates, (iv) haematological recovery, (v) coma recovery in cerebral malaria, and (vi) resolution of organ dysfunction and metabolic acidosis in severe malaria.\textsuperscript{128} *In vivo* resistance generally manifests initially as a slowing of the parasite and fever clearance time - patients take longer to get better. This is then followed by an increase in the rate of recrudescence in non-immune patients with uncomplicated malaria; clinicians will notice patients returning to the clinic for retreatment of their malaria. In endemic areas this manifests first in young children who have less immunity and greater parasite burdens. As resistance increases treatment failure rates increase, and the median time to recrudescence shortens. Eventually patients are encountered whose parasitaemia does not clear, although they may improve symptomatically e.g. because of the anti inflammatory effects of chloroquine. Finally, with high grade resistance there is no or little clinical or parasitological response to treatment and patients are at risk of developing severe malaria.
In high transmission areas, an increasing prevalence of anaemia may be the first sign of worsening drug resistance with a concomitant increase in blood transfusion requirements.\textsuperscript{21, 129}

1.7.3 \textbf{Prophylaxis breakthrough}

Although not a formal test of resistance, prophylaxis breakthrough in the presence of adequate, prophylactic drug levels is evidence of reduced parasite sensitivity because parasites have emerged at drug concentrations above the MIC of fully sensitive parasites. Confirmation of the latter requires formal efficacy testing. As with treatment, appropriate dosing is a critical factor before ‘prophylactic resistance’ can be diagnosed. This is illustrated well by the case of mefloquine.

When first tested, mefloquine dosed every two weeks, was as effective as weekly mefloquine in semi immune Thai adults in 1980.\textsuperscript{130} However, by 1984, there were a large number of failures using biweekly MQ.\textsuperscript{131} Mefloquine prophylactic failures also occurred in West Africa when the recommended regimen was weekly mefloquine for four weeks then bi-weekly.\textsuperscript{132, 133} Breakthrough parasitaemias of 1 to 1.4/100 person-months were documented in long term users during the phase of the biweekly dosing. These occurred at a mean of 12 days after the last MQ dose when whole blood MQ levels were low, ranging from 62 to 398 ng/ml. Weekly mefloquine was subsequently introduced with a marked drop in prophylaxis failures, 0.2/100p-m. More detailed PK work in a different cohort of long term travellers documented clearly lower mean Day 14, plasma MQ trough levels compared to Day 7: 888 vs. 1188 ng/mL, supporting the use of the weekly mefloquine.\textsuperscript{134}

The importance of measuring drug levels to diagnose prophylactic failures was also shown in a study of 16 French travellers to West Africa.\textsuperscript{101} Some took the recommended dose (250 mg weekly), others half this dose, as recommended by the travel clinic to reduce toxicity, whilst others were not fully compliant. Plasma MQ levels, taken between 3 and 26 days after the last MQ dose in nine \textit{P. falciparum} failures, ranged from 345 to 3,000 ng/ml. At the time of the study, the plasma MQ level that was considered adequate to suppress \textit{P. falciparum} by the drug manufacturer was between 200-300 ng/ml. These nine failures were considered to be due to mefloquine resistance. However, more detailed PK MQ data led to a revision of these drug levels. Lobel \textit{et al} established that the prophylactic effectiveness of MQ was critically dependent on MQ levels. They
measured whole blood MQ concentrations (ratio of whole blood: serum/plasma is 1:1.28) and estimated that 99% prophylactic efficacy (PEf) could be achieved with whole blood MQ levels $\geq 915$ ng/ml, 95% with $\geq 620$ ng/ml, and 90% with $\geq 462$ ng/ml. These data show that simply attaining a level above a certain threshold is insufficient for diagnosing prophylactic failure due to resistance. Accordingly, some of the French 'prophylactic failures' were clearly due to inadequate MQ levels. In another report of MQ prophylaxis failures in four travellers to West Africa, two had plasma MQ levels measured at the time of patent parasitaemia; these were 507 ng/ml and 105 ng/ml. Both travellers said they were compliant but the traveller with a level of 105 ng/ml stopped his MQ after three weeks (one week early) on return to France. In vitro data ($^3$H-hypoxanthine microtest method) from three cases showed reduced MQ sensitivity. The measured $IC_{50}$ values were $> 30$ nmol/L (35, 38, 82 nmol/L). Three were treated successfully with therapeutic doses of MQ; the fourth was treated with CQ. Prophylactic failure in these travellers was probably due to a combination of reduced parasite sensitivity to MQ, poor compliance, and/or poor drug absorption. The successful use of MQ therapy excludes strictly defined parasite resistance as the cause of these failures.

Current recommendations for MQ prophylaxis vary. Some authorities recommend starting MQ one week before entry into the endemic area whilst others three weeks; the rationale for the latter is to detect MQ toxicity. Both recommendations, however, are too short to achieve steady state MQ levels which are attained after 6 to 7 weeks of weekly dosing. During this time, mean MQ levels are significantly lower than those obtained after a loading dose of mefloquine (250 mg/d x 3 days) at the start of prophylaxis. The loading dose has not gained favour with civilian travellers but has been used and been well tolerated by soldiers. An important PK finding is the wide interindividual absorption of MQ. After 18 weeks of MQ prophylaxis, plasma MQ concentrations (taken 2 days post last dose) in 87 Dutch Marines ranged from 242-1,918 with a mean of 979 ng/ml. Corresponding whole blood levels were 159-1,671 and 788 ng/ml. In a small cohort of 15 travellers, the post, first dose (250 mg), plasma, $C_{\max}$ values ranged from 412-956 ng/ml; at steady state the trough MQ levels also varied ~ two fold: 888-2080 ng/ml. For some MQ users, therefore, prophylaxis failures will occur despite full compliance. A reasonable PK marker of which is a CMQ:MQ ratio $\geq 2:1$ (CMQ is the non active carboxyacid metabolite of mefloquine).
1.7.4 *In vitro resistance - description and principles*

*In vitro* laboratory methods assess the ability of differing concentrations of antimalarial drugs to inhibit the growth of malaria parasites in culture and is a measure of the intrinsic drug susceptibility of a given malaria isolate. Most *in vitro* work has centred on *P. falciparum*. *P. vivax* is very difficult to culture (C Ohrt, personal communication). Parasite growth inhibition is assessed either biochemically or morphologically (rings to schizonts). The WHO Mark III *in vitro* test assesses the inhibition of *P. falciparum* from ring forms to schizonts.\(^{139}\) Patient's blood is inoculated into plates that are pre-dosed with different concentrations of antimalarial drug; the control well contains no drug. Giemsa-stained slides are prepared and read after 24 to 48 hours of culture. The degree of parasite maturation (number of schizonts) is compared with that of the control well. Although, this test has the advantage of requiring minimal equipment, it is cumbersome and lacks precision. Incubating to 48 hours improved results in Indonesia.\(^{140}\) Other *in vitro* tests rely on the use of radiolabelled \(^3\)H-hypoxanthine, malaria parasite lactate dehydrogenase (pLDH), and the detection of the histidine rich protein-II (HRP-II). Hypoxanthine, a purine, is essential for the synthesis of DNA. Measuring the uptake of radiolabelled \(^3\)H-hypoxanthine as a marker of parasite growth is the basis of this *in vitro* test.\(^{141}\) It is not widely used because of the use of radio-labelled material (raising disposal problems) and the need for expensive equipment (a beta-counter). The basis of the pLDH microtest is the production of pLDH by asexual falciparum parasites. It is adapted for field use and requires minimal equipment e.g. spectrophotometer. It yields quantitative results, is unaffected by the immune status of the host, and correlates well with the \(^3\)H-hypoxanthine *in vitro* method.\(^{142}\) The HRP-II *in vitro* relies on the detection by enzyme-linked immunosorbent assay (ELISA) of the HRP-II protein that is produced by *P. falciparum*. It is reliable, easy to perform, and correlates well with the isotopic \(^3\)H-hypoxanthine assay, and the WHO schizont maturation tests \((R = 0.959; P < 0.0001)\).\(^{143}\)

The inhibition of parasite growth as a function of drug concentration is sigmoid in shape (Appendix 6). Drug activity is usually expressed as follows:

- Maximum drug effect \((E_{\text{max}})\) = maximal inhibitory effect of drug (the top of the sigmoid curve); greater drug concentrations do not produce more parasite inhibition.
- \(\text{MPC} = \text{minimum parasiticidal concentration; the lowest drug concentration that produces the maximum effect (}E_{\text{max}}\)
• IC\textsubscript{50}, IC\textsubscript{90}, IC\textsubscript{99} = drug concentration at which 50%, 90%, and 99% of growth inhibition occurs relative to that of the control.

• Minimum inhibitory concentration (MIC) = lowest drug concentration that prevents the growth of asexual parasites.

One of the major problems with \textit{in vitro} tests is the determination of the threshold IC\textsubscript{50} values that distinguish sensitive from resistant parasites. There are currently no fully validated cut-off points for assessing \textit{in vitro} resistance. Therefore, the positive predictive value of a given IC\textsubscript{50} for predicting \textit{in vivo} resistance is unknown\textsuperscript{119} Certain drug levels have been proposed by the WHO (Mark III \textit{in vitro} test) and the Walter Reed Army Institute of Medical Research (WRAIR) to distinguish sensitive from resistant parasites (Table 1.2).

Table 1.2 \textit{In vitro} drug concentrations used to determine drug resistance (Source: WHO and WRAIR).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Drug concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant*</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>≥ 8*</td>
</tr>
<tr>
<td>Sulfadoxine</td>
<td>≥ 1,000</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>≥ 12.5</td>
</tr>
<tr>
<td>Quinine</td>
<td>≥ 256</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>≥ 64</td>
</tr>
<tr>
<td>Amodiaquine</td>
<td>≥ 4</td>
</tr>
<tr>
<td>Halofantrine</td>
<td>-</td>
</tr>
</tbody>
</table>

* values from the WHO in pmol/well
† values from WRAIR in ng/ml

The WHO values for sensitive infections have been validated but not those for resistant infections. (P. Ringwald, personal communication). To circumvent the difficulty of using cut off values, evidence of emerging or increasing drug resistance over time is present if the median IC\textsubscript{50} increases over time using the same methodology. As resistance develops, the curve shifts to the right and eventually the E\textsubscript{max} will fall. Large shifts of the curve are associated with point mutations that cause a marked reduction in parasite susceptibility e.g. cytochrome \textit{bc1} gene (involved in the electron transport chain) and
atovaquone resistance, dhfr and dhps genes and sulfadoxine/pyrimethamine resistance.\textsuperscript{89} In vitro tests have been used primarily to conduct surveillance of drug resistance and to support the findings of clinical trials. Monitoring trends is their most useful application.\textsuperscript{118}

1.7.5 Drug pharmacokinetics and the measurement of resistance

The in vivo assessment of drug resistance should, where possible, include measuring drug concentrations to determine whether adequate drug absorption has taken place - a prerequisite for meeting the WHO definition of resistance.\textsuperscript{75} Interpretation of these measurements requires an appreciation of drug pharmacokinetics. Drug disposition in the humans, namely, absorption, distribution, metabolism, and elimination define the PK properties of a given drug. The following PK parameters are routinely measured and reported:

- $T_{\text{max}}$ = the time taken to reach maximum concentration
- $C_{\text{max}}$ = the maximum drug concentration at $T_{\text{max}}$
- Distribution half life ($T_{1/2a}$) = the time taken for half the drug to disappear from blood because of distribution
- Elimination or terminal half life ($T_{1/2\beta}$) = the time taken for half the drug to disappear from the blood due to its metabolism and excretion
- Residence time = total time a drug is present in man and $\approx 3 \times T_{1/2\beta}$
- Volume of distribution (Vd) = a measure of drug distribution in body compartments
- Area under the curve (AUC) of a plot of drug concentration over time
- Therapeutic range = the concentration of drug that confers antimalarial activity within a safety margin
- Free plasma concentration = unbound drug concentration in plasma
- Protein binding = the degree to which drugs bind to plasma proteins e.g. albumin, $\alpha$-1 acid glycoprotein
- Total drug concentration = the sum the free and protein bound drug concentrations
In malaria, it is the asexual parasites that cause clinical disease. Although intraerythrocytic, they are exposed to drug concentrations that correspond closest to the free (unbound) plasma concentration rather than the red cell concentration (derived from measurements mainly in uninfected erythrocytes). It is unbound drug that enters parasitised red cells to exert its pharmacological action.\textsuperscript{83} Effective antimalarial therapy requires continual drug activity over at least four life cycles i.e. > 6 days.\textsuperscript{85} Extravascular drug distribution and tissue penetration are not important for parasite killing. Drugs with long half lives are dosed once e.g. mefloquine (T\textsubscript{1/2} 2-3 weeks), sulphadoxine (10 days) / pyrimethamine (4 days) or once daily e.g. chloroquine (1-2 months). Shorter half life drugs e.g. halofantrine (3-5 days), lumefantrine (3-6 days), and atovaquone (1-3 days) need to be given over several days. Drugs with the shortest half lives need dosing over seven days e.g. quinine (16 hours in patients) and artemisinin derivatives (1 hour).

Malaria itself may affect drug kinetics. Vomiting results in reduced drug absorption. There is contraction of the volume of distribution, especially for drugs that are highly bound to α-1 acid glycoprotein like quinine. The acute inflammatory response results in an increase in α-1 acid glycoprotein and an increase in the total quinine concentration. Reduced drug clearance due to impaired hepatic and renal function results in the prolongation of the half life e.g. the T\textsubscript{1/2} of quinine is 11h (volunteers), 16h (uncomplicated malaria), and 18h (severe malaria).\textsuperscript{145}

1.7.6 The problem of defining a therapeutic range for chloroquine

Effective antimalarial treatment depends upon drug concentrations within the therapeutic range acting for a sufficient length of time to kill all malaria parasites without undue toxicity to the host. Determining the therapeutic range, particularly its lower limit, is problematic because parasite sensitivity varies, interindividual CQ absorption varies widely, and immunity synergises with drug action. Currently the therapeutic range for chloroquine remains undefined for any malaria species (most work has focussed on \textit{P. falciparum} and \textit{P. vivax}). In addition, the precise PK PD parameters determining parasite killing remain uncertain.\textsuperscript{85} Three PK PD parameters are considered important: (i) the MIC, (ii) the MPC, and (iii) the AUC. For chloroquine, and other long half life drugs, the AUC which lies above the MIC is important and is closely related to the time chloroquine concentrations exceed the MIC and the MPC. Therefore, the extent of chloroquine absorption and its duration of action are important. The C\textsubscript{max} appears less important
because concentration dependent killing of parasites by chloroquine is not a significant PK-PD factor for producing parasitological cure. In Gambian children with severe malaria, higher peak parenteral (sc, im, iv) CQ concentrations did not result in more rapid fever or parasite clearance times compared to chloroquine delivered by nasogastric tube.\textsuperscript{146} Measuring the drug concentration at the time of recurrent parasitaemia is informative in terms of giving an idea of the MIC for that particular infection.

In one study of 39 semi immune Tanzanian children, Hellgren \textit{et al} examined the PK-PD relationship of CQ and uncomplicated falciparum malaria, using resistance (RI/II/III) as the PD endpoint.\textsuperscript{147} They estimated the \textit{in vivo} MICs from the mean, plasma CQ concentrations on the day before and after either RII parasites increased in number or RI parasites reappeared. The median (range) MICs were 147 (44-673) nmol/L [\(\equiv 44 (13-226)\) ng/ml] for a RI response, and 790 (444-869) nmol/L [\(\equiv 237 (133-261)\) ng/ml] for a RII response, providing supporting pharmacological evidence for increased parasite resistance. This small study found a non significant \((P=0.058)\) lower AUC in the RII cases \((n=7)\) compared to the RI cases \((n=19)\) but a significantly lower C\(_{\text{max}}\), suggesting PK factors (in this case absorption) might have been influenced by the disease itself. For field trials, to obviate the need for multiple sampling, the C\(_{\text{max}}\) can be used as the PK surrogate marker for the AUC. This was done in the chloroquine doxycycline treatment trial presented in this thesis and in many \textit{in vivo} tests conducted in Indonesia by the US Navy. The C\(_{\text{max}}\) of orally administered CQ (25 mg/kg) occurs at 51 hours.\textsuperscript{148}

Determining the therapeutic range for \textit{P. vivax} malaria is also problematic because of the changing sensitivity of \textit{P. vivax} over time. A rough estimate of the lower end of the therapeutic range could be the minimum, Day 2 C\(_{\text{max}}\) that cures patients, assessed by Day 28. In a small study of Swedish patients \((n=15)\) with non-falciparum malaria, the minimum Day 2, total whole blood CQ level after standard dose CQ was 1.76 \(\mu\text{mol/L} \equiv 528.5\) ng/ml. On Days 4 and 7, the respective levels were 1.48 \(\mu\text{mol/L} \equiv 444.4\) ng/ml and 1.09 \(\mu\text{mol/L} \equiv 327.3\) ng/ml.\textsuperscript{148} A similar result was obtained in 23 Vietnamese with CQ sensitive \textit{P. vivax} (517 ng/ml) and in 25 Indonesians with CQ sensitive vivax and falciparum infections (472 ng/ml); the latter study did not distinguish between vivax and falciparum patients.\textsuperscript{149,150}

Research conducted in 1948 provides key data on the PK characteristics of CQ in vivax malaria. Berliner and colleagues studied the dose response of experimentally
induced, McCoy strain (from Tallahassee, Florida, USA) *P. vivax* malaria in 25 American volunteers. They classified the therapeutic effects in three ways: (i) Class I - no certain effect, (ii) Class II - temporary suppression of parasitaemia and/or fever, and (iii) Class III - 'permanent effect', the absence of parasitaemia for 14 days for *P. vivax*, followed by a positive reinoculation to indicate continued host susceptibility to infection. The total doses of CQ administered orally ranged from 130 to 600 mg. Mean plasma CQ concentrations, measured in 21 subjects, ranged from < 1 to 69 µg/L (median 12 µg/L). Of the 25 volunteers, there were two Class I, seven Class II, and 16 Class III responses. The critical mean plasma concentration that distinguished a Class III from a Class II effect was 10 µg/L over four days. Taking this seminal work and the PK data in monkey models, the minimum plasma concentration of chloroquine required to cure vivax malaria is considered to be 15 µg/L. This is approximately equivalent to a whole blood, TCQ concentration of 120 ng/ml. Following standard dose CQ in 15 Swedish travellers, the mean, Day 28 TCQ concentration was 99 ng/ml, a level that suppressed *P. vivax*. For the purpose of defining the MIC for *P. vivax*, a level of 100 ng/ml has been proposed by Baird. Field studies conducted in Indonesia have used this total CQ concentration as a marker of *P. vivax* resistance. A recurrent vivax parasitaemia that occurs after supervised, standard dose CQ when the TCQ concentration is ≥ 100 ng/ml, is considered evidence of resistance whether it is a new infection, relapse, or recrudescence.

1.8 Antimalarial drug resistance and drug combinations

Drug resistance may be inevitable but the rate of its development can be altered. The importance of treating an infection adequately and minimising selective pressures are crucial. The idea that resistance could be overcome, delayed or prevented by combining drugs with independent modes of action is not new. This approach has been used to treat other infections such as subacute bacterial endocarditis, leprosy, and tuberculosis. The use of drug combinations in malaria therapeutics has long been advocated by Peters.

The main reason for using drugs with different modes of action is to reduce the probability of treatment failure due to resistance. On the assumption that resistance results from spontaneous genetic mutations, then the probability that a parasite is simultaneously resistant to two drugs with independent modes of action is the product of the individual parasite mutation frequencies. If 1 in 10⁶ parasites is resistant to drug A, and 1 in 10⁶ is resistant to drug B, then 1 in 10¹² will be simultaneously resistant to A and B. If all these

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mutants result in a resistant parasite, then the chance of selecting a drug resistant mutant is reduced a million fold by using both drugs together. The sequential deployment of the two drugs is much less effective because it fails to exploit the multiplicative reduction in selection risk. Combinations could theoretically delay the selection of drug resistance and interrupt the spread of established resistance. If a patient acquires an infection already resistant to drug A, then drug B should still be effective on its own and vice versa. If both drugs have matching PK profiles, then the mutual protection provided is high because the parasite will 'see' both drugs. It would, therefore, be more effective to deploy the two drugs together from the outset rather than waiting for resistance to develop to either drug.155

Data on resistance evolution and the use of malaria combinations are few. Evidence from Vanuatu, an island of mixed vivax/falciparum transmission, suggests that the deployment of chloroquine and S/P in combination has resulted in low rates of resistance to either drug. (A. Schapira, personal communication). By contrast, there are ample data demonstrating increased treatment efficacy with antimalarial drug combinations that have different modes of action e.g. antimalarial and antibiotic combinations, and artemisinin based combinations.

Mefloquine/artesunate cured > 95% of patients with highly resistant falciparum malaria acquired on the Thai-Burmese border at a time when mefloquine had a failure rate of ~ 25%.156 In Gabon, clindamycin improved the efficacy of a 3 days course of quinine from 32% to 88%, and that of standard dose chloroquine from 9% to 70%.157 Combining doxycycline or tetracycline with quinine, amodiaquine, or mefloquine, resulted in treatment efficacies exceeding 94% against falciparum malaria in Thailand.158

Three days of doxycycline plus standard dose chloroquine in Gabonese adults resulted in a cure rate of 75% in an area where the background, adult CQ resistance rate was 36%; all CQ-D failures were RI.163 164 This CQ-D cure rate was, however, significantly lower than those of halofantrine (100%) and CQ-clindamycin (97%) in the same study.

Despite promising results from controlled trials, deploying combinations is not without its challenges. In 1984 the triple combination of mefloquine plus S/P was introduced for the treatment of multidrug resistance falciparum malaria in Thailand with the aim of delaying the onset of resistance to mefloquine. However, this strategy failed
because *P. falciparum* was already highly resistant to S/P.\textsuperscript{7} Recent data on the deployment of artesunate/mefloquine in the refugee camps along the Thai-Burmese border, where prescribing is well controlled, has resulted in sustained high cure rates, reduced infection rates of falciparum malaria, and seen a fall in the median IC\textsubscript{50} for mefloquine.\textsuperscript{94} This encouraging scenario needs to be tested in other areas where the patterns of malaria transmission are different.

Developing countries, like Indonesia, have a pressing need to tackle the substantial burden of drug resistant malaria. Using currently available drugs that are inexpensive with user friendly regimens is one way forward while waiting for newer drugs to be developed or efficacious combinations to be affordable. Although S/P is a useful inexpensive drug for chloroquine resistant *P. falciparum*, it lasted only five years in the low transmission areas of Thailand following its deployment.\textsuperscript{7} S/P resistance is present in Africa and has been documented in Indonesia.\textsuperscript{39,165} Furthermore, S/P is a poor choice for treating *P. vivax* because it is intrinsically slow to clear vivax parasitaemia, and resistance by *P. vivax* is emerging in Thailand and Indonesia.\textsuperscript{54,166}

In Papua combining S/P with CQ as an interim malaria control measure against *P. falciparum* and *P. vivax* would be sub optimal because of the high degree of CQ resistance already present to both species, and the emergence of S/P resistance.\textsuperscript{39,46} Inexpensive, alternative treatments to chloroquine are certainly needed for chloroquine-resistant falciparum and vivax malaria in north-eastern Papua. Doxycycline, produced locally, at a cost of some US$3 per 100 capsules (52 cents for 7 days) might be a potential partner to chloroquine which costs about seven US cents for an adult treatment.

### 1.9 The treatment of CQ resistant *P. vivax*

Little attention has been given to the alternative treatment of chloroquine resistant or sensitive *P. vivax*. In Papua, CRPV has been treated with halofantrine alone (8 mg/kg x 3 doses), and chloroquine combined with two dosing regimens of primaquine (10 mg/kg/d x 28d or 2.5 mg/kg/d x 3d).\textsuperscript{20} The Day 28 cure rates with halofantrine, either primaquine regimen, and chloroquine alone were, respectively, 94%, 85%, and 22%. In Thailand, CQ sensitive *P. vivax* was treated with several drugs: S/P, mefloquine, quinine, artesunate, and artemether. Cumulative cure rates were assessed after 30 days. For chloroquine and mefloquine, the cumulative cure rates were 100% due to their long duration of action. The S/P cure rate was low (22%) and due to resistance. For the short
acting drugs, relapse occurring within a few weeks confounded the cure rates which appear modest at ~ 45%, despite rapid parasite clearance.  

1.10 Malaria prophylaxis

1.10.1 Review of the current recommendations for malaria prophylaxis

Antimalarial prophylactic recommendations for travellers from non endemic areas are published by national and international authorities. The current British guidelines are based on several essential elements: (i) the country to be visited, (ii) the type of travel e.g. ecotourism, business, (iii) local resistance patterns, (iv) the risk of contracting malaria, (v) the prophylactic efficacy of current drugs, (vi) drug tolerability, (vii) contraindications to the use of prophylactic drugs, and (viii) their ease of use. Recommended prophylactic regimens are weekly chloroquine alone or combined with daily proguanil, weekly mefloquine, daily doxycycline, and daily atovaquone/proguanil (Malarone®). Tafenoquine and primaquine are under development as prophylactic agents.

1.10.2 Assessing the quality of the research data

Studies conducted in certain populations such as residents from malaria endemic countries and soldiers may not be directly applicable to travellers because malaria acquired immunity enhances drug action, and young, healthy soldiers tend to report fewer side effects than travellers. The best quality research data come from randomised, placebo controlled trials but are costly, and difficult to conduct. Retrospective studies e.g. using post travel questionnaires are easier to conduct, and are able to collect data on very large numbers of travellers. Although subject to bias because responders may be different to non responders and recall may be imprecise, they still provide useful data. Case reports of drug toxicity are difficult to evaluate in terms of drug relationship because the evidence is circumstantial. Never the less, they alert clinicians to the possibility of rare and, possibly, serious adverse effects. Assessing the risk of drug related toxicity is crucial for formulating prophylactic policy because toxicity is associated with poor adherence, disrupted holidays, and malaria. Very large clinical trials are required to assess prospectively the risk of rare side effects and to detect the relative risks of low frequency events between different prophylactic regimens. This is not achievable during trials for drug registration. A system of pharmacovigilance operates once a drug has been registered and widely used. In the UK, pharmacovigilance operates through the Yellow
Card System. However, it does not capture all the pertinent data because of under reporting due to failure of both physician and traveller to suspect, appreciate, and report toxicity. In addition, because background rates of adverse events are generally unknown, a cause and effect relationship with the antimalarial drug cannot be established firmly. The latter is a particular problem for users of long term prophylaxis because symptoms and complaints will occur naturally that are not drug related.\textsuperscript{172}

1.10.3 \textbf{Assessing the risk of acquiring malaria}

Malaria transmission varies widely in different regions, countries, and within countries. Assessing the risk requires the continued collection and interpretation of sound epidemiological data. Maps are available that stratify countries based on the risk of acquiring malaria; one such map is published by the WHO (Appendix 9). The risk of acquiring malaria depends on various factors, including length of stay, urban vs. rural travel, awareness of the benefits of preventative measures, use of personal protective measures, and drug compliance. Quantifying the risks of acquiring malaria has come from several studies of travellers and Peace Corps volunteers.

Data of British travellers from the mid 1980s, showed Oceania, principally Papua New Guinea, to be have the highest malaria risk, affecting 41/1,000 travellers per year; most of these cases (37/1,000) were due to vivax malaria.\textsuperscript{173} Risk estimates were 1.72/1,000 for East Africa, 3.75/1,000 for West Africa, 2.26/1,000 for India, 1.77/1,000 for Pakistan, 0.12/1,000 for Latin America, 0.02/1,000 for SE Asia, and 0.01 for the Middle East. Figures within West Africa varied markedly and were 5.55/1,000 for Nigeria, 7.79/1,000 for Ghana, and 0.028/1,000 for the Gambia. For travellers to East Africa, the risk was high in the first week, falling over the next 3 weeks but rising thereafter. Business travellers had the highest risk. Poor drug compliance compromised the prophylactic efficacy of all regimens and was greatest in adult men. Poor compliance with chloroquine/proguanil was associated with a 2.5 and 5 fold increase in the risk of acquiring \textit{P. falciparum} malaria for travellers to West and East Africa, respectively. In East Africa, poor compliance offered very little protection. Another report on risk trends of travellers to West Africa between 1986 and 1988 showed that immigrants from West Africa living in Britain who visited their families had the highest risk with attack rates rising from 0.4% (’86) to 0.8% (’87) to 1.4% (’88) for Ghana, and 0.4%, 0.6%, and 0.8% for Nigeria, respectively.\textsuperscript{174} These figures were higher than those for East Africa which
remained stable at a rate 0.2%. A parallel situation was also seen in a study of imported malaria into the USA from 1985 to 1988; there was a stable risk in East Africa and a rising risk for West Africa. The reasons for this rise were related to increasing CQ resistance, increased transmission in West Africa, and poor use of chemoprophylaxis. Risk data from tourists (n=139,164) visiting Kenya for less than one year, mostly up to four weeks were 1.2%/m for those who did not take prophylaxis and 0.8% for those on CQ-P. In an earlier study of ~ 42,000 travellers who took no prophylaxis, the risks per month for East and West Africa were 1.52% and 2.42%, respectively. For Peace Corps volunteers who took long term weekly chloroquine while living in several west African countries, the overall risk of malaria acquisition was ~ 3.5%/m which probably corresponds to a risk of 7%/month without prophylaxis, assuming a prophylactic efficacy of 50% for CQ.

Deaths due to malaria have occurred in travellers who did not take or comply with prophylaxis or who took chloroquine alone. Given a case fatality rate in Europe of 0.5-1%, the estimated risk of dying from malaria for travellers is approximately 1 in 4,000.

1.10.4 Definition of prophylactic efficacy and effectiveness

When measured prospectively, the prophylactic efficacy (PEf) of a given drug compares the risk of malaria acquisition between placebo and the active drug. The PEf is the percent reduction of malaria by the active drug and is computed by the formula PEf = 1-(I_{drug}/I_{placebo}). I is the malaria incidence, measured as the cumulative incidence (proportion) within a certain time span, or the incidence rate (number of infections per person-year). The PEf has a point estimate and a 95% confidence interval (CI). Retrospective studies have estimated the prophylactic effectiveness using the same formula whilst other studies have simply reported the effectiveness of a given regimen e.g. the proportion of individuals who remain parasite free after a given time period of unsupervised prophylaxis. There is no generally accepted cut off value that determines whether a given drug will be registered and recommended as prophylaxis. Clearly, the higher the PEf, particularly it's lower 95% CI, and the lower the toxicity, the better. Prophylactic regimens will change over time as new drugs are registered, serious toxicities become apparent, and as the resistance patterns of malaria change. A synopsis of the PEf of the main prophylactic regimens is presented below.
1.10.5 Prophylactic efficacy and effectiveness of commonly used regimens

1.10.5.1 Chloroquine and Chloroquine/proguanil

Several small studies of expatriates living in Africa have documented the poor prophylactic effectiveness of chloroquine against *P. falciparum*. In the early 1980s, a retrospective questionnaire study administered to 821 expatriates living in Dar Es Salaam showed that chloroquine was ineffective and associated with a malaria incidence rate of 16.9/p-y, a rate similar to no prophylaxis. CQ/P was considerably better (2.2/p-y). In Cameroon, CQ/P was reasonably effective in expatriates. Over 18 months, nine (11.5%) of 78 subjects developed falciparum malaria compared to 26 (50%) of 52 subjects who took no prophylaxis (P<0.0001). CQ/P or proguanil alone were the best regimens used by expatriates living in Malawi; 8.5% of users on these regimens developed malaria within one year compared to 22% for CQ alone. The largest published, retrospective series is of 145,003 travellers to Kenya between 1985 and 1991. Using data from 139,164 travellers who stayed for <1 year, CQ/P prevented 72% (95% CI 56-82) *P. falciparum* infections compared to no prophylaxis; the corresponding figure for weekly mefloquine was 91% (95% CI 85-94). Amongst US Peace Corps volunteers in West Africa on long term prophylaxis, CQ alone resulted in 3.1 to 3.8 malaria attacks/100 person-months (p-m); the figures for CQ/P were 1.7 to 2.1/100 p-m. In a prospective study in 1992/93, CQ/P had a PEf of only 54% compared to placebo in malaria immune western Kenyans aged 9-14 years. In 1992/93, amongst Italian soldiers (n=4,800) deployed to Mozambique, the incidence rate of falciparum malaria was 17/1000 p-m in those taking CQ/P compared to 1.8/1000 p-m for those on mefloquine, a nine fold increase in risk. French soldiers (n=107) using CQ/P (administered as a daily, fixed dose combination) or doxycycline while on mission in an unspecified central African country for three months suffered an incidence rate of falciparum malaria of 3.1/100p-m. Most (87 of 107) of the malaria was in a group of soldiers who had recently arrived. Their malaria incidence rate was 5.3/100p-m for CQ/P users and 6.3/100p-m for doxycycline recipients. Poor compliance was reported by ~40% of the prophylactic failures. Difficult field conditions made the implementation of personal protective measures (e.g. insect repellents, bed nets) problematic and contributed to the risk of acquiring malaria. A prospective trial of CQ/P and atovaquone/proguanil (A/P) in western travellers has recently been conducted (See 1.10.5.4).
Chloroquine alone as prophylaxis has little role. It may be used in areas of *P. vivax* predominance and where there is no reported falciparum resistance. CQ/P may be used as in areas of limited and low grade chloroquine resistance and for travellers with contraindications or intolerance to the other main regimens.

1.10.5.2 **Mefloquine**

Weekly mefloquine (250 mg in adults) provides very effective prophylaxis in areas where mefloquine resistance is not a problem. Efficacy and effectiveness exceeding 90% have been reported in non immune Indonesians, and travellers to East and West Africa. Whilst prophylactic failures have been reported occasionally with weekly MQ, its introduction in 1993 has resulted in a substantial fall in the number of malaria cases of imported malaria into the UK from East Africa concomitant with declining use of CQ/P. A similar fall in malaria cases was seen in Peace Corps volunteers stationed in West Africa. In the mefloquine resistant areas of SE Asia, mefloquine has been less effective.

Thai soldiers (n=1,307), deployed on the Thai-Cambodian border for 22 weeks, had a malaria failure rate of 3.2 cases/100 p-m; 2.2 for *P. falciparum*, 1 for *P. vivax*. Compliance was high, averaging 91% over the course of the study, but prophylactic failures due to poor compliance were calculated as 0.3/100 p-m (falciparum) and 0.2/100 p-m (vivax). At the time of prophylactic failure, 32 soldiers had serum MQ levels of 26 to 2,515 ng/ml with a mean of 950 ng/ml. Nine had levels < 500 (< 391 whole blood) ng/ml, 23 > 500 ng/ml, and 16 had levels > 1,000 ng/ml. Halofantrine failed to cure (RI resistance) seven of the 32 soldiers. All 7 RI failures had had serum MQ levels > 500 ng/ml. The mean MQ IC$_{50}$ of those who failed halofantrine was significantly higher than that of the halofantrine treatment successes: 23.8 ng/ml vs. 12.5 ng/ml. There was also a positive correlation between the MQ and halofantrine IC$_{50}$. These data demonstrate cross resistance between mefloquine and halofantrine and sound evidence of MQ resistance as the cause of some of these prophylaxis failures. Similarly, of 2,289 Dutch marines stationed in Cambodia for six monthly tours of duty, 59 developed 64 attacks of malaria (*P. falciparum* n=42, *P. vivax* n=22), giving a cumulative incidence of 2.6%. Most soldiers, 86.3% (n=1,975) reported full compliance. Eight of 11 falciparum isolates tested had evidence of *in vitro* mefloquine resistance (methodological details not given). Malaria occurred during deployment (*P. falciparum*, n=31), and after return to Holland: (i) *P.*
falciparum failures (n=11) 2-10 weeks, and (ii) P. vivax (n=22) 1-18 months after leaving Cambodia, consistent with data in returned UK travellers. In non immune Indonesian soldiers stationed in north-east Irian Jaya for 13 weeks, mefloquine was highly effective at preventing falciparum and vivax malaria, achieving prophylactic efficacies of 100% (87-100) and 100% (83-100), respectively. A prospective, comparative study with A/P has recently been reported (See 1.10.5.4). Mefloquine prophylaxis is recommended for Africa, Latin America, and parts of Asia where there is chloroquine but no MQ resistance.

1.10.5.3 Doxycycline

Doxycycline is effective against drug resistant P. falciparum and P. vivax malaria. It lacks appreciable causal activity and has no activity against liver hypnozoites. Prophylactic studies have been conducted in SE Asia, New Guinea island, and Africa.

On the Thai-Burmese border, daily doxycycline (n=95) was compared to weekly chloroquine (n=93) in refugee children and adolescents aged 10-15 years. Doxycycline (100 mg/d) was administered to those ≥ 40 kg and 50 mg/d to those < 40 kg for nine weeks. Doxycycline failures numbered 5 over 597 person-weeks; CQ failures were 31 over 488 p-w. In another trial in the same population, standard [100 mg (n=66)] and half dose (n=77) doxycycline were compared to placebo (n=80) given over six months. Both regimens were more effective than placebo for preventing falciparum and vivax parasitaemia. The failure rate against P. falciparum was low ~ 0.2/100 p-w for both doxycycline regimens but the 100 mg dose was more effective at preventing vivax malaria (0.3 vs. 1.4/100 p-w). Two doxycycline (50 and 100 mg) regimens (n=243) were compared to one tablet of weekly pyrimethamine 12.5 mg /dapsone 100 mg [Maloprim® (n=123)] in Thai soldiers deployed on the Thai-Cambodian border. Drug administration was not supervised. Low rates of compliance and corresponding low efficacy rates were documented for all three regimens over 17 weeks. Compliance was 79% and 73% for the Maloprim® and doxycycline regimens, respectively. The cumulative failure rates for both malaria species were 19% (100 mg), 32.5% (50 mg), and 52% (Maloprim®). The higher doxycycline dose was significantly better at preventing falciparum but not vivax malaria compared to the 50 mg regimen. In another trial of 77 Thai soldiers, conducted over 80 days, falciparum attack rates were low (3.9%) during the first 40 days but increased to 9.5% by study end. The P. vivax cumulative incidence was
1.3% at both time points. Declining doxycycline effectiveness was associated with poor compliance.194

In Cambodia, doxycycline combined with chloroquine (300 mg/w) was effective in Australian soldiers. Over two years, there were eight cases (Pf=2, Pv=6) of malaria in 600 men, giving a cumulative incidence of 1.3% (0.65% over one year).195 The French experience of doxycycline in Cambodia was similar. There were three cases of malaria in 703 soldiers stationed in south-west Cambodia for just over three months, giving an attack rate of 0.43%; 93% reported full compliance by questionnaire.196 In a randomised, double blind, placebo controlled trial, doxycycline had a protective efficacy of 96.5% (81-100) against *P. falciparum* and 100% (83-100) against *P. vivax* in non immune Indonesian soldiers in north east Irian Jaya.136 In Papua New Guinea (PNG), there were no cases of *P. falciparum* in 115 Australian soldiers on daily doxycycline (100 mg) who were exposed to malaria for up to six weeks. Prophylaxis was discontinued only three days after leaving the endemic area; 15 cases of *P. vivax* occurred two to three weeks later.197 Daily doxycycline (100 mg) combined with low dose primaquine (7.5 mg/d) was given to 53 Australian soldiers two days before entering PNG and continued for three days after leaving. There were no cases of malaria whilst in PNG but three soldiers developed falciparum malaria three weeks after stopping prophylaxis and 15 developed vivax malaria between three and 40 weeks.198 The premature stopping of doxycycline was a risk factor for malaria.

In Africa, doxycycline prophylaxis has been studied in Kenya, Somalia, and central Africa. In Kenya, doxycycline (50 mg/d or 100 mg/d) had a prophylactic efficacy of 84% (66-92%) and 92.6% (79.9-97.5%) in malaria immune children and adults, respectively.181 199 In Somalia, there were three cases of malaria (Pf=1, Pv=2) in 900 Australian soldiers who took doxycycline for four months, giving a cumulative incidence of 0.33%.195 However, during the deployment of US forces to Somalia, doxycycline (n=52) appeared less effective than mefloquine (n=344) for preventing malaria with 5.49 (95% CI 1.78-12.82) failures/person-weeks vs. 1.15 (95% CI 0.03-6.41) for mefloquine. Although this difference was not statistically significant, the proportion of soldiers reporting full compliance was significantly less (*P* < 0.0001) in the doxycycline recipients (81%) compared to those taking MQ (98%).200 Soldiers were at an increased risk of developing malaria if they did not fully comply with prophylaxis (2.4 fold), did not roll
down their sleeves in the evening (2.2 fold), or who did not use bed nets (2.6 fold). Some prophylactic failures occurred despite adequate drug levels of mefloquine or doxycycline. Of the 13 mefloquine recipients who developed malaria, eight had a CMQ:MQ ratio ≥ 2, and five had serum MQ levels > 620 ng/ml, of whom one had a level of 1,067 ng/ml. Of the 20 doxycycline failures, only 10 had measurable levels. Five had very low levels, 0.1 ng/ml, and five had levels ranging from 1 to 7.5 ng/ml, consistent with compliance. In a follow up study of malaria that developed in 106 US Marines who had returned to the USA from Somalia, 97 (87%) had P. vivax, 8 (7%) P. falciparum, 6 (5%) mixed vivax/falciparum infections, and 1 P. malariae. Compliance for the necessary four weeks post return was reported by only 56%. A surprising finding was that only half of the soldiers had received sufficient doxycycline or mefloquine or primaquine to complete prophylaxis. In French soldiers deployed to Gabon and the Central African Republic, the effectiveness of daily doxycycline (as hyclate salt) was similar to that of daily CQ/P despite the fact that doxycycline had superior prophylactic efficacy. The malaria attack rates (AR) over four months were 1/171 (0.6%) for doxycycline and 13/270 (4.8%) for CQ/P, giving an eight fold increased risk of acquiring malaria on CQ/P. However, withdrawals because of gastrointestinal upset were 6.4% for doxycycline and none for the CQ/P group, giving an adjusted AR of 7% vs. 4.8%, respectively (P=0.3).

Doxycycline is recommended for use in areas of multidrug resistant malaria, and as an alternative for individuals unable to take mefloquine or atovaquone/proguanil.

1.10.5.4 Atovaquone/proguanil

This fixed dose combination comprises atovaquone 250 mg and proguanil 100 mg (A/P) and has recently been introduced as a daily prophylactic drug. A/P has the advantage over other antimalarials because it processes causal prophylactic activity allowing prophylaxis to start one day before entry to and finish one week after leaving the endemic area.

In clinical trials A/P had high rates of protective efficacy 98% (91.9-99.9) in malaria immune Africans from Kenya, Gabon, and Zambia. One trial has assessed the prophylactic efficacy of A/P (n=148) relative to placebo (n=149) in Papuan adults with limited malaria acquired immunity. In Papua, the PEf was 96 (72-99)% and 84 (44-95)% against falciparum and vivax parasitaemia, respectively. Two trials have
also been conducted in western travellers comparing A/P with either CQ/P or mefloquine. Travellers in both studies spent a mean of 22 weeks in the endemic areas; the majority travelled to Africa 63% (CQ/P study) and 79% (MQ study). A/P was effective in both studies. Of the 501 A/P recipients (CQ/P study), one (0.2%) developed *P. ovale* and three (0.6%) of 507 CQ/P recipients developed *P. falciparum*. In the A/P-MQ study, there were no cases of prophylaxis failure with either regimen.

A/P is recommended for prophylaxis against multidrug resistant *falciparum* malaria for short trips and for travellers who cannot tolerate doxycycline or mefloquine.

### 1.10.6 Adverse effects of prophylactic regimens

Drug related toxicity is a crucial element for recommending a particular prophylactic drug. Clinicians know that patients will not take a drug that makes them feel unwell. This is particularly true for the healthy travellers for whom the risk-benefit ratio requires both very safe and highly tolerable regimens. Rare toxicity may not be evident from randomised trials. Indeed, approximately 100,000 subjects would be needed in such trials to detect a toxicity rate of 1 in 10,000.

#### 1.10.6.1 Chloroquine and chloroquine/proguanil

Chloroquine, alone or combined with proguanil, has been in use for many years. It is generally well tolerated, and is considered safe in pregnancy. Commonly reported symptoms are generally mild and include gastrointestinal upset, headache, malaise, dizziness, visual disturbance, mouth ulcers, itching, and insomnia. In one open label, prospective study in Scandinavian travellers, adverse effects reporting rates associated with CQ/P were low and considered mild: nausea (3%), diarrhoea (2%), and dizziness (1%). Retrospective, post travel studies have generally recorded considerably higher rates. The large series of Steffen et al reported the side effects of several prophylactic regimens by questionnaire of 145,003 travellers to Kenya most of whom (97.6%) stayed for up to 4 weeks.

Non serious side effects were assessed in 89,902 travellers who used no prophylaxis (*n*=4,026), CQ 300 mg weekly (*n*=3,354), CQ 600 mg weekly (*n*=3,646), CQ/P (*n*=20,150), sulfadoxine/pyrimethamine (*n*=8,673), and mefloquine (*n*=50,053). Side effects were reported frequently by users of all prophylactic regimens and by 5.3% of travellers not on prophylaxis. The corrected rates (reported rate less 5.3) were 18.5% (CQ300), 17.2% (CQ600), 30.1% (CQ/P), 11.6% (S/P) and 18.7% for mefloquine.
Overall, ~ 50% of side effects were considered subjectively mild, 36-40%, moderate, and 10-14% severe. Nausea was the commonest side effect for all regimens, reported by 11-19% of the CQ regimens and 12% by the MQ recipients. The rates for nausea (18.8%) and for mouth ulcers (7.9%) were the reasons for the overall excess of side effects in the CQ/P group compared to the other regimens. Dizziness was reported by more mefloquine (7.6%) than by chloroquine (5-6%) users, whereas the opposite trend was noted for insomnia. No differences were found for depression and headaches. Serious neuropsychiatric side effects, consisting of convulsions, acute psychosis, and severe vertigo, were reported rarely by the CQ, CQ/P, and MQ groups. The risk of any of these reactions was estimated as 1 in 13,600 for CQ and 1 in 10,600 for MQ.

In a telephone survey of returning British travellers who had used prophylaxis for a median of seven weeks, the reporting of any side effect was similar for CQ/P (n=1,181) and MQ (n=1,214) users, some 40%. Most side effects were considered 'trivial' and did not interfere with daily activities. Mild GI upset was reported by more of the CQ/P group: 16.3% vs. 12.5% (P=0.009) but the reporting of any neuropsychiatric (NP) event was more likely in the MQ group: 333 (27%) vs. 189 (16%) RR=1.7 (1.5-2). There were eleven travellers, ten women and one man, who reported disabling neuropsychiatric side effects, defined as preventing the traveller from undertaking the activity for which she/he had made the journey. One traveller with a family history of epilepsy, considered a contraindication by the authors, was excluded from the analysis. Reported disabling NP rates were 0.7% (n=9) for MQ compared to 1 (0.08%) CQ/P users (P=0.02). These findings were broadly similar to a postal survey of visitors to the Kruger National Park in South Africa, an area of chloroquine resistance. Travellers on CQ/P (n=2,488) reported significantly more nausea (15.1% vs. 12.2%), diarrhoea (6.3% vs. 3.7%) and mouth ulcers (2.9% vs. 0.5%). Significantly more MQ (n=1,300) recipients reported neuropsychiatric side effects: light headedness (7.5% vs. 4.8%), vivid dreams (2.8% vs. 1.7%), depression (2.7% vs. 1%), and anxiety (0.5% vs. 0.01%). Mouth ulcers were also problematic in a cohort study of 470 British soldiers in Belize; 37% (142/382) of CQ/P users reported mouth ulcers compared to 24% (21/88) who took proguanil alone [RR=1.56 (95% CI 1.05-2.31)].

There has been experience with the fixed combination of chloroquine and proguanil. This combination is used in several European countries, notably France and
Switzerland. CQ/P was well tolerated by 194 French travellers. Gastrointestinal side effects were the most commonly reported side effects, affecting ~10% of travellers. Non serious neuropsychiatric effects were reported by 2%, a significantly lower rate compared to the mefloquine users, 11.5% (n=183).

In a cohort of French soldiers, none of the CQ/P users (n=270) stopped their prophylaxis compared to 11 (6.4%) of 171 soldiers on doxycycline hyclate. It was felt that the formulation of the doxycycline was a factor in this GI intolerance. By contrast, CQ/P was significantly less well tolerated and less well complied with than doxycycline monohydrate in 522 French soldiers deployed to Gabon or Chad. The following side effects were reported more frequently (P < 0.05) in the CQ/P arm vs. the doxycycline arm: (i) epigastralgia, (ii) diarrhoea, (iii) mouth ulcers, (iv) urticaria, (v) sun sensitisation, (vi) skin desquamation, (vii) pruritus, and (viii) skin rashes (morphologies not specified). Despite the poorer tolerability, there were no withdrawals from the CQ/P group but 15 from the doxycycline group because of drug intolerance [gastrointestinal effects (n=9), headache (n=5), and skin rash (n=1)]. However, the authors caution that withdrawal should not be viewed as an indicator of doxycycline tolerability for several reasons: (i) the soldiers knew which prophylaxis they were taking, (ii) they were also aware of the previous poor gastrointestinal tolerability experienced by French soldiers, and so were more anxious about possible doxycycline toxicity, (iii) there was a bias in favour of stopping doxycycline because CQ/P could be used as an alternative whereas no such alternative existed with the CQ/P group. Of interest is that skin sensitisation was more common in the CQ/P than the doxycycline recipients, a finding consistent with a study in travellers from Switzerland, Germany, and Israel.

As prophylaxis, very few serious adverse reactions have been reported with CQ. Generalised convulsions have been reported rarely in travellers. In a case series of four women who developed clonic-tonic fits, one had a past history of tonic-clonic and complex partial seizures, and another a history of absence seizures. The latter and another woman had EEG evidence of a reduced seizure threshold.

Chloroquine induced skin rashes are rare and have included urticaria, lichen planus, photosensitivity, a pustular eruption, bullous pemphigoid, toxic epidermal necrolysis, exfoliate dermatitis, and exacerbation of psoriasis. Deafness
has been reported rarely with chloroquine generally in association with long term use in rheumatology patients, and following intramuscular injection for malaria. Contraindications to CQ prophylaxis are (i) epilepsy, (ii) known allergy, (iii) severe renal disease, (iv) severe hepatic disease, and (v) a history of generalised psoriasis (WHO recommendation). The prophylactic dose of CQ should be reduced in patients with mild or moderate renal failure to avoid drug accumulation. Chloroquine should not be used in patients severe renal failure (serum creatinine > 700 μmol/L). Data are lacking on the optimal CQ prophylactic dose in liver impairment. In 'mild' liver disease, standard CQ dose appears to be safe.

Chloroquine is listed as a drug that can be used in patients with acute porphyria (Cardiff Porphyria Service: www.uwcm.ac.uk, accessed January 2003). However, it is considered hazardous in patients with porphyria cutanea tarda at the doses used for treatment or prophylaxis because severe toxicity may be caused by the mobilisation of porphyrins. The question of chloroquine exacerbating psoriasis remains unanswered because psoriasis fluctuates naturally. Chloroquine has been used successfully for patients with psoriatic arthritis. Never the less, there have been a number of case reports of exacerbations of psoriasis coincident with CQ as antimalarial prophylaxis, and CQ or hydroxyCQ as treatment for rheumatoid and psoriatic arthritis. For clinicians, psoriasis should be viewed as contraindication to CQ prophylaxis but as a relative contraindication for treatment.

1.10.6.2 Mefloquine

The prophylactic dose is 250 mg weekly (adults) and 5 mg/kg for children; the recommended treatment dose is 15 mg/kg or 25 mg/kg in areas of MQ resistance.

As treatment, MQ tolerability is generally good. Several dose related symptoms are note worthy such as anorexia, nausea, and vomiting, dizziness, inability to walk unaided, and insomnia. Early, MQ induced vomiting is another significant problem of treatment especially in young children and adults > 50. The tolerability of mefloquine is improved appreciably by splitting the higher dose (15 followed by 10 mg/kg) and delaying the administration dose to the second day of treatment in a three day regimen with artemesunate.

As prophylaxis, the proportion of travellers reporting any side effect varies considerably between trials but has ranged between 18-40% in the larger retrospective,
questionnaire studies. The majority (~90%) are, subjectively, of mild or moderate severity and self limiting e.g. nausea, diarrhoea, headache, strange dreams, insomnia, dizziness, and mood changes. Much attention has focussed on the NP effects of mefloquine. A neuropsychiatric event describes any central nervous system (CNS) or psychiatric symptom. A minor NP event includes headache, dizziness, light headedness, paraesthesiae, insomnia, vivid and unpleasant dreams, irritability, and nervousness. A serious NP event encompasses the following principal diagnoses: convulsion, disturbance of consciousness, inability to walk unaided due to vertigo or ataxia, psychosis, disorder of affect, acute neurosis, and acute confusion. Predisposing factors are a past history of neuropsychiatric disorders, recent (within two months) MQ exposure e.g. treating a MQ prophylactic failure with MQ, previous MQ related NP event, previous treatment with psychotropic drugs, and MQ used in the recovery phase of severe malaria. A family history of epilepsy has been reported by some travellers who developed fits while on MQ prophylaxis but it is unclear if this is a risk factor. Data on NP AEs associated with MQ prophylaxis come from studies of different study designs of tourists and soldiers. Reports to the manufacturer as part of pharmacovigilance complement formal trials. Essential points from these studies are reviews are: (i) many (40%) NP side effects occur soon after the first dose and 75% are manifest by the third dose, (ii) most are of mild or moderate severity and resolve completely, (iii) some require medical management including hospitalisation, (iv) less than 2% of patients have sequelae, (v) women are more likely to experience NP AEs than men, (vi) MQ in soldiers (fit, young men) is well tolerated, including the loading dose of MQ (250mg/d x 3d), (vii) in prospective studies, MQ related NP side effects were broadly similar to other antimalarials or placebo, (vii) long term MQ prophylaxis is well tolerated, and (viii) the risk of serious NP AE during prophylaxis is estimated to be 1 in 10,600 for MQ and 1/13,600 for CQ.

In a meta-analysis of controlled trials, mefloquine users were more likely to withdraw from studies compared to placebo but not when compared to other prophylactic regimens. There are limited data assessing psychomotor function, motor function, action requiring fine co-ordination, and balance and hearing. Boudreau et al found no compromise of performance of American soldiers who took weekly MQ (preceded by the loading dose) or chloroquine as prophylaxis. Sleep disturbances, increased dream activity,
and depressed feelings were detected in the MQ group.\textsuperscript{135} In a double blind, randomised, cross over, placebo controlled study of 23 Swiss trainee pilots (mean age 27), MQ was given as a loading dose, followed by weekly dosing for three weeks. This regimen was tolerated by all subjects except one who complained of dizziness, diarrhoea, and flu like symptoms during the loading dose phase; he was withdrawn from the study. There were no significant differences in flying performance, psychomotor functions, and postural sway between the two arms. Poorer sleep quality was reported by the MQ users who had a lower mean reduction in sleep time of 34 minutes that was not statistically significant. Similar findings were reported in another placebo controlled trial of MQ prophylaxis and the effect of alcohol driving car. There was no impairment of driving in a cohort of young volunteers who had blood levels of alcohol of between 0.3-0.5 mg/ml (the legal limit in many countries is 0.8 mg/ml).\textsuperscript{241} In 10 volunteers, MQ had no effect on audiometry and vestibular functions assessed using posturography and the recording of nystagmus.\textsuperscript{242} There is one report of impaired hearing in three travellers who took MQ as prophylaxis.\textsuperscript{243}

Two studies have examined tolerability of long term prophylaxis. Mefloquine was well tolerated in 2,289 Dutch Marines stationed in Cambodia for six months.\textsuperscript{188} One battalion of 754 was given a loading dose of mefloquine, 250 mg daily for three days. Seven (0.3%) sought medical care for serious adverse events: convulsions (n=2), myoclonus (n=1), severe dizziness (n=2), and skin rash (n=2). Six changed to doxycycline and one was given MQ bi-weekly with good effect. By questionnaire, 609 (30.2%) of 2,015 soldiers reported voluntarily 820 complaints; these were mostly headache, difficulty in concentration, weakness, dizziness, and nausea. Mefloquine taken weekly was also well tolerated by 802 American Peace Corps volunteers of whom 152 used mefloquine for more than one year.\textsuperscript{133} The reporting of side effects by volunteers decreased significantly over time from 44% after 4 months use to 19% after more than one year of use, suggesting that mild symptoms were well tolerated. Only 7 (0.9%) stopped prophylaxis because of MQ related toxicity. These data show that MQ tolerability was acceptable over the medium to long term.

Cutaneous toxicity with mefloquine as treatment or prophylaxis is rare. In a review of published data from 1983-1997, only 74 cases of any skin reaction were reported, making skin toxicity rare. These reactions included itching, red maculopapular
rashes, urticaria, cutaneous vasculitis, exfoliate dermatitis, SJS, toxic epidermal necrolysis, and facial bullous lesions. Maculopapular rashes (30%) and pruritus (4-10%) were two most commonly reported reactions.\textsuperscript{244} Six cases [prophylaxis (n=3), treatment (n=3)] of severe and three of mild itching occurred in those using Fansimef (MQ+S/P). MQ is not generally associated with cardiovascular toxicity. Reports of sinus bradycardia and sinus arrhythmia are more likely to be related to resolution of malaria, especially in young fit individuals. There have been reports of complete AV block with mefloquine treatment and atrial flutter with 1 to 1 conduction, and AV conduction through an aberrant pathway with mefloquine as prophylaxis.\textsuperscript{245} \textsuperscript{246} \textsuperscript{247} Acute intravascular haemolysis has been reported with malaria treatment in expatriate Europeans.\textsuperscript{248} \textsuperscript{249} Agranulocytosis has also been reported.\textsuperscript{250} MQ causes transient elevation of transaminases but is rarely associated with hepatitis.\textsuperscript{251}

Mefloquine should not be given to individuals with known allergies to mefloquine or quinine nor to those with previous MQ related, serious toxicity. Prophylactic contraindications include: (i) epilepsy, (ii) psychiatric disease e.g. depression, bipolar affective disorder, any psychotic disease, severe anxiety neurosis, (iii) the first trimester of pregnancy (women should avoid becoming pregnant for 3 months post MQ use), and (iv) severe hepatic disease. A family history of epilepsy is not considered a contraindication.\textsuperscript{227} Renal failure is not a contraindication to MQ prophylaxis; MQ PK values were similar to normal volunteers.\textsuperscript{252} Halofantrine should not be used to treat mefloquine prophylaxis failures because of the risk of cardiotoxicity. Treatment contraindications are: (i) epilepsy, (ii) psychiatric disease, (iii) mefloquine treatment within the past two months, (iv) the recovery phase of severe malaria, (v) pregnant women in the first trimester (unless no other choice is available), and (iv) concurrent halofantrine treatment. Mefloquine as treatment or prophylaxis is not recommended for persons performing activities requiring fine co-ordination and spatial discrimination e.g. pilots. On the question of individuals with cardiac disease, the BNF advises caution.\textsuperscript{227}

1.10.6.3 MQ use in pregnancy and breast feeding women

Mefloquine prophylaxis after the first trimester was highly effective in Malawi and in western Thailand.\textsuperscript{253} \textsuperscript{254} \textsuperscript{255} As treatment, MQ alone or in combination with artesunate has been used on the Thai-Burmese border because multidrug resistant \textit{P. falciparum} limits therapeutic choice.\textsuperscript{256} However, in a retrospective study, MQ alone as

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treatment was associated with a higher risk of still birth compared to either quinine (Q) monotherapy or other antimalarial drugs (OT). The proportions of still births were (i) 9/200 (4.5%) MQ recipients vs. 10/633 (1.6%) Q recipients, OR=4.72 (95% CI 1.7-12.7), vs. 12/873 (1.4%) of women who received other drugs, OR=5.1 (95% CI 2-13.1). Mefloquine was not associated with an increased risk of abortion, low birth weight, neonatal neurological retardation or congenital malformations. Interestingly, the prospective, prophylactic study of 159 women (weekly MQ after week 24 of gestation) found a non significant increased risk of still birth in the MQ recipients: 7% vs. 2.6% [RR=2.63 (95% CI 0.86-8.08)]. This result may have been due to an inadequate sample size and does not exclude the possibility of a positive association between mefloquine and stillbirth, especially in view of the later, retrospective, treatment study. In Malawi, the rate of still births with mefloquine (750 mg, followed by 250 mg MQ weekly) was 3.6% (37/1,015), a rate similar to that of chloroquine recipients, 3.8% (119/3,132). Approximately 60% of women in both arms reported side effects, notably, itching, dizziness, and GI upset. The MQ recipients were more likely to complain of dizziness but of less itching (details not given). One MQ recipient developed self limiting, acute confusion [0.09% (95% CI 0-0.55)].

In a post marketing survey of 1,627 Western woman who had been exposed to mefloquine pre or post pregnancy, there were 32 babies with congenital malformations, and 79 women had spontaneous abortions. All congenital abnormalities occurred in women who had taken mefloquine before becoming pregnant or within the first trimester of pregnancy. In a retrospective study of accidental MQ exposure as prophylaxis in US Servicewomen (n=72), spontaneous abortions numbered 12; all occurred between 6 to 12 weeks of gestation. In the analysis, the authors excluded women with an unknown pregnancy (n=19) or foetal (n=17) outcome, giving an abortion rate of 12/36 [33.3% (95% CI 19.5 - 49.8)]. There were no congenital abnormalities in the 23 live births and no stillbirths.

Mefloquine as prophylaxis or treatment in pregnancy requires careful assessment. Although the risk benefit ratio is difficult to define, MQ use should clearly be beneficial. MQ prophylaxis should not be used in the first trimester unless there is a pressing need to travel to a malaria endemic area. In fact the WHO advises pregnant women not to travel to malaria endemic areas. In view of the limited data suggesting a risk of congenital
abnormalities in women exposed to MQ before they become pregnant, the recommendation that pregnancy should be avoided for three months after the end of MQ prophylaxis should be followed. Mefloquine is secreted into breast milk in small amounts, the activity of which is unknown.\textsuperscript{260} Circumstantial evidence suggests that adverse effects do not occur in breast-fed infants whose mothers are taking mefloquine (International Standard Prescribing Information. Roche, May 1999). Mefloquine use on the Thai-Burmese border has not been associated with apparent ill effects in breast fed infants. (R. McGready, personal communication).

1.10.6.4 Doxycycline

Doxycycline prophylaxis or treatment is generally well tolerated and serious toxicity is rare. In placebo controlled trials, the overall tolerability of doxycycline has been comparable or even better than placebo. In Irian Jaya, Indonesian soldiers on doxycycline reported any symptom less frequently than placebo recipients (rate ratio D:P for all symptoms = 0.64, $P < 0.001$) because the latter reported significantly more mild CNS symptoms e.g. poor sleep, dizziness, and headache (detailed frequencies of each symptom are not stated in the paper). Although the doxycycline recipients reported less frequently any GI symptom, the D:P rate ratio was not significant.\textsuperscript{136} Doxycycline recipients reported nausea if they consumed doxycycline without food, a finding consistent with other studies.\textsuperscript{195} In this study, doxycycline was better tolerated than mefloquine. Three other placebo controlled trials have reported tolerability data in less detail. In Kenyan children, the mean number of reported symptoms per subject [nausea (3.3 vs. 4.9), abdominal pain (7 vs. 8), diarrhoea (1.2 vs. 1), fever (5.3 vs. 5.8), headache (7 vs. 6)] were similar between placebo and doxycycline arms.\textsuperscript{181} In another trial in Kenyan adults, the tolerability of doxycycline was similar to that of placebo but one female on doxycycline had repeated episodes of vaginal candidiasis and was withdrawn from the study.\textsuperscript{199} In Thai refugees of all ages the reporting frequency of any GI symptom was highest in the 100 mg doxycycline group (49/p-y); this frequency was significantly higher than the 50 mg group (20/p-y) but not compared to placebo (32/p-y). The placebo group reported more fever, headache, and dizziness compared to doxycycline.\textsuperscript{192}

In 623 travellers, participating in a double blind comparison with the three other main prophylactic drugs and answering a symptoms questionnaire, the doxycycline group (n=153) tolerated doxycycline monohydrate well. Although this study was not
sufficiently powered to detect small differences in reported symptoms between the arms, certain inferences can be drawn. Moderate gastrointestinal symptoms (9 vs. 20%, P=0.0006) and moderate skin symptoms, defined as itching, or abnormal reddening of skin (3 vs. 8%, P=0.08) were reported less frequently compared to the CQ/P group.\textsuperscript{217}

In a retrospective questionnaire of 383 Australian travellers (male=200), who used doxycycline for a mean of 4 weeks, a total of 82 (21.4\%) reported symptoms that were considered by the travellers to be drug related; 25 (6.5\%) reported these as troublesome (interfered with normal activity), and 22 (5.7\%) stopped doxycycline.\textsuperscript{262} The specific reasons for stopping doxycycline were not documented. Two symptoms of note were vaginal itch (as a proxy for vaginal thrush), reported by 17 (9.3\%) women, and red skin, consistent with mild photosensitivity, that was reported by 22 (5.7\%) travellers. Abdominal pain, nausea or vomiting, and diarrhoea were reported by some 8\%, 15\%, and 12\%, respectively.

Doxycycline induced upper and or lower gastrointestinal symptoms are important side effects that have resulted in discontinuation of prophylaxis: 3 and 6.4\% in two studies of French soldiers.\textsuperscript{203, 216} Upper GI intolerance is commonly reported, affecting between 10 to 35\% of subjects.\textsuperscript{198, 200, 203, 216} Studies of doxycycline for the prevention of traveller's diarrhoea have reported broadly similar rates (4-12\%) of upper GI upset.\textsuperscript{261} Heartburn secondary to oesophagitis is well described and may be reduced if doxycycline is taken with meals and not before sleep.\textsuperscript{263, 264} Oesophageal ulceration causing severe hiccups, heartburn, and interference with sleep, was reported in a 51 years old man following doxycycline as adjunct treatment for malaria.\textsuperscript{265} Doxycycline induced diarrhoea was reported by \sim 18 and \sim 20\% of French soldiers, a rate higher than the 3\% reported in American outpatients prescribed short course doxycycline.\textsuperscript{216, 266} In a double blind, double dummy study comparing doxycycline with mefloquine in soldiers on exercise in Thailand, rates of diarrhoea in both groups was similar, 49 (58/119) and 48 (64/134)\%, respectively. The authors concluded that doxycycline in Thailand, where many of the common pathogens causing traveller's diarrhoea are resistant to tetracyclines, doxycycline conferred no benefit in preventing diarrhoea but did not also increase risk of diarrhoea.\textsuperscript{267} Antibiotic induced colitis due to \textit{Clostridium difficile} is a well recognised complication of antibiotic use.\textsuperscript{268} Laboratory confirmed \textit{C. difficile} diarrhoea secondary to doxycycline as prophylaxis for malaria was reported in three Australian travellers. One stopped
prophylaxis but the others continued doxycycline despite the diarrhoea. The risk of acquiring *C. difficile* diarrhoea during doxycycline prophylaxis is unknown.

Doxycycline enhances the phototoxicity of sunlight. This photosensitive reaction is an exaggerated sunburn response that is dose dependent and results in skin redness, and blistering, especially in fair skinned individuals, after excessive exposure to sunlight. The reported incidence of photosensitivity in doxycycline users varies. In a retrospective questionnaire, 22 (5.7%) of 383 Australian travellers reported 'red skin'. Mild photosensitivity occurred in 4.5% of 125 Australian soldiers taking 50 mg of doxycycline daily and weekly CQ for just under 13 weeks. Doxycycline caused photosensitivity in 21.2% (n=52) American soldiers compared to 5.2% (n=344) on mefloquine (*P* < 0.01) but this does not appear to have resulted in drug withdrawal. Photosensitivity was reported by five of seven Caucasian men who were exposed to intense sunlight on a sailing holiday. They took 100 mg of doxycycline daily to prevent travellers' diarrhoea. These five complained of severe pruritus and stinging that recurred within minutes of reexposure to the sun. Six developed nail discoloration which progressed to photo-onycholysis (nail separation) in five. Photo-onycholysis is a well recognised but rare reaction of the tetracyclines. Non severe, doxycycline induced skin rashes have included toxic erythema, urticaria, and fixed drug eruptions. Data are few on the risk of developing a non severe rash with doxycycline; it was 4% in a study of 113 American outpatients. Severe cutaneous reactions are rare and include erythema multiforme, toxic epidermal necrolysis, vasculitis, and a generalised pustulosis.

Neurological toxicity is unusual but light-headedness (a minor NP event) was significantly more common in doxycycline recipients (19.2%) compared to those on mefloquine (10.5%). As a class, severe tetracycline induced CNS toxicity is rare. Benign intracranial hypertension has been reported in five women on long term tetracycline (250 mg daily) for acne, and in two female travellers on doxycycline prophylaxis. In the travellers, appropriate management resulted in the return of normal vision in one woman but the other woman developed optic atrophy with consequential reduced central acuity, and poor colour and peripheral vision. Her loss of vision was estimated at 70%.

Longitudinal data on the use of the tetracyclines for treating acne have been summarised in early reviews at a time when doxycycline was not commonly used.
All tetracyclines were well tolerated with a small excess of reported symptoms compared to placebo (7.9% vs. 0.9%). Demeclocycline, the tetracycline with the greatest phototoxic potential, caused all the cases of photosensitivity, and accounted for some 40% of all reported toxicity. In a clinical series of 130 women who were taking tetracycline for acne, 16 (12.3%) developed vaginal candida; a doubling of the background rate of some 6% that was found in a study of non diabetic, non pregnant women not on antibiotics. Candidiasis developed after a mean of seven weeks (range 1-20). Ten of the 16 restarted tetracycline two of whom had a recurrence.

Contraindications to doxycycline use include: (i) pregnancy, (ii) breast feeding, (iii) children under 12 years (< 8 in the US literature), (iv) a history of tetracycline allergy, (v) severe hepatic disease, and (vi) systemic lupus erythematosus. Doxycycline is not contraindicated in renal disease. Travellers should be advised to use sunscreen in sunny environments. Women on the combined oral contraceptive pill should be warned of the theoretical risk of contraceptive failure due to functional impairment of bacteria that are involved in the recycling of ethinyloestradiol from the large bowel. The BNF states this risk only applies to a short course of doxycycline and that additional contraceptive measures should be used. However, these additional measures are not needed if doxycycline use exceeds three weeks.

1.10.6.5 Atovaquone/proguanil

Atovaquone/proguanil is well tolerated as treatment or prophylaxis. The most commonly reported adverse events in clinical trials have been anorexia, nausea, vomiting, dyspepsia, abdominal pain, diarrhoea, headache, and coughing. The frequencies of some of these symptoms were similar to other antimalarials (treatment or prophylaxis) or placebo (prophylaxis). However, in one treatment trial of Thai adults, vomiting occurred in 14 of 91 A/P recipients of whom 13 (14.3%) required retreatment; this compared to 2 of 91 MQ patients, none of whom required retreatment (P < 0.001). Vomiting was also a frequent symptom in 63 treated Gabonese adults (29%) and 25 travellers (44%). From pooled data, overall rates of early vomiting (within one hour) were 8% in adults and 11% in children. However, repeat vomiting after retreatment was low (< 1%). As treatment, transient elevation of liver enzymes has been documented.

Two prophylactic studies in travellers have compared A/P with CQ/P or MQ. Overall, A/P was better tolerated than either comparator regimen. The majority of
reported symptoms were of mild severity in both studies. GI complaints (nausea, vomiting, and abdominal pain) were reported by significantly more of the 511 CQ/P recipients (20%) compared to 12% of the 511 A/P recipients. Rates of mild/moderate neuropsychiatric side effects (headache, dizziness, abnormal dreams) were similar between both groups. One CQ/P recipient had a convulsion. One A/P recipient developed an 'allergic reaction' that required study withdrawal. No further details are given but it must be assumed this was a serious A/P related adverse event. In the A/P-MQ study, rates of GI symptoms in both groups were similar [16% (A/P, n=493)] versus 19% [MQ, n=483]). MQ recipients had significantly higher rates of NP complaints (strange vivid dreams, insomnia, dizziness/vertigo, anxiety, and depression). For both groups, the majority of AEs were mild. Moderate (interfered with daily activity) or severe (medical advice sought) AEs that were considered drug related and resulted in drug discontinuation numbered 6 (1.2%) in A/P group and 24 (4.9%) in the MQ group (P=0.001); 19 of the 24 MQ withdrawals were due to NP events. A/P was also well tolerated in adult transmigrasi who reported symptoms with similar frequencies compared to placebo recipients except for stomatitis (0.8/p-y vs. 0.01/p-y) and back pain (1.1/p-y vs. 0.7/p-y). Overall, a high proportion in both groups (A/P-94%, placebo-91%) reported at least one symptom over 20 weeks.\footnote{207}

A/P should be taken with food to reduce GI upset and increase drug levels. Contraindications to A/P use include: (i) pregnancy (lack of safety data), (ii) known allergy or severe toxicity to either component, (iii) severe renal disease (defined as < 30 ml/min by the manufacturer) for both prophylaxis and treatment. A/P may accumulate if used as prophylaxis in severe renal disease (www.gsk.com accessed July 2002). Proguanil may cause a megaloblastic bone marrow toxicity in renal failure patients.\footnote{286} There are no data of A/P use in liver disease.

1.10.7 Perspective on antimalarial chemoprophylactic toxicity

In prescribing prophylaxis, clinicians need to take into account the malaria resistance patterns, potential drug toxicity, drug contraindications, ease of use (compliance), and the probability that a given regimen might need to be changed whilst the traveller is overseas for a short or long stay. Several trials have assessed some of these issues but have used different definitions of adverse events, and sample sizes. This makes direct comparisons difficult.
Drug withdrawal rates in soldiers have been reported in prospective studies and have been generally low (< 7%), for the three main regimens: doxycycline, CQ/P, and MQ. Rates of doxycycline discontinuation have been 0.6% (n=900), 1.7% (n=600), 3.2% (n=125), and 5.6% (n=53) in Australian soldiers, 6.4% in 171 French soldiers, 3.8% of 52 American soldiers, and 0% (n=486) and 2.6% (n=77) Thai soldiers. Mefloquine discontinuation rates were 0.3% of 344 American soldiers, 0% in 750 Thai soldiers, and 1.6% of 1,580 Italian soldiers, and 4.9% in travellers. Only 1.4% (54/3,760) of Italian soldiers stopped CQ/P prophylaxis whilst deployed in Somalia. In two studies of travellers, rates of withdrawal for A/P were low at 0.8 and 1.2%. One of 148 (0.7%) adult transmigrasi withdrew from A/P prophylaxis because of the development of a serious cutaneous reaction which was thought to be possibly drug related.

An important study of malaria prophylaxis has been published recently in travellers. Its importance lies in the fact that the four main regimens are compared directly. Although the study did not achieve its stated sample size, it has still provided important comparative data. Furthermore, the study was a randomised, double blind, study with a placebo run-in phase. The main outcome measures were the reporting rates of adverse events, assessed subjectively. Of 623 recruited non-immune travellers (~ 50% female) to sub-Saharan Africa: 153 each received either doxycycline, mefloquine, or the fixed combination chloroquine and proguanil, and 164 received A/P.

Overall, many travellers reported adverse events, even in the placebo group. The CQ/P arm had the highest proportion of mild to moderate adverse events: 69/153, 45% (95% CI 37%-53%), followed by mefloquine: 64/153, 42% (34%-50%), doxycycline: 51/153, 33% (26%-41%), and atovaquone and proguanil: 53/164, 32% (25%-40%); P=0.048 (3 degrees of freedom). The MQ (12%) and CQP (11%) recipients tended to report higher rates of more severe events than the A/P (7%), or doxycycline (6%) recipients (P=0.137, 3 degrees of freedom). Neuropsychiatric adverse events were significantly higher in the mefloquine group: 37% vs. 30% for CQ/P vs. 24% for doxycycline, vs. 20% for A/P (P=0.003). The highest proportion of moderate or severe skin problems were reported in the CQ/P arm: 8% vs. 3% (doxycycline) vs. 2% (A/P) vs. 1% (MQ), P=0.013. Women reported significantly more neuropsychiatric, gastrointestinal, and skin problems than men.
A total of 22 participants withdrew from the study because of adverse events or because allocation concealment had to be broken. Although rates were low, and differences did not achieve statistical significance (P=0.42), they appear to be least in the A/P group (n=3) 2% (0-4%) vs. 3% (0-6%) for doxycycline (n=5) vs. 4% (1-8%) for MQ (n=6), and 5% (2-9%) for the CQ/P group (n=8). The grade of adverse event was known in 18 subjects. AEs were mild in five participants, moderate in eight, and severe in five.

This study confirmed the low rates of withdrawal from prophylaxis of any regimen but that A/P and doxycycline appeared to be better tolerated than MQ and CQ/P. Of interest was the high rate of reported skin reactions with CQ/P. These were chiefly photosensitivity (other diagnoses were not stated), a side effect that is emphasised for doxycycline. In long term users of prophylaxis, adverse events are generally reported most often in the early phase of prophylaxis and stabilise over time.\textsuperscript{287} Withdrawal rates for long term mefloquine users were low in Dutch marines (0.3%), and American Peace corps workers (0.7%).\textsuperscript{133,188}

Taken together, these data show that drug withdrawal rates due to adverse events or drug toxicity (where causality has been established) are low. The majority of travellers and soldiers tolerate the different prophylactic regimens.

1.11 Pharmacology of chloroquine

Chloroquine, developed in the 1930's, has been the standard antimalarial drug for the past 50 years. It is also used for treating rheumatoid arthritis, exploiting its intrinsic anti inflammatory properties, and amoebic liver abscess. Standard dose CQ for the treatment of all malaria species is 10 mg/kg once on Days 0 and 1, and 5 mg/kg on Day 2. The dose for prophylaxis for adults is 300 mg of CQ base per week.

1.11.1 Mechanism of action

Chloroquine is a mix of two enantiomers. The CQ (-) enantiomer is less active than CQ (+) against CQ resistant strains \textit{in vitro}.\textsuperscript{288} MonodesethylCQ, the main CQ metabolite, has less antimalarial activity than CQ. Chloroquine is concentrated in the acidic food vacuole of parasitised red cells where it interferes with haem polymerisation, a natural detoxifying process for the parasite. The anti inflammatory effects are due to the inhibition of TNF-alpha release from macrophages and the attenuation of TNF release by glycosylphosphatidylinositol (GPI), the putative malaria 'toxin'.\textsuperscript{289}
1.11.2 Pharmacokinetics

1.11.2.1 Absorption

Chloroquine is rapidly absorbed when administered orally, by subcutaneous, or intramuscular injection, and by intravenous infusion. The $T_{\text{max}}$ after the first oral dose is 2-5h, and 51h after 25 mg/kg taken over 48 h. Oral bioavailability is high (~ 80%) and is increased with food but reduced in kwashiorkor. There is wide interindividual absorption of CQ which varies two to six fold for plasma and up to 11 fold for whole blood.

1.11.2.2 Distribution

Chloroquine has a large volume of distribution because of extensive binding to tissues. Just under two thirds (58-64%) of CQ binds to protein. Because equilibration is slow compared to absorption, there is a tendency to develop transiently high and, potentially, toxic peak concentrations after parenteral administration. Chloroquine must never be given by bolus iv injection.

1.11.2.3 Metabolism

About 50% of chloroquine is metabolised in the liver to monodesethylchloroquine (DCQ) and several secondary metabolites.

1.11.2.4 Excretion

Urinary excretion is the main mechanism of elimination; a mean of 42-47% of chloroquine is excreted unchanged and 7-12% is excreted as DCQ. The terminal half life of chloroquine is long; low concentrations of CQ persist for 1-2 months. Following CQ prophylaxis, half life estimations were 45-55 days (CQ) and 59-67 days (DCQ) with mean residence times of 20 and 35 days, respectively. In one study of Nigerian children treated with standard dose CQ, the mean plasma CQ levels on Day 7 were 38% those of Day 3, giving a half life of 3-4 days during the first week. Renal failure significantly prolongs the elimination half life, increases the AUC, and is also associated with less conversion of CQ to DCQ. Dose reduction is required only for prophylaxis. PK parameters differ between healthy subjects and malaria patients. In one Thai study, patients with vivax malaria had a significantly higher $C_{\text{max}}$, $\text{AUC}_{28\text{days}}$, and longer $T_{1/2}$ than healthy controls.
1.11.3 Toxicity

Chloroquine is usually very well tolerated when used for malaria treatment or prophylaxis (see above). More toxicity is associated with the higher doses used over prolonged periods for rheumatic diseases. Commonly reported symptoms include headache, malaise, dizziness, blurred vision, difficulty focussing, mild GI upset, and itching. Itching is more common in African patients and may affect up to ~ 50% of patients. Itching is described as a widespread prickling sensation mostly affecting the palms, soles, perineal region, and scalp. It starts within 7 to 30 hours after the first dose of CQ, reaches a peak of intensity between 4 to 12 hours later, and lasts up to 48 hours. Antihistamine treatment is not usually very effective. By contrast, a retrospective study of Thai or Burmese patients with vivax malaria found itching was uncommon, affecting ~ 2% (23/1189), mild in severity, and responded to antihistamine treatment. Less common side effects include lightening of skin colour, rashes, reversible hair bleaching, and hair loss. ECG effects have been documented in three volunteers given prophylaxis followed by a therapeutic dose (25 mg/kg total dose). The ECG changes recorded were prolongation of the QTc interval, and a reduction in the height of the T wave. Postural hypotension has also been reported. Severe toxicity such as neuromyopathy, toxic epidermal necrolysis, bone marrow toxicity, and retinopathy is rare. Irreversible CQ induced retinopathy is a complication of long-term, high-dosage therapy. Cumulative doses of 1g base/kg body weight or 50-100 g base total have been associated with retinal damage. At malaria prophylactic doses, potential eye toxicity is a significant concern only for long term (> 5 years) users. Patients may complain of temporal scotomas (words disappear when reading, only half an object is seen), and misty vision. Retinal signs include: pale optic disc, arteriolar narrowing, peripheral retinal depigmentation, macular oedema, retinal granularity and oedema, and retinal pigmentary changes consisting of a circle of pigmentation and central pallor, the so called 'doughnut' or 'bull's eye' macula. CQ induced, punctate corneal opacities occur in 30-70% of rheumatology patients within a few weeks of treatment; they do not correlate with retinal changes. Symptoms are manifest in 50% and comprise photophobia, visual halos around lights, and blurred vision. Case reports have documented hearing loss in association with long term chloroquine or hydroxychloroquine use.
1.11.4 Overdose toxicity

Chloroquine has a low safety margin; a one time dose of 30 mg/kg may be lethal.\(^{304}\) Severe clinical features are of rapid onset (1-3 hours), such as vomiting, drowsiness, convulsions, visual disturbances, hypokalaemia, (serum K\(^+\) < 3mmol/L), shock, cardiorespiratory arrest, and death. The case fatality rate (CFR) is 10-30\%.\(^{305}\) Diazepam is a specific antidote.

1.11.5 Use in pregnancy and breast feeding women

Chloroquine is considered safe for use as treatment and prophylaxis throughout pregnancy.\(^{253} 306 307 308\) Two small case series (n=21, n=35) have reported normal visual fields and slit light examinations in children who were exposed to CQ or hydroxyCQ (for maternal rheumatoid arthritis or SLE) \textit{in utero} for time periods of one to nine months. Eight children were also breast fed.\(^{309} 310\)

Chloroquine is excreted into breast milk in small quantities and has mean T\(_{1/2}\) of 8.8 days. The estimated fraction of the maternal dose that a breast feeding baby would receive was 0.7% of the daily dose and 4.2% over nine days.\(^{311} 312\) CQ can be safely given to breast feeding mothers.

1.11.6 Drug interactions, cautions, and contraindications

Several drug interactions are important. Antacids reduce CQ absorption, and cimetidine inhibits CQ metabolism resulting in increased concentrations. CQ increases the risk of ventricular arrhythmias with concurrent amiodarone use. HydroxyCQ increases the concentration of digoxin; this may also apply to CQ. Cyclosporin concentrations are increased by chloroquine. In practice, there are few contraindications to using chloroquine: known allergy, or unacceptable toxicity from previous use (see also 1.26.1). Chloroquine may increase the symptoms of myasthenia gravis.

1.12 Pharmacology of doxycycline

Doxycycline, a tetracycline, is a well established antibiotic that is used to treat a variety of infections e.g. scrub typhus, bacterial exacerbation of chronic bronchitis, and brucellosis. Doxycycline is a standard malaria prophylactic drug. It is also used in combination for treating falciparum malaria and has replaced tetracycline because of its twice daily dosing and low cost (US$ ~ 3/100 generic capsules in Indonesia).
1.12.1 Mechanism of action

The tetracyclines are bacteriostatic antibiotics; they inhibit ribosomal protein synthesis. In malaria parasites, tetracycline is believed to act in the parasite mitochondrion where it inhibits dihydroorotate dehydrogenase, an electron transport enzyme important for pyrimidine synthesis. Doxycycline has also been shown to inhibit the production of nucleoside- (e.g. ATP) and deoxynucleoside 5'- (e.g. dATP) triphosphates which are required for malaria parasite DNA replication. Doxycycline acts principally against the asexual forms of the malaria parasite. Causal activity is not consistent and there is no hypnozoite activity. In vitro, the mean (95% CI) IC$_{50}$ of doxycycline against African isolates of *P. falciparum* was high, 11.3 (9.5-13.4) μmol, reflecting its weak activity. The IC$_{50}$S between CQ sensitive and resistant stains of *P. falciparum* were similar. These findings were consistent with an *in vitro* study of Thai isolates.

1.12.2 Pharmacokinetics

1.12.2.1 Absorption

Absorption is rapid from the duodenum after oral administration and is linearly related to dose; bioavailability is 95%. After a single oral dose of 100 or 200 mg, the C$_{\text{max}}$ was 1.7-5.7 mg/L, and the T$_{\text{max}}$ 2-3.5 hrs. Unlike tetracycline, the absorption of doxycycline is not greatly affected by food or milk.

1.12.2.2 Distribution

Doxycycline is lipophilic and widely distributed in tissues. The Vd is ~ 53-134 L/kg and the volume of the central compartment is 22 L/kg. The variation in Vd values is partly due to the different doxycycline salts studied. The highest tissue concentrations are found in the liver, kidneys, and digestive tract. The tissue to serum ratio is 2:1 and is stable over time. Plasma protein binding is between 80-90% and is reduced in children with malnutrition; the latter results in increased total and non renal elimination, a decrease in the AUC, and a shortening of the T$_{1/2p}$.

1.12.2.3 Metabolism

Metabolism of doxycycline occurs in the liver, as evidenced by a decrease in the AUC with the concomitant administration of rifampicin, a known inducer of cytochrome P450. However, no doxycycline metabolites have been found in blood, urine, or faeces.
1.12.2.4 Excretion

Doxycycline excretion is via the GI tract (60-70%) and the kidneys (30-40%). Renal clearance (mix of glomerular filtration of unbound doxycycline and renal absorption) is ~2L/h and is enhanced by alkalinisation. Faecal elimination is primarily through gut wall diffusion; biliary excretion contributes to a lesser extent. Intra-inestinal doxycycline is chelated with calcium and magnesium ions which render it unabsorbable and less potent as an antibiotic. Non chelated doxycycline is reabsorbed and is recycled (enterohepatic cycling). The elimination half life is 15-25 hours; it is unchanged in the elderly and dose reduction is not necessary. In all degrees of renal failure, the AUC and half life remain the same or are slightly increased. Drug accumulation has not occurred with repeat dosing because decreased protein binding results in increased clearance via the liver and gut.

1.12.3 Toxicity

At recommended doses for prophylaxis, treatment, and in combination with antimalarial drugs, doxycycline is well tolerated (see above).

1.12.4 Overdose toxicity

There are few data on the overdose toxicity of doxycycline. Reported symptoms include nausea, vomiting and abdominal pain. Severe toxicity is thought unlikely. One case report describes a male who took 1 gm of doxycycline daily for 12 years. He developed hepatic dysfunction (hepatic necrosis and cholestasis), grey/blue patches on his shins, anaemia, leukopenia, and Wenckebach phenomenon on ECG. These effects were reversible.

1.12.5 Use in pregnancy and breast feeding women

Tetracyclines should not be used in pregnancy (see below). Little published data exist on tetracycline excretion into breast milk. Minocycline concentration peaked at 0.8 mg/L following 200 mg, representing 25% and 75% of the measured levels in amniotic fluid and the umbilical cord, respectively. The amount of tetracyclines absorbed by the breast fed infant is considered small because of chelation with calcium (Ca^{2+}) ions in breast milk.

1.12.6 Drug interactions, cautions, and contraindications

Significant interactions include anticonvulsants (increased metabolism, reduced blood levels, shortened T_{1/2p}), wine (reduced AUC), beer (delayed absorption, increased
T1/2, antacids (reduced absorption), ferrous salts (reduced absorption), and rifampicin (increased clearance). The dose of doxycycline should be increased if co-prescribed with anticonvulsants.

Known tetracycline allergy is a contraindication. Tetracyclines cause brown discoloration and abnormalities of bone growth during periods of tooth formation. They cross the placenta and have caused foetal abnormalities in animals. Therefore, they are contraindicated during pregnancy, breast feeding, and in children less than 12 years (<8 in the US). Doxycycline is listed by the British National Formulary as a drug that is unsafe for use in acute porphyria and should not be used. Exacerbation of SLE has been described with the tetracyclines and should not be used. Data on the use of doxycycline in patients with liver disease are lacking. However, as a class, tetracyclines can be hepatotoxic and should not be used in such patients. Intravenous tetracycline is contraindicated because it has caused acute liver necrosis and death in pregnant and non-pregnant adults.

1.12.7 Tetracyclines alone for the treatment of P. falciparum and P. vivax

There are few data on the treatment of P. falciparum and P. vivax with doxycycline or tetracycline. Both drugs have modest activity against both species. In experimentally induced malaria infections in non-immune American volunteers, seven days of doxycycline (100 mg bid) cured 9/9 men with low parasitaemic P. falciparum malaria whereas five days of doxycycline resulted in 4/4 cases of recrudescence that occurred between 19 to 27 days. In the same study, Chesson strain vivax malaria (the New Guinea strain) was treated with tetracycline or doxycycline. Twelve American volunteers were given tetracycline as 2 gm daily for seven (n=4) or 14 days (n=2), and 4 gm daily for seven days (n=6). All cleared their parasites but eleven had recurrent vivax parasitaemia between Days 21 to 44; the twelfth man was given primaquine on Day 33. Doxycycline was given to two men (100 mg bid x 4 or 6 days) with similar results. In another volunteer study, tetracycline was given to 29 volunteers in doses of 250 mg qid for 3½ (n=3), 5 (n=6) or 7 days (n=20). All three (3½ d regimen) and one from each other group had parasite recrudescence.

Tetracycline (250 mg qid for 10 days) cured 12 of 16 asymptomatic, low parasitaemic, P. falciparum patients of whom four had recently failed chloroquine (RI resistant). The other four developed symptomatic disease within 72 hours and had to be
given rescue treatment. Malaysian children, aged two months to eight years, with chloroquine resistant falciparum malaria had a higher cure rate when treated with seven days of doxycycline (4 mg/kg) compared to four days: 22/26 (84.6%) vs. 4/9 (44.4%), P=0.01. Five Gambian children aged 1½ to 5 years cleared their parasites with high doses of tetracycline, 125 or 250 mg qid for five days. In all these studies, tetracycline and doxycycline produced slow parasite (PCT) and fever (FCT) clearance times against both malaria species. The PCTs and FCTs for *P. falciparum* ranged from 4 to 8 and 1 to 8 days, respectively; corresponding figures for *P. vivax* were 4 to 10 and 4 to 9 days, respectively.

1.12.8 Use of the tetracyclines as adjunct treatment of *P. falciparum* malaria

A number of studies have reported the use of tetracycline or doxycycline in combination with standard antimalarials achieving efficacious cure rates of ~ 95%. Twenty nine (96.6%) of thirty Thai adults were cured by three days of quinine and ten days of tetracycline. Similar results also from Thailand were obtained in other studies that used seven days of tetracycline or doxycycline combined with quinine (3 or 7d), mefloquine (25 mg/kg total dose), or amodiaquine. In Gabon, doxycycline for three days combined with one day of quinine (3 doses) resulted in a cure rate of 91% (32/35) compared to 38% (14/37) for quinine alone; all treatment failures were RI. The corresponding figures for doxycycline/CQ and CQ alone were 75% (27/36) and 36% (15/42) in two other studies. However, a clinical trial of Thai adults with falciparum malaria assessing the efficacy of chloroquine with tetracycline against falciparum malaria was prematurely abandoned because two patients with RIII resistance became seriously ill with high fever and rising parasite counts. The investigators concluded that this combination was potentially dangerous and recommended against its use for treating chloroquine resistant falciparum malaria.

1.13 Pharmacology of Azithromycin

Azithromycin is an azalide antibiotic with a similar structure to erythromycin and clarithromycin. It has broad antimicrobial activity and is used to treat a number of infections, including community-acquired pneumonia, travellers' diarrhoea, trachoma, genital *Chlamydia trachomatis*, and *Mycobacterium avium-intracellulare* complex
(MAC). The dose of azithromycin varies with the clinical indication but for routine use, the adult dose is 1.5 gm given taken over three days.

1.13.1 Mechanism of action

Azithromycin inhibits ribosomal protein synthesis in bacteria, resulting in a predominantly bacteriostatic effect. A post antibiotic effect of several hours has been demonstrated against respiratory bacteria. In malaria parasites, protein synthesis is also inhibited but the precise details are unknown. Like doxycycline, azithromycin results in a reduced production of nucleoside-5'-triphosphates and 2'-deoxynucleoside-5'-triphosphates.315

In vitro, azithromycin has weak antimalarial activity against CQ resistant and sensitive P. falciparum, comparable to the other antimalarial antibiotics.331 332 In one in vitro experiment, the MIC was higher at 48 h (6.2 and 8.7 μg/ml) compared to 96 h (0.08 and 0.04 μg/ml) for two falciparum isolates. The marked reduction in the MIC values between the first and second asexual erythrocytic cycles suggests a slow onset of action.333 An additive or synergistic effect was seen with chloroquine and quinine.334

1.13.2 Pharmacokinetics

The PK profile of azithromycin is one of rapid absorption, extensive tissue uptake, followed by slow release that results in a long half life. Compared to erythromycin, azithromycin has lower serum and AUC values, greater tissue penetration, and a longer terminal half life.

1.13.2.1 Absorption

Azithromycin is absorbed rapidly from the gastrointestinal tract, reaching peak plasma concentrations after approximately 2½ hours. In studies of different regimens of orally administered azithromycin to healthy adult volunteers (e.g. 500 mg on Day 0, followed by 250 mg/d x 4 or 9d, 500 mg once only on Day 0), the measured pharmacokinetic parameters were: (i) a low bioavailability of 37% (probably related to incomplete absorption), (ii) Cmax 0.41-0.5 mg/L, (iii) Tmax 2.2-2.5 hours, (iv) a mean serum concentration at 24 hours of 0.05 mg/L, (v) a T1/2α ~ 4 hr, (vi) mean T1/2β ~ 57 and 68 h (the T1/2β depended on when sampling was done), (vii) half lives in tissues were 2.3 days (prostate), and 3.2 days (tonsils), (viii) AUC0-72 = 4.3 mg/L·h (stat dose only), (ix) AUC0-24 = 2.6 mg/L·h, (x) AUC0-4 = 3.39 mg/L·h, (xi) Vd = 31 L/kg, (xii) tissue:serum ratio > 100 at 12 h, and (xi) a high plasma clearance of 630 ml/min.335 336 Compared to
young volunteers, older subjects had (i) a significantly slower $T_{\text{max}}$ (3.8 vs. 2.5 h post first dose), and (ii) a significantly higher AUC on Day 5 (2.7 vs. 2.1 mg/Lh) but not Day 1 (3.8 vs. 2.5 mg/Lh).

### 1.13.2.2 Distribution
Azithromycin is widely distributed in tissues. Protein binding is mostly to $\alpha_1$-acid glycoprotein and is inversely related to the serum concentration. Binding is $\sim 50\%$ and 7.1% at serum concentrations of 0.02-0.05 and 1 mg/L, respectively. Its high tissue (intracellular) penetration is a therapeutic advantage against intracellular pathogens like *Chlamydia* and *Toxoplasma* species. There is also high penetration into white blood cells and macrophages but there are no data on its penetration into normal or parasitised red cells.

### 1.13.2.3 Metabolism
Azithromycin is metabolised in the liver but not by the cytochrome P450 isoenzymes. The main metabolic reaction is N-demethylation of the desosamine sugar of the macrolide ring; others include O-demethylation, hydrolysis, and hydroxylation. There is no significant interaction with theophylline, digoxin, and warfarin.

### 1.13.2.4 Excretion
Biliary excretion of mostly unchanged drug is the main route of elimination; $\sim 6\%$ of an oral dose appears unchanged in urine. In mild to moderate renal failure (GFR 10-80 ml/min), the $C_{\text{max}}$ and $AUC_{0-120}$ increased modestly by 5.1 and 4.2%, respectively; corresponding values in severe renal failure (GFR < 10 ml/min) were 61% and 35%. In mild or moderate hepatic impairment, PK differences with normal volunteers were inconsistent. The $T_{1/2\beta}$ was increased modestly in volunteers with moderate hepatic impairment. Dose reduction in mild or moderate renal or hepatic disease is unnecessary.

### 1.13.3 Toxicity
Standard dose azithromycin, administered over 3 to 5 days to children (30 mg/kg total dose) or adults (1.5 gm total dose), is well tolerated. A total of 12% report any symptom. Less than 10% of patients report any gastrointestinal symptom [nausea or vomiting (3.4%), abdominal pain (2.5%), diarrhoea (3.6%)]. The majority of patients, 93%, reported mild or moderate side effects. Unacceptable toxicity resulted in drug discontinuation in 0.7-1.3% of azithromycin treated patients.\(^{337} 338 339 340\) This tolerability
is comparable or better than other antibiotics that were used for the same clinical indications.\textsuperscript{341} \textsuperscript{342} \textsuperscript{343} \textsuperscript{344} High dose azithromycin e.g. 1 gm for adult genital chlamydia, 20 mg/kg in paediatric trachoma, is also well tolerated.\textsuperscript{345} \textsuperscript{346} However, tolerability is worse when azithromycin is used for longer periods against MAC in both AIDS and non-AIDS patients.\textsuperscript{347} \textsuperscript{348} \textsuperscript{349} \textsuperscript{350} Doses of 1.2 gm weekly or 300–600 mg/day are associated frequently with side effects, especially GI symptoms. In a study of MAC prophylaxis, 1.2 gm of azithromycin was administered weekly to 85 patients for a mean of 400 (30-985) days. Seventy one (78.9\%) reported at least one GI symptom, a 2-4 fold increase compared to placebo. GI intolerance resulted in six (7\%) patient withdrawals at a median time of 112 days.\textsuperscript{348}

Several small studies (total n 46 patients), have identified reversible hearing loss as an important side effect in 13-17\% of patients with AIDS or non-AIDS related MAC. Audiogram confirmed hearing loss has usually been in the speech frequency range and lasted between two and eleven weeks.\textsuperscript{349} \textsuperscript{350} \textsuperscript{351} \textsuperscript{352} There is, however, one published report of irreversible, high frequency hearing loss in a 37 years old woman who took 750 mg of azithromycin.\textsuperscript{353}

In the Kenyan prophylaxis study, both azithromycin groups (250 mg/d or 1gm/w) reported symptoms that were similar to the placebo group. There was also no evidence of toxicity on routine clinical haematological or biochemical testing.\textsuperscript{199} In large clinical series, changes in haematological and biochemical parameters have been unremarkable. Transient neutropenia (1.5\%) and transient, mild elevations of liver transaminases (1.5-1.7\%), alkaline phosphatase (0.3\%), bilirubin (0.7\%) have been reported in a minority of patients. Significant hepatotoxicity has occurred rarely e.g. hepatitis as part of a systemic hypersensitivity syndrome, and cholestasis.\textsuperscript{354} \textsuperscript{355} Serious azithromycin related toxicity e.g. pseudemembranous colitis, erythema multiforme, Churg-Strauss syndrome, cutaneous vasculitis, pustular rash, Henock-Schönlein purpura, and anaphylaxis is also rare.\textsuperscript{337} \textsuperscript{338} \textsuperscript{339} \textsuperscript{356} Anaphylaxis may reappear after apparent successful treatment without reexposure to the drug. Therefore, treatment for anaphylaxis may be required for several days. There are no published data on azithromycin overdose.

\textbf{1.13.4 Use in pregnancy and breast feeding women}

Azithromycin is not licensed for use in pregnancy but there is accumulating experience with the treatment of genital \textit{Chlamydia trachomatis}. Azithromycin is well
tolerated and has not caused untoward toxicity for both mother and foetus.\textsuperscript{357} \textsuperscript{358} Azithromycin is excreted into breast milk in small amounts but its effect on infants is unknown.\textsuperscript{359} Breast feeding should not, therefore, be considered a contraindication.

1.13.5 Drug interactions, cautions, and contraindications

Antacids reduce the absorption of azithromycin. Because azithromycin is not metabolised by cytochrome P450, there are far fewer metabolic interactions compared to erythromycin e.g. theophylline, fluconazole, carbamazepine, and cyclosporine. Nelfinavir increases azithromycin serum concentrations. There is also no significant interaction with warfarin (displacement from protein binding). Azithromycin has few contraindications; namely, known macrolide allergy, and severe liver disease. Caution should be exercised in patients with mild hepatic or renal impairment but dose reduction is unnecessary. There are no dose recommendations for patients with severe renal disease. Because the safety database in pregnancy is limited, azithromycin cannot yet be recommended. Never the less, azithromycin can be used if there are no safer alternative drugs.

1.13.6 Antimalarial activity

1.13.6.1 Preclinical studies

Against blood forms of \textit{P. berghei} in mice, azithromycin was more active than erythromycin and its activity was increased by chloroquine.\textsuperscript{360} Azithromycin was more potent than doxycycline in a murine experiment of causal prophylaxis against \textit{P. yoelii}; the effect was dose dependent.\textsuperscript{361} In mice and monkey experiments, azithromycin had schizonticidal activity against \textit{P. berghei} and \textit{CQ resistant P. falciparum}, respectively. It also enhanced the antimalarial activity of quinine, halofantrine, and artemisinin.\textsuperscript{362}

1.13.6.2 Clinical studies

Two human challenge experiments demonstrated a prophylactic effect of azithromycin and its causal activity. Four volunteers were given supervised azithromycin for 7 days (500 mg on Day -2, then 250 mg/d for Days -1 to +5). Mosquito challenge with a chloroquine resistant strain of \textit{P. falciparum} was done on Day 0. Three volunteers remained malaria free; the one who developed malaria did not have measurable azithromycin serum levels, consistent with poor absorption. Fifteen control subjects, who did not receive azithromycin, developed malaria 9 to 12 days after sporozoite challenge.\textsuperscript{363} In the second study, a prophylactic effect against the initial 7 day period of solely liver infection was determined by loading subjects with azithromycin prior to
malaria challenge on Day 0 and giving azithromycin for 7 days after challenge. The regimen was 500 mg on Day -14, followed by 250 mg/d from Day -13 to Day +7. Four of 10 subjects did not develop patent parasitaemia. In a second cohort (n=10), azithromycin was continued for 28 days after challenge. None of the 10 subjects became infected. For each cohort, two concomitantly challenged control subjects did not receive azithromycin and all developed patent parasitaemia on Days 9 to 13. Azithromycin was partially effective against liver parasites and highly effective against blood stage parasites.

When azithromycin was used for treating Gambian children with trachoma, there was a coincidental reduction in spleen rates, parasite counts, and the number of episodes of febrile parasitaemia due to *P. falciparum*. A placebo controlled prophylactic study against *P. falciparum* was conducted over ten weeks in Kenyan adults from western Kenya. Daily (250 mg) or weekly (1 gm) azithromycin, and daily doxycycline (100 mg) were tested. Using cumulative incidence, the prophylactic efficacies were: (i) daily azithromycin 83% (68-91), (ii) weekly azithromycin 64.2% (41.1-77.1), and (iii) doxycycline 92.6% (79.9-97.5). Although the PEfs of azithromycin were not significantly different, these data suggest that daily dosing was better than weekly dosing.

1.14 Rationale for the chloroquine doxycycline study

This study was undertaken as a joint collaborative effort between the Indonesian Ministry of Health, the Indonesian Navy, and NAMRU-2. At the time, the first line drug for malaria was chloroquine for all provinces of Indonesia. SP was the second line choice for chloroquine resistant *P. falciparum* and there was no official second line choice for chloroquine resistant *P. vivax*. Aside from CQ and SP, only primaquine, quinine, mefloquine and Fansimef were registered in Indonesia at the time of the study. Well known antibiotics e.g. the tetracyclines, the penicillins, co-trimoxazole, chloramphenicol were also registered.

By 1995, it was clear that Indonesia had areas of multidrug resistant *P. falciparum* as well as CRPV. Other parts of south east Asia were also known to harbour multidrug resistant malaria; the best well characterised area was, and still is, the western border of Thailand. Standard (15 mg/kg) and high (25 mg/kg) dose mefloquine had become ineffective, the latter falling to about 60% efficacy by 1994.94 In 1993, three days of artesunate plus high dose mefloquine had achieved a cure rate of some 98%.156 This
combination subsequently rescued mefloquine and is currently (2005) the first line treatment for drug resistant *P. falciparum*.

The predominant view regarding the treatment of malaria in 1995 was still to use monotherapy. Experience with combinations like artesunate plus mefloquine or standard antimalarial drugs with antibiotics had been limited mostly to the research setting. In addition, quinine alone or in combination with tetracycline sufficed for drug resistant malaria. Although artesunate and other artemisinins had been used in China for many years, there appears to have been a reluctance to embrace this class of drugs in the 1990s. This is certainly also the case for Indonesia. It is also fair to point out that although the problem of drug resistance was known, effective and long lasting solutions were not obvious. The failed experience of Fansimef in Thailand may have also dampened the enthusiasm for using drug combinations and there were no longitudinal studies which showed convincingly that a given combination had held resistance in check and / or reduced malaria transmission. This information only became widely available in 2000 when the experience from the Thai Burmese border was published.94

Therefore, for the Indonesian Ministry of Health, options were limited. Mefloquine was expensive, associated with unpleasant side effects that might limit acceptability by the population, and not registered in Indonesia. Halofantrine was also expensive and unregistered, and at this time reports were emerging of fatal cardiotoxicity, rendering a poor choice for widespread use. Atovaquone / proguanil have never been registered in Indonesia and had only been registered in early 1990s in other countries. Cost precluded its widespread use despite its good efficacy and tolerability. The question of using artemisinins was never debated as a serious option because little was known about this class of drugs and they were not widely available. Amodiaquine, a drug widely used in Africa, has never been used as drug policy in Indonesia and was, therefore, never considered an option.

Clearly, an easy and readily applicable option would be to use drugs that were well characterised, inexpensive, registered and widely available in Indonesia. One option was to examine the possible benefit of adding doxycycline to chloroquine for both falciparum and vivax malaria, cogniscent that this combination could not be used in children under 8 years or in pregnant women. There was adequate documentation from Asia, mostly Thailand, and Africa that tetracycline and doxycycline increased cure rates
when combined to chloroquine and quinine. The cost of this combination for an adult would increase the total cost to about 50 US cents, a not unreasonable price. Therefore, this combination was chosen and tested in adult patients.

1.15 Rationale for the azithromycin prophylaxis study

The azithromycin prophylaxis study was part of a drug registration project that was a partnership between Pfizer, the US Army, US Navy, the Indonesian Army, and the Indonesian Ministry of Health. At the time of the study, there were three widely available and internationally registered drugs for malaria: chloroquine/proguanil, doxycycline, and mefloquine. Atovaquone / proguanil was in development and was registered as a prophylactic in 1998.

With only three prophylactic drugs, there was certainly a need for more and better drugs. The usefulness of chloroquine/proguanil is severely limited by the spread of chloroquine resistance that continues. This combination had already been relegated to second choice by 1995 and is currently not recommended by some countries. Although never formally tested in Indonesia, its prophylactic efficacy is likely to be very low, possibly no better than placebo in north east Papua. Mefloquine is certainly efficacious in areas where there is high sensitivity to this drug but there is a cloud over its tolerability and a perception that is not well tolerated despite the documented evidence. Doxycycline is highly effective and generally well tolerated but its clinical indication does not include small children or pregnant women. The antimalarial properties of azithromycin were well described in animal work, human challenge experiments, indirect field experience in trachoma treatment, and a prophylactic study in malaria immune Kenyans.

Azithromycin is registered for use in adults and children down to the age of six months. For use in pregnancy, the US FDA classifies azithromycin as a B drug (reproduction studies show no evidence of harm to the fetus but adequate and well controlled trials in pregnancy are lacking). Azithromycin has been used to treat sexually transmitted diseases in pregnant women without apparent untoward effects for both mother and foetus but experience is still young. Azithromycin is secreted into breast milk in small amounts and can be given to breast feeding mothers.

Malaria chemoprophylaxis and has traditionally been the domain of non immune travellers but there is currently increasing interest in prophylaxis and intermittent preventative treatment in pregnant women in malaria endemic countries. For drug
registration, demonstrating high prophylactic efficacy in a non immune population is better than trials conducted in malaria immune individuals. Indeed, this was the request of the US FDA for this study (D. Braitman, personal communication). Therefore, if the measured prophylactic efficacy of azithromycin in a population of low or no immunity were high against *P. falciparum* malaria, and its tolerability good, then azithromycin would be a substantial addition to the small number of antimalarial prophylactic drugs currently available. Azithromycin could be used in individuals of all ages, breast feeding mothers, and in those with certain illnesses e.g. epilepsy, mild to moderate renal failure, depression, other major psychiatric illnesses, and those with doxycycline induced photosensitivity. There is a potential role for azithromycin in pregnant women but more safety data are required before this recommendation can be made.

The decision to allow azithromycin to be used in Indonesia was made by the Indonesian National Ethics Review Committee and the Indonesian Army Ethics committee. There was less enthusiasm from the Ministry of Health because the likely high cost of the drug would have precluded its widespread use. However, the Indonesian Army was keen have another prophylactic option and were happy to have close ties with the US Navy. Cost was less of an issue (See also Ethical Issues in the Discussion section).

1.16 Study aims

The aims of the studies were to assess: (i) azithromycin as a potential antimalarial prophylactic drug, and (ii) the combination of chloroquine and doxycycline as alternative therapy to chloroquine against chloroquine resistant *P. falciparum* and *P. vivax*.

1.17 Study objectives

The objectives were to: (i) measure the prophylactic efficacy of azithromycin and doxycycline relative to placebo for preventing *P. falciparum* and *P. vivax* parasitaemia, and (ii) evaluate the efficacy and tolerability of chloroquine combined with doxycycline for treating acute, uncomplicated *P. falciparum* and *P. vivax*. 
Chapter 2 - Materials and Methods

2.1 Study site - Jayapura and Arso region

2.1.1 General description

Irian Jaya, renamed Papua in 2000, forms the western half of New Guinea island (Appendix 2). It is geographically diverse with central highlands that peak at ~ 5000m, lowlands, and areas of dense tropical forestation. Jayapura is the provincial capital and is located on the north east coast. Jayapura has a population of 210,000. The Greater Jayapura area extends for approximately 60 km around the city centre and includes several small towns [Abepura (population of 46,000), Sentani (pop. 30,000)], and several transmigrant settlements. There is one public (Rumah Sakit Umum) and two military hospitals. The RSU has 373 beds, and functions as a regional referral centre. Annual visits to the hospital total approximately 130,000 outpatients, with almost 14,000 inpatients. There are also several government and private medical clinics, laboratories, and pharmacies located in the area. Antimalarial and other drugs may be purchased easily without a prescription.

The Arso region is a rural area situated south east of Jayapura. It is a low land swamp that is surrounded by forested hills. The plain stretches south for 50 km from Sudarso bay in the north and is between 20-40 km in breadth. Several small tributaries of the Tami river cross the plain. Arso is hot and wet all year round. Temperatures range from 20°C to 35°C. The relative humidity is usually approximately 80%. Total annual rainfall is estimated at 3,400 mm (134 inches). It is highest during November and March; there is no dry season. The northern part consists mostly of forested areas ('hutan') and a large palm oil plantation of some 10,000 hectares dominates the extreme south of the plain. Local Irianese and transmigrasi (Indonesian immigrants from other parts of Indonesia) live together in small settlements constructed by clearing the hutan and erecting simple wooden houses. Most settlements do not have basic amenities e.g. running water, electricity, and telephone lines. There is, however, a reasonable public health infrastructure in Arso. Free, primary health care is provided by health workers (called mantrees) with basic training, and the larger settlements have government run clinics (puskesmas). The majority of villagers use these government clinics for health
care. There are no private clinics in rural Arso but some small shops do sell antimalarial as well as other drugs.

2.1.2 Population - Irianese, transmigrants, soldiers

The population of Jayapura and its surrounding rural environs consists of indigenous Irianese and transmigrasi. The rural based Irianese and transmigrasi are subsistence farmers. The transmigrasi volunteer to immigrate to Irian under a government scheme, the Transmigration Programme that resettles people from other parts of Indonesia. Most are farmers from rural Java where malaria transmission is low.6 The Irianese have had life long exposure to malaria and naturally acquired, malaria immunity. By contrast, the transmigrasi have had little or no malaria exposure and correspondingly little or no immunity. The transmigrasi who were recruited for the azithromycin prophylaxis study had lived in the village of PIR V for 15 months. As per Indonesian Ministry of Health (MoH) policy, they had received free chloroquine prophylaxis for the first three months of their arrival that was distributed by the mantrees. Bed nets, usually not impregnated, were used in PIR V by individual families but there was no government run bed net programme.

The Indonesian Army soldiers serve one year of duty from their home bases in other parts of Indonesia. Soldiers are stationed in small military posts and encampments that are situated near villages throughout Arso. Like the transmigrasi, they have had limited or no malaria exposure. The recruited soldiers (prophylaxis study) had arrived 6 months earlier from Lampung, southern Sumatra, an area of low malaria incidence (F. Laihad, personal communication). Upon arrival in Arso, the soldiers were issued with non impregnated bed nets and were given several anti malaria prophylactic drugs, doxycycline, sulfadoxine/pyrimethamine, or chloroquine. There did not appear to be a consistent prophylaxis policy. All were changed to doxycycline by the research team whilst preparing for the azithromycin study.

2.1.3 Malaria characteristics in Jayapura and Arso region

2.1.3.1 Epidemiology in Jayapura

Surveys conducted in the 1970s in Jayapura and Abepura showed low malaria prevalence rates of 10% and 20% in 1970 and 6% and 18% in 1979, respectively.57 Two thirds of cases were due to *P. falciparum*. There are no recently published malaria epidemiological data for Jayapura but the impression from local hospital physicians is
that urban malaria is not a significant problem; the majority of malaria cases seen are from the rural areas.

2.1.3.2 Epidemiology in Arso - data from the Dutch era

There are ample malaria data from the rural environs of Jayapura, particularly for Arso. In the 1950s Dutch malariologists classified the malaria endemicity based on parasite rates rather than spleen rates because the latter was deemed a better reflection of infection risk (Table 2.1).\textsuperscript{367} Interestingly, they noted that adult Irianese had high spleen rates (78%) in the Nimboran valley (60 km south west of Jayapura) an area of holoendemic malaria (parasite rate ~ 95%). This finding contrasted with holoendemic Africa. Accordingly, they classified holoendemic malaria as the African type (low spleen rates in adults) and the New Guinea type (high adult spleen rates).

<table>
<thead>
<tr>
<th>Transmission intensity</th>
<th>APR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoendemic</td>
<td>0 - 10</td>
</tr>
<tr>
<td>Mesoendemic</td>
<td>11 - 50</td>
</tr>
<tr>
<td>Hyperendemic</td>
<td>51 - 75</td>
</tr>
<tr>
<td>Holoendemic</td>
<td>&gt; 75</td>
</tr>
</tbody>
</table>

Malariometric surveys conducted in 1958 in two villages in Arso before the pyrimethaminised salt campaign showed an overall malaria prevalence of 58% of 1,450 individuals examined. Rates were much higher in children compared to adults and were highest in children under five, just over 90%. Rates of between 35 and 45% were found in adults aged 15 and above. Counting mixed infections twice (rates not stated), the species breakdown was 31% for \textit{P. falciparum}, 22% for \textit{P. vivax}, and 19% for \textit{P. malariae}. The corresponding figures obtained six months into the salt campaign were 17%, 2%, and 0%. Overall malaria prevalence rates decreased to 22% from 58% pre salt campaign.\textsuperscript{67}

2.1.3.3 Epidemiology in Arso - recent data

During 1987 and 1993, malariometric surveys were conducted by the US Navy in indigenous Irianese and transmigrasi who lived in new as well as well established settlements. Malaria prevalence and spleen rates varied between villages but were
generally high (Table 2.2). *P. falciparum* predominated in most villages. *P. malariae* and *P. ovale* were detected rarely.

Table 2.2 Malaria prevalence rates in transmigrants of all ages from Java who had resettled in several villages in the Arso region of north east Papua. Surveys conducted by the US Navy. Data from Cdr. JK. Baird, US Navy.

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>N*</th>
<th>*P. falciparum†</th>
<th>*P. vivax†</th>
<th>Pf:Pv</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PIR I</td>
<td>Nov 1987</td>
<td>188</td>
<td>37</td>
<td>15</td>
<td>2.5:1</td>
<td>52</td>
</tr>
<tr>
<td>PIR II</td>
<td>Jan 1989</td>
<td>224</td>
<td>30</td>
<td>37</td>
<td>0.8:1</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Mar 1990</td>
<td>215</td>
<td>19</td>
<td>17</td>
<td>1.1:1</td>
<td>36</td>
</tr>
<tr>
<td>PIR V</td>
<td>Jun 1991</td>
<td>689</td>
<td>63</td>
<td>23</td>
<td>2.7:1</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>Mar 1992</td>
<td>553</td>
<td>41</td>
<td>15</td>
<td>2.7:1</td>
<td>56</td>
</tr>
<tr>
<td>PIR VI</td>
<td>Jun 1991</td>
<td>257</td>
<td>25</td>
<td>26</td>
<td>1:1</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Mar 1992</td>
<td>477</td>
<td>15</td>
<td>12</td>
<td>1.25:1</td>
<td>27</td>
</tr>
<tr>
<td>PIR IV</td>
<td>Mar 1993</td>
<td>216</td>
<td>30</td>
<td>14</td>
<td>2.1:1</td>
<td>44</td>
</tr>
</tbody>
</table>

* N=number surveyed
† prevalence rate as %

### 2.1.4 Epidemiological surveys in PIR I

Arso PIR I village was established in 1986. At the time, the population numbered 960 of whom 45% were Irianese and 55% were transmigrasi. A malaria prevalence survey was conducted in 1987 by screening 473 (49%) villagers; 213 were Irianese, the rest (260) were transmigrasi. The overall prevalence of malaria was ~ 45% (n=213) with a falciparum to vivax ratio of 2:1. Overall, the transmigrasi (~ 51%) had significantly higher rate than the Irianese (~ 37%). Older transmigrant children (age ≥ 11) and adults (age > 15) had higher rates of *P. falciparum* than Irianese. Rates in transmigrant (~ 38%) and Irianese (~ 29%) children < 11 years had rates of *P. falciparum* that were not significantly different. The prevalence rates for *P. vivax* were similar between both groups; only transmigrasi adults (~ 13%) had significantly higher rates than the Irianese adults (~ 4%). None of the villagers carried *P. vivax* gametocytes but 25/258 (9.7%) of transmigrasi carried falciparum gametocytes compared to 5 (2.3%) of 215 Irianese (*P = 0.001*). Gametocytaemia was always associated with asexual parasitaemia and was 2¼ fold (95% CI 1.1-6.7) more likely in the transmigrasi [25/94 (26.6%)] than the Irianese [5/51 (9.8%)]. In this survey, malaria prevalence rates declined with increasing age for both groups. Rates were less for the older Irianese, consistent with a
greater degree of acquired immunity. Malaria infected Irianese also had lower gametocyte carriage rates, suggesting the development of immunity against sexual stages of the malaria parasite.

In a later study in PIR I, malaria attack rates and incidence rates were measured during the high and low transmission seasons. Subjects aged > 15 received radical cure for malaria and were monitored weekly by blood film for 20 weeks during the high transmission season (May to October) and for 12 weeks during the low transmission season (January to April). The \textit{P. falciparum} malaria incidence rates in the low season were significantly higher in the transmigrants (3.4/p-y) compared to the Irianese (1.6/p-y) but were similar in the high season, 4.6/p-y vs. 4.1/p-y, respectively. Cumulative incidence data were consistent. During the low transmission season, 50\% of the transmigrants developed falciparum parasitaemia by week 10 and 43\% of the Irianese were positive by week 17, for a relative risk of 1.65 (95\% CI 1.17-2.34). During the high transmission season study, 50\% of the transmigrants and 50\% of the Irianese were \textit{P. falciparum} positive by week 6 and week 9, respectively, for a relative risk (transmigrasi:Irianese) of 1.1 (0.85-1.47). Comparing the risk of falciparum parasitaemia within each group, the transmigrasi had similar attack rates in the high [70\% (43/61)] and low [64\% (42/66)] seasons. The Irianese had a two fold increased risk in the high season compared to the low season: 63\% (29/46) vs. 32\% (16/50), RR=1.97 (1.24-3.12). The attack rates for \textit{P. vivax} were similar between both groups during the high and low seasons: 27\% (18/66, transmigrasi) vs. 32\% (16/50, Irianese), and 11\% (7/61, transmigrasi) vs. 8.7\% (4/46, Irianese), respectively.

Epidemiological data from other villages obtained during clinical trials following radical cure have shown variable results compared to those of PIR I. During one year of malaria surveillance in Arso PIR XI, the cumulative incidence of malaria was ~ 48\% for \textit{P. vivax} and ~ 36\% for \textit{P. falciparum}; the measured incidence rates were ~ 0.5/p-y for each species. In a prophylactic study conducted in non immune Indonesian soldiers, stationed throughout Arso, the incidence rates were 3.3/p-y for \textit{P. falciparum} and 2.5/p-y for \textit{P. vivax}. 

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2.1.5 Age, clinical immunity, and malaria

Data to support the hypothesis that protective malaria acquired immunity in malaria naïve, transmigrasi adults may develop over a short time span of two years come from several epidemiological and serological studies.

One study obtained detailed malaria prevalence data at 15 time points over the course of five years in six villages in Arso: PIR I, II, IV, V, VI, and Nimborokrang (Genyem subdistrict). Some 4,550 Giemsa-stained thick films were examined and sample sizes ranged from 91 to 701 people (mean 304). The Javanese transmigrants in these villages had been resident for between three weeks to six years. In the newly established villages (Arso PIR I, II, Nimborokrang), malaria prevalence rates decreased markedly with increasing age beyond 6-10 or 11-15 years in transmigrants resident for more than 16-24 months.

In the second study, conducted in PIR I, five parameters of malaria infection were examined: (i) individual slide positivity rate (proportion of blood films positive for that individual during surveillance), (ii) median time to first positive slide, (iii) degree of parasitaemia, (iv) proportion of subjects with symptomatic parasitaemia, and (v) antibody levels of ring-infected erythrocyte surface antigen (RESA). The transmigrasi had been resident for 1-25 months. Compared to transmigrasi children, the adult transmigrants had lower malaria prevalence rates, longer median time to first parasitaemia, lower mean parasitaemias, fewer reported symptoms, and higher RESA levels. Similar changes were also seen in the Irianese. However, compared to the transmigrasi of all ages, the Irianese had significantly lower malaria prevalence rates, longer median times to first parasitaemia, and lower rates of symptomatic parasitaemia. The overall malaria prevalence was significantly lower in the Irianese (23%) compared to the transmigrasi (31%), as were the rates of symptomatic parasitaemia: 16% (n=128) vs. 28% (n=233). The 'benefit' of life long exposure was estimating using the Irianese-transmigrasi difference in slide positivity rates. An interesting species dependent difference was observed. For \textit{P. falciparum} the benefit was approximately 20% across all age groups but for \textit{P. vivax}, the benefit was age dependent, ranging from \sim 5\% in the under fives to \sim 80\% in adults. Taken together these malariometric data suggest that the transmigrasi had acquired protective immunity against \textit{P. falciparum} but not little or no immunity against \textit{P. vivax} over approximately two years since their arrival in Irian Jaya.
Supporting antibody data for an age dependent immune response were found in a study of 30 residents of PIR I (10 adult Irianese, 10 adult transmigrasi, and 10 transmigrasi children with 45 months of malaria exposure).\textsuperscript{372} Essential findings were similar patterns of the disruption of red cell rosettes and agglutination for all groups. The mean MSP2 and RESA antibody titres were similar between both adult groups but significantly lower in the transmigrasi children. In another study, antibody titres to two antigens R32LR (a peptide containing sequences from the \textit{P. falciparum} circumsporozoite repeat region) and MSP19 (a proteolytic fragment of MSP-1) were measured in 66 transmigrasi adults and children after 6 and then 14 months of malaria exposure. Antibody titres rose with time but were independent of age.\textsuperscript{373} The short period of exposure may account for these contrasting findings to those of Reeder \textit{et al.}

\textbf{2.1.6 Clinical study of newly arrived transmigrasi}

This study was conducted in 1992 when Arso PIR IV, population of 1,059, was newly established.\textsuperscript{374} Within four months of their arrival, the prevalence of \textit{P. falciparum} in the transmigrasi was 72\%, and the monthly cumulative incidence of visits to the puskesmas for clinical malaria was 81\%. Ninety percent of the malaria was due to \textit{P. falciparum}. The rates for emergency evacuation to hospital because of clinically severe malaria (usually cerebral signs) was highest in young and middle aged adults (range 16-40), accounting for half of the total. By six months, the risk of hospital referral was significantly higher for adults compared to children: 23.2\% (148/639) vs. 8.6\% (36/420), giving a relative risk of 2.7 (95\% CI 1.9-3.8). Total deaths numbered 8, six adults and two children, for an overall risk of death of 0.8\%. Of those evacuated, the CFR was 4.4\% (8/184). As a result of the deteriorating situation in PIR IV, the public health authorities treated all cases of malaria with chloroquine and primaquine and sprayed the houses with insecticide with good effect. The difference in susceptibility to severe disease between the adults and children represents a difference in the immune response following primary exposure to \textit{P. falciparum}.

\textbf{2.1.7 Entomology}

Entomological studies from Arso PIR I showed that \textit{Anopheles koliensis} was the predominant vector, accounting for 92\% of the anopheline catch; \textit{An. punctulatus} and \textit{An. farauti} comprised 5.7\% and 3.2\%, respectively. Entomological inoculation rates for \textit{P
varied between 0.018 and 0.39 infective bites/person/night (≈ 6.6 and 142/p-y) during the low and high transmission seasons, respectively.²⁶⁹

2.2 Clinical trials conducted in Jayapura and Arso

2.2.1 Chloroquine for Plasmodium falciparum

Meuwissen documented the early clinical indication of reduced chloroquine sensitivity in the 1960s by recording prolonged parasite clearance times of 144 hours (6 days) in 12 (41%) of 29 local residents following the administration of standard dose chloroquine.⁵⁷ By the mid 1970s, chloroquine resistant falciparum malaria was confirmed in Jayapura. Seven (26%) of 27 patients had recurrent parasitaemia (RI resistance) on Days 11 (n=1) and 21 (n=6).⁶⁸ By 1982, high grade clinical chloroquine resistance was documented in Jayapura using the 7 day in vivo test. Of 16 patients, seven failed to clear their parasites by Day 7: RIII (n=2), RII (n=2), RI (n=3).¹⁴⁰ All 16 isolates also showed evidence of in vitro resistance using an extended 48 hour WHO micro test (schizont development in CQ concentrations ≥ 5.7 pmol/L). High grade CQ resistance (7 day in vivo test) was also found in Arso PIR I and II in 1987 and 1992.³⁷⁵ Fifty of 92 patients had RIII (n=21) or RII (n=29) resistance. In vitro data of eleven isolates showed evidence of resistance to chloroquine (n=9), amodiaquine (n=7), and pyrimethamine (n=6). Data from 1995 from the village of PIR V, cumulative failure rates with CQ were high - 55% (Day 7), 83% (Day 14), and 94% (Day 28).³⁷⁶

2.2.2 S/P and mefloquine for Plasmodium falciparum

Pyrimethamine resistance was first reported from Arso several months into the medicated salt campaign.⁶⁷ Pyrimethamine (≈ 50 mg for adults on days 0, 4, 9) failed to clear P. falciparum in 23/48 (49%) Arso residents; all failures occurred in children < 12 years. At the start of the campaign, the same regimen cleared parasites in all patients (n=96). Proguanil (≈ 300 mg/d for adults x 8d) resistance was also found; some patients with persistent parasitaemia also failed to respond to an increased dose of 600 mg/d for a further 5 days. S/P resistance was first reported in 1979 in Jayapura. At the time when the recommended dose of S/P was 15 mg/kg, the failure rates were in 37 adults were 17% (D7), 37% (D15), and 63% (D 21).⁴³

Two studies conducted in Jayapura in the mid 1980s revealed all grades of resistance in a small proportion of patients who received either S/P alone or as Fansimef, a fixed combination of S/P and mefloquine. Four (5.2%; S/P=2, Fansimef=2) of 77
patients failed to clear their parasites by Day 7. The two S/P failures were RII and early RI (parasites cleared by Day 2 but returned on Day 3); the Fansimef failures were RII and RIII. Serum drug concentrations confirmed drug absorption. *In vitro* cultures (WHO microtest) showed evidence of resistance to MQ in 7 of 31 isolates.\(^4\)\(^5\) In another study of 18 S/P treated patients, two had RI resistance on Days 14 and 21.\(^3\)\(^7\)\(^7\) The mean (range) parasite and fever clearance times in hours were 52.2 (24-144) and 13.8 (0-56), respectively. In Arso PIR I and II, 16 (27%) of 59 patients failed S/P treatment by Day 7 with a striking age related difference. Children aged 18 months accounted for 14 of the failures, for a failure rate of 58% (14/24).\(^3\)\(^7\)\(^5\) In Genyem in 2000, S/P had an overall clinical failure rate of 20% in 46 patients of all ages.\(^4\)\(^6\)

### 2.2.3 Chloroquine resistant *Plasmodium vivax*

In a prospective treatment and prophylactic study, Murphy *et al* demonstrated unequivocal evidence of chloroquine resistant *P. vivax* in children and adults.\(^1\)\(^2\) Of 46 patients of all ages with *P. vivax* malaria who were treated with chloroquine, ten (22%) developed recurrent vivax parasitaemia by Day 14; all were children younger than 11 years, 7 of whom were younger than 4 years. A second course of chloroquine was given to seven of these failures, resulting in initial parasite clearance but a recurrence in five (71%) within 14 days. The 20 patients who were aparasitaemic by Day 14 were given weekly chloroquine prophylaxis for eight weeks. Three were excluded from the analysis because of reduced compliance. Nine (53%) developed breakthrough parasitaemia despite adequate chloroquine levels. The median time to parasitaemia was 5.3 weeks.

In a comparative clinical trial, standard dose chloroquine had a failure rate of 44% (n=48) at Day 14 and 78% (n=45) by Day 28. Corresponding rates for chloroquine plus primaquine (2.5 mg/kg) were 0% (n=21) and 16% (n=19), and 0% (n=19) and 6% (n=17) for halofantrine. Four of the chloroquine recipients failed to clear parasites by Day 7, indicating high grade failure.\(^20\) An *in vivo* test in 1995 also documented high, cumulative failure rates of CQ: 38% (D7), 58% (D14), and 83% (D28).\(^3\)\(^7\)\(^6\) Indirect evidence of chloroquine resistance was found in Javanese transmigrasi from several villages in Arso (PIR I, VIII, X, & XI). When screened most of those with either clinical vivax disease [35/56 (63%)] or asymptomatic vivax parasitaemia [(106/112 (95%)] had total chloroquine concentrations ≥ 100 ng/ml; CQ levels ranged from 0 to 8,342 ng/ml.\(^3\)\(^7\)\(^8\)
2.2.4 Prophylaxis trials

Three prophylactic studies against vivax and falciparum malaria have been conducted in Arso: (i) daily primaquine vs. placebo vs. chloroquine, (ii) alternative day primaquine vs. daily CQ, and (iii) mefloquine vs. doxycycline vs. placebo.

Primaquine (30 mg/d, n=44) had a prophylactic efficacy of 95% (95% CI 57-99) against *P. falciparum* and 90% (58-98) against *P. vivax*. Chloroquine and placebo failure rates were high over 52 weeks: 29.7% (n=40) and 35.8% (n=42), respectively, for *P. falciparum*, and 39.6 and 47.7%, respectively, for *P. vivax*. In the open label study, primaquine (30 mg thrice weekly) provided 74% (23-91) and 90% (25-99) protection against *P. falciparum* and *P. vivax*, respectively, compared to weekly CQ. There were 5 prophylaxis failures (4 Pf, 1 Pv) in the primaquine recipients over 677 person-weeks compared to 30 (18 Pf, 12 Pv) in the CQ arm (796 p-w). In both studies, primaquine was administered with food and was well tolerated. Mean levels of methaemoglobinaemia were significantly higher in the primaquine arm [5.8% (1.4-13%)] compared to the placebo [0.7% (0-3%)] and CQ [0.7% (0-3%)] arms. These levels were not associated with respiratory symptoms or cyanosis. One week after primaquine was stopped, mean methaemoglobin levels fell significantly to 2.4% (0-4.5%). The findings in Arso were similar to another study of primaquine in Armopa (200 miles west of Jayapura). Primaquine had a prophylactic efficacy over placebo of 88 (48-97)% for *P. falciparum*, and > 92 (> 37-99)% for *P. vivax*.

Doxycycline and mefloquine were highly effective against both malaria species over 13 weeks. Of 67 doxycycline recipients, there was only one case of falciparum breakthrough, and no cases in the 68 mefloquine recipients. The placebo arm (n=69) had 30 cases of falciparum and 23 cases of vivax parasitaemia. The prophylactic efficacy of doxycycline was 98% (88-100) against *P. falciparum*, and 100% (90-100) against *P. vivax*; the respective figures for mefloquine were 100% (93-100) and 100% (91-100).

2.3 Azithromycin prophylaxis study

2.3.1 Study design, site, and participants

This was a randomised, double-blind, placebo-controlled study to estimate the prophylactic efficacy of daily azithromycin against *P. falciparum* and *P. vivax* malaria and to assess toxicity. Doxycycline served as a positive control because of its high prophylactic efficacy in the same area. The study took place in Arso from July 1996 to
January 1997. Indonesian soldiers and villagers from PIR V took part in the study. The decision to use soldiers and civilians was made in order to reach the sample size and to include women subjects. Gender mainstreaming was a consideration. Had the study started when it should have done, the PIR V civilians would have only had one year of malaria exposure. Based on the incidence rate data from Arso, an expected mean of four infections per person would have occurred during this time. This amount of malaria exposure was thought to induce only a small amount of malaria immunity.

2.3.2 Ethics

Written, informed consent was obtained from all volunteers. The study was conducted according to Indonesian Ministry of Health, Indonesian Army, United States Navy and Army regulations governing the protection of human subjects.

2.3.3 Sample size and randomisation

The statistical framework for determining the sample size was based on the objective of estimating the protective efficacy of azithromycin in order to rule out a level < 70%. To reduce the number assigned to placebo and to increase power, a 2:1 allocation (A:P) was used. Assuming an attack rate of 60% over 20 weeks, and an anticipated 85% reduction with azithromycin, a total of 195 subjects (130 azithromycin, 65 placebo) would be required to have 80% power to rule out a protective efficacy of < 70% (5% type I error; one-sided). The number to receive doxycycline was the same as placebo. To allow for dropouts the final target number of volunteers to be randomised was 300. The trial was also designed to collect detailed information on drug tolerability but was not powered to detect differences in side effects of low frequency events or small differences between events. The randomisation list was computer generated and numbered in sequence starting with 001. Blocked randomisation with an average block size of four (computer generated with variable block size) was used to assign the three study drugs, azithromycin, placebo, doxycycline to achieve an expected 2:1:1 ratio (2A:1P:1D) after every four subjects were randomised. Following radical cure, subjects were sequentially assigned a study number that started at 001.

2.3.4 Pre-study assessment and radical curative treatment

The screening of potential subjects consisted of: (i) medical history, (ii) physical examination, (iii) malaria smear, (iv) complete blood count (Coulter, Miami, Florida, USA), (v) biochemistry: Na+, K+, creatinine, AST, alkaline phosphatase, and total
bilirubin (Kodak, Rochester, New York, USA), (vi) qualitative glucose-6-phosphate dehydrogenase (G6PD) activity (G6PD spot test, Sigma, USA), (vii) urine dipstick (Boehringer, Germany), and (viii) urine pregnancy test (βHCG test pack, Abbott, Illinois, USA). Eligible subjects were healthy males or non-pregnant females, aged 18 to 55 who completed radical cure. Exclusions were: (i) clinically significant disease, (ii) hepatic disease, (iii) splenectomy, (iv) hearing impairment (abnormal Rinné or Weber test), (v) glucose-6-phosphate dehydrogenase (G6PD) deficiency (G6PD spot test, Sigma, USA), (vi) known study drug hypersensitivity, and (vii) residence for more than 18 months in Arso. Enrolled subjects were given radical cure to eliminate blood and liver forms of malaria using a regimen of concurrent quinine sulphate [10 mg/kg twice daily (thrice daily with positive parasitaemia), 4 days], doxycycline (100 mg twice daily, 10 days) and primaquine base (30 mg daily, 14 days). In previous studies in Irian Jaya, this regime has been used successfully to clear patent parasitaemia and prevent recurrent parasitaemia for at least two weeks post treatment (T. Richie, unpublished observations).

2.3.5 Blinding and drug packaging

No field based investigator knew the randomisation code which was securely stored in individually sealed envelopes. Study code was broken only if a subject had a serious adverse event necessitating study withdrawal. Study drugs and identical placebo (azithromycin/placebo red tablets, white doxycycline/placebo capsules) were supplied in blister packs by Pfizer Central Research, Groton CT, USA. Drug packs were produced that contained a one week supply of both azithromycin/placebo and doxycycline/placebo, with two extras of each drug to use in case of vomiting within one hour of study drug administration.

2.3.6 Conduct of clinical trial

Radical cure was initiated over seven weeks. The administration of study drugs commenced the day after radical cure (Day 0). All doses were given using the double dummy method, the simultaneous administration of active drug/s and placebo/s or double placebos. On Day 0, all subjects received one doxycycline (100 mg)/placebo capsule and a loading dose (three tablets) of azithromycin (750 mg)/placebo. Based on a computer simulated model, the loading dose of azithromycin would attain 90% of steady state serum and tissue levels by Day 1 (C. Ohrt, personal communication). Thereafter, one azithromycin (250 mg)/placebo and one doxycycline (100 mg)/placebo were given daily
over a follow up period of 20 weeks. Drinking water and sweet biscuits were provided with each dose. Drug administration and consumption were witnessed and signed for by a health worker and the subject. If a soldier was absent, his post commander was given study drugs, sweet biscuits, and a record book. The post commander was responsible for administering and recording drug administration. Soldiers away on patrol were given a two weeks supply of study drugs. Drug forms were inspected daily, supplemented by periodic drug counts and supervisory field visits. Thick and thin malaria smears were made every week. Additional slides were made if subjects became ill in between times. Giemsa-stained slides were read by microscopists, unaware of subject symptom status. Two or more asexual malaria parasites defined a positive slide, after reading 200 thick smear fields at x 1000 magnification. All positive slides and 10% of all negative slides were confirmed by a second microscopist in a blinded fashion. Discrepant slide results were subject to a third blind read. Parasitaemia (number/µL) was quantified using a standard approximation method; namely, the number of asexual forms per 200 wbc x 40. All subjects with parasitaemia were interviewed by a study doctor. One or more of the following defined symptomatic parasitaemia: fever, chills, sweating, myalgia, headache, anorexia, nausea, vomiting, abdominal pain, and diarrhoea.

2.3.6.1 Health care during the trial & rescue treatment

The Indonesian soldiers were posted in a number of small encampments and were visited daily by team members for drug administration and the collection of safety data. In addition, there was a mobile medical service comprising Indonesian Army and civilian doctors who made daily rounds in two vehicles. Not all posts could be visited every day but it is a fair assumption that all camps would have been visited two to three times per week by the mobile medical teams. These teams were able to issue a limited supplies of medicines to treat common illnesses. All soldiers, irrespective of whether they were in the study, were seen and treated. The puskesmas in PIR V was provided with a full time team doctor, a microscope and all necessary accessories, and a limited supply of medicines (as above). All medical care was freely provided for all the villagers and not just those in the study. Any subject who was found to have malaria was treated with quinine and doxycycline.
2.3.7 End points

The primary efficacy endpoint was the first occurrence of slide proven parasitaemia (prophylaxis failure). Follow-up time for efficacy analysis was defined as the time from Day 0 to the date of prophylaxis failure or the date of study completion. For those not completing scheduled follow-up i.e. withdrawal or protocol violations, follow-up time was measured to the date of the last negative slide. Reasons for non-malaria withdrawals were: (i) serious adverse event, (ii) unsupervised drug administration >14 days, (iii) consumption of non-study anti-malarial drug, (iv) two doses of study drug/placebo missed within 7 days, and (v) voluntary drop-out.

2.3.8 Assessment of safety and tolerability

Tolerability was assessed by recording symptoms and performing clinical and laboratory tests. Symptoms were recorded by questionnaire and in response to the question 'Any symptoms?' The questionnaire consisted of 26 symptoms: twenty five specific symptoms: anorexia, nausea, vomiting, heartburn, mild abdominal pain, severe abdominal pain, diarrhoea, severe diarrhoea (> 5 motions/day), mild headache, severe headache, 'pusing' (translated as dizziness/muzzy head), hearing loss, tinnitus, paraesthesiae, blurred vision, hallucinations, difficulty sleeping, mild itching, severe itching, rash, cough, fever, chills, sweats, myalgia, and 'Others.' The questionnaire was administered on Days 0 and 1 for loading dose tolerability, and monthly thereafter for symptom trends. Symptoms in response to the open question were recorded daily by the health workers. At study end, a health questionnaire (general health, certain GI symptoms, and hearing) was completed. Hearing was assessed clinically using the Rinne and Weber tuning fork tests at enrolment and study end. Other clinical examinations were performed as required e.g. to assess an intercurrent illness. A complete blood count and routine biochemistry were done at enrolment, one month later, and at study end.

2.3.9 Adverse event classification

An adverse event (AE) was defined as a new symptom, physical sign or illness that developed during the study. AE severity was classified as (i) mild: able to conduct activities of daily living, no/little treatment required, (ii) moderate: affecting activities of daily living, treatment required, or (iii) severe: affecting activities of daily living, treatment and bed rest or hospital admission required. A serious adverse event was defined as lethal, life threatening, and/or requiring hospital admission.381
2.3.10 **Statistical analysis**

All randomised volunteers (n=300) were included for analysis of efficacy and drug safety/tolerability. Data were double entered, discrepancies resolved, and end points finalised before the data bases were locked and the randomisation code broken. Epi Info 6.02 (Centers for Disease Control and Prevention, Atlanta, Georgia), Minitab 11 for Windows (Minitab Inc., State College, PA), and Statxact 3 for Windows (CYTEL Software Corp., Cambridge, MA) were used for data management and analysis.

### 2.3.10.1 Efficacy

Incidence (density) rates (IR) were calculated as the number of malaria cases divided by the total person-years of follow-up. The protective efficacy (PEf) was defined as the percent reduction in the incidence rates of malaria: \(1 - \frac{IR_{drug}}{IR_{placebo}}\). Confidence intervals (CIs) for PEf (1−ratio of two Poisson variables) were calculated using the exact conditional distribution. The Kaplan-Meier method was used to estimate the cumulative risk and standard error of developing either falciparum or vivax malaria during follow-up; the follow-up time was considered censored if one malaria species occurred before the other. Cumulative risk curves were compared by the log rank test.\(^{382}\)

### 2.3.10.2 Tolerability

Reported symptoms in the drug/placebo groups were compared in terms of: (i) the proportion of subjects with a new symptom one day after the loading dose, and (ii) the proportion of subjects who reported at least once any symptom at the daily checks, (iii) the incidence rate of daily symptoms reported (total number of symptoms/total person-time for safety/tolerance follow-up). Fisher's exact or chi-squared tests were used to assess differences (2 arms or 3 arms) of independent proportions. Koopman's method was used to compute 95% CI for relative risk measures.\(^{383}\) Because of the disproportionately high loss to follow-up of the placebo arm because of malaria, comparison of proportions (active drug vs placebo) of subjects reporting daily symptoms was performed only for the first four weeks of follow-up. To account for differences in follow-up, incidence (density) rates (IR) of reported symptoms were computed and compared (as above). Follow-up time for assessing daily reported symptoms was calculated taking into account (i) the days subjects were absent for questioning (e.g. soldiers on patrol), and (ii) symptoms due to malaria (symptoms reported within 7 days of a positive slide). Where no symptoms were reported (daily reported symptoms), an adjustment was made to estimate the rate
ratios and relative risks. For the rate ratios, this was done by adding a \( \frac{1}{2} \) only to the numerator counts but and not to the person time data i.e. \( 0 + \frac{1}{2} \), and \( N + \frac{1}{2} \). For the relative risks, a \( \frac{1}{2} \) was added to both numerator counts and denominator samples sizes i.e. \( \frac{0 + \frac{1}{2}}{\text{sample size } A + \frac{1}{2}} \) divided by \( \frac{N + \frac{1}{2}}{\text{sample size } B + \frac{1}{2}} \).

Analysis of variance (ANOVA) was used to assess overall differences between arms for laboratory data. Student's t-test (unpaired or paired) was used to assess differences between two means (between or within arms). The Mann-Whitney test was used to compare median parasite counts or other highly skewed data. Because many comparisons were performed, it was decided to flag all comparisons that achieved a nominal \( P \) value of 0.05 (95% CI for relative risk or rate ratio measures that excluded 1) and to report much of the basic data.

2.4 Chloroquine-doxycycline combination study

2.4.1 Study design, site, and participants

This open clinical trial compared chloroquine combined with doxycycline, to chloroquine or doxycycline alone for the treatment of uncomplicated falciparum malaria and vivax malaria. The study took place from October 1995 to January 1998 in the Rumah Sakit Umum and Indonesian Navy hospitals in Jayapura. Participants were civilians and Indonesian Navy personnel who lived in Jayapura or in the surrounding area of Arso.

2.4.2 Ethics

Written, informed consent was obtained from all volunteers. The study was conducted according to Indonesian Ministry of Health, Indonesian Army, United States Navy and Army regulations governing the protection of human subjects.

2.4.3 Sample size

The sample size calculations were based on the assumptions that the frequencies of resistance (RI/RII/RIII) were 0.5 for chloroquine alone (CQ) and 0.05 for the combination arm (CQD), and 0.4 for doxycycline (D). Using a two-sided alpha of 0.05, sample sizes of 18 per arm (CQ vs. CQD) would detect these resistance frequencies with a probability of 0.89. Although not the primary aim of the study, a comparison of D vs. CQD, with the same sample size of 18 per arm would detect the resistance frequencies with a power of 0.73.
2.4.4 Patient assessment and selection

Potential subjects were referred to the research team by local physicians and medically screened: (i) medical history, (ii) physical examination, (iii) malaria smear, (iv) urine pregnancy test (β HCG test pack, Abbott, Illinois, USA), and (v) qualitative glucose-6-phosphate dehydrogenase (G6PD) activity (G6PD spot test, Sigma, USA). Eligible subjects were healthy males or non-pregnant females aged 15 to 50 years with either uncomplicated falciparum malaria or vivax malaria. Specific exclusions included mixed infections, symptoms or signs of severe and complicated malaria, malaria treatment within 7 days of presentation, and known study drug hypersensitivity.

2.4.5 Conduct of clinical trial

Enrolled patients were admitted to the hospital and sequentially randomised to treatment (CQ, CQD, D, CQ, etc). This was later changed to a system of using labelled tickets that were selected randomly by the team physician. Patients with falciparum malaria were arbitrarily classified as 'mild': parasitaemia 0.25% (≡ parasite density 12,500/μL), or 'moderate': parasitaemia > 0.25-< 3% (≡ parasite density > 12,500-< 150,000/μL). This artificial division was designed so that no randomised 'moderate' patients would receive doxycycline alone, due to concern over its slow onset of action.

Patients randomised to chloroquine (Malarex®, Dumex, Indonesia) were treated with chloroquine base: 10 mg/kg once on Days 0 and 1, and 5 mg/kg once on Day 2 (CQ and CQD arms). The dose of doxycycline (Pfizer, USA) was 100 mg every 12 hours for 7 days (D and CQD arms). All drugs doses were administered and documented by ward nurses. Patients were hospitalised for 28 days. The wards were screened with mosquito mesh on the windows and subjects slept under mosquito nets. Symptoms were recorded daily. Oral temperatures were taken every 8 hours during the first week, and daily thereafter. Treatment failures were treated with quinine (10 mg/kg thrice daily for 4 days) and doxycycline (100 mg twice daily for 10 days). Patients with vivax malaria were given primaquine base (30 mg/day for 14 days) at the time of hospital discharge.

Giemsa-stained malaria smears were read daily until negative; thereafter, three times per week until Day 28. A positive smear was defined as one or more asexual form/s seen after examining 200 thick smear fields under x 1,000 magnification. The parasite count (number/μL) was quantified using the measured total white cell count (WCC). Where this was missing, the total WCC was assumed to be 8,000/μL. Filter paper (No. 1
Whatman, Fairfield, NJ) blots for chloroquine drug levels were made on Days 0, 3, and 28. One hundred µL of whole blood were drawn from a finger stick into a capillary tube and spread evenly onto the filter paper. Blots were air dried, stored in individual plastic bags at ambient temperatures until analysed. Chloroquine and desethylchloroquine were measured in whole blood by HPLC, using a published method. The sum of chloroquine and desethylchloroquine is reported as the total whole blood chloroquine (TCQ) concentration (ng/ml).

2.4.6 End points

The parasitological end points were classified as sensitive (S) or resistant, graded as RI, RII, or RIII. For vivax malaria, RI cases were classified as Rl/relapse because both are clinically indistinguishable. Genotyping by the polymerase chain reaction (PCR) was performed on the recurrent and original (Day 0) parasitaemias for both species by examining MSP1 and MSP2 (falciparum) and MSP1 and circumsporozoite (CS) genes for P. vivax. The secondary clinical end points were: (i) the fever clearance time (FCT/hours): the time taken for the patient to become and remain afebrile (oral temperature 37°C recorded at least four consecutive times), (ii) the parasite clearance time (PCT/days): the number of days taken for a malaria smear to become negative for at least two consecutive days, and (iii) the proportion of patients remaining symptomatic (reporting either fever, chills, headache, myalgia, weakness, anorexia, or nausea) on Days 3 and 7.

2.4.7 Statistical analysis

Data were double-entered, validated and analysed using Epi Info 6.04b (Centers for Disease Control and Prevention, Atlanta, GA, USA) and SPSS for Windows 8.0 (SPSS, Chicago, IL, USA). Proportional data were analysed using chi squared. Laboratory data were analysed using the student's paired/unpaired 't' test or ANOVA or the corresponding non-parametric tests, as appropriate. All statistical tests were two sided and a $P$ value of 0.05 was considered statistically significant. The efficacy analysis efficacy excluded patients who were lost to follow up or withdrew from the study before Day 28.

2.5 Safety monitoring for both clinical trials

At the time when both studies were conducted, there was no ethical requirement to establish a Drug Safety and Monitoring Board. Never the less, both studies were
monitored closely. There were medical monitors assigned to both studies. There was a strict reporting system for serious adverse events. These were reported to colleagues and collaborators from the US Navy, the US Army, and the Indonesian Ministry of Health. Regular updates and a final report of all serious AEs were provided. In the azithromycin trial, the breaking of study code was done only after consultation with the above mentioned colleagues.
Chapter 3 - Results

3.1 Azithromycin prophylaxis study

3.1.1 Enrolment and withdrawals

Of the 364 registered volunteers, 48 did not meet entry criteria and 16 failed to complete radical cure (Figure 3.1). Three hundred volunteers were randomised to the three drug/placebo arms: azithromycin (n=148), doxycycline (n=75), and placebo (n=77). Subjects' enrolment characteristics and baseline laboratory values were similar between the three study arms (Table 3.1). All subjects had normal Rinne tests but one (A arm) had Weber lateralisation, consistent with a sensineural hearing impairment (a protocol violation). The majority of subjects were male, 286/300 (95.3%), of mean age 27. Young soldiers accounted for 225 of the 286 (78.7%) male subjects. Many participants [144/300 (48%)] reported at least one attack of malaria since arriving in Irian Jaya. Certain markers of recent malaria exposure were significantly higher in the civilians compared to the soldiers (see Sub-group analysis).

One hundred and fifty four subjects (51.3%) completed the study as per protocol without acquiring parasitaemia. Eighty seven (29%) developed parasitaemia and 59 (19.6%) withdrew from the study for other reasons (Figure 3.1). The median follow up time, in weeks (range), for all subjects in each arm was: doxycycline 16.1 (2.4-20.1), azithromycin 15.8 (1.1-20.1) and placebo 7.6 (0-18.6) weeks ($P < 0.00001$).
Figure 3.1 Trial profile of the azithromycin prophylaxis study.
Table 3.1 Baseline characteristics of subjects enrolled in the malaria prophylactic study of azithromycin, doxycycline, and placebo.

<table>
<thead>
<tr>
<th></th>
<th>Azithromycin</th>
<th>Doxycycline</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>148</td>
<td>75</td>
<td>77</td>
</tr>
<tr>
<td>Male</td>
<td>142</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Female</td>
<td>6</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Age, median (range)</td>
<td>27 (18-52)</td>
<td>27 (21-54)</td>
<td>26 (20-50)</td>
</tr>
<tr>
<td>Weight, kg, median (range)</td>
<td>60 (43-75)</td>
<td>58 (39-83)</td>
<td>59 (42-75)</td>
</tr>
<tr>
<td>Reported malaria, mean (SD)*</td>
<td>3.8 (3.7)</td>
<td>3.8 (4.4)</td>
<td>3.2 (3.2)</td>
</tr>
<tr>
<td>Splenomegaly, % (n)</td>
<td>12.8 (19)</td>
<td>8 (6)</td>
<td>10.4 (8)</td>
</tr>
<tr>
<td>Positive malaria slides, % (n)</td>
<td>25.7 (38)</td>
<td>28 (21)</td>
<td>29.9 (23)</td>
</tr>
<tr>
<td>Hb, g/dl†</td>
<td>14.3 (1.5)</td>
<td>14.2 (1.2)</td>
<td>14.1 (1.7)</td>
</tr>
<tr>
<td>Platelet count†</td>
<td>195 (54)</td>
<td>195 (55)</td>
<td>190 (52)</td>
</tr>
<tr>
<td>Creatinine, mg/dl†</td>
<td>1.1 (0.2)</td>
<td>1.1 (0.1)</td>
<td>1.1 (0.1)</td>
</tr>
<tr>
<td>AST, IU/L†</td>
<td>29 (10)</td>
<td>28 (10)</td>
<td>28 (10)</td>
</tr>
<tr>
<td>Total bilirubin, mg/dl†</td>
<td>0.6 (0.2)</td>
<td>0.6 (0.1)</td>
<td>0.7 (0.2)</td>
</tr>
</tbody>
</table>

* Reported malaria illnesses within one year of enrolment, not microscopically confirmed.
† Laboratory values are mean (standard deviation).

3.1.2 Parasitaemia end points

By study end, there were 87 cases of parasitaemia: 55 P. falciparum, 29 P. vivax, and 3 cases of mixed infection; the latter were counted as P. falciparum, the dominant species in each case. The number of cases of parasitaemia (% of randomised) in each arm was: placebo 56/77 (72.7%), azithromycin 28/148 (18.9%), and doxycycline 3/75 (4%). The cumulative incidence of parasitaemia as a function of time is shown in Figure 3.2. The curves are significantly different comparing either drug arm with placebo.
The majority of prophylaxis failures, 75.8% (66/87), had symptomatic parasitaemia. By species, these symptomatic infections were 47/58 (81%) for *P. falciparum*, and 19/29 (65.5%) for *P. vivax*. The distribution by drug arms was not significantly different (*P*=0.14): (i) placebo, 45/56 (80.3%: 27 Pf, 18 Pv), (ii) azithromycin, 20/28 (71.4%: 19 Pf, 1 Pv), and (iii) doxycycline, 1/3 (33.3%: 1 Pf). The mean number of symptoms reported by the 66 symptomatic prophylaxis failures was 3.8; of whom 55 (83.3%) reported >1 symptom. The three most common symptoms were headache 51/66 (77.2%), myalgia 41/66 (62.1%), and fever 33/66 (50%). The median falciparum parasitaemia was significantly higher in the placebo arm compared to the azithromycin arm: 1,480 (60-32,920) vs. 240 (80-700) μL (*P*=0.023).

### 3.1.3 Prophylactic efficacy

Compared to placebo, the prophylactic efficacy of azithromycin against *P. falciparum* was 71.6% (50.3-83.8) and 98.8% (93.1-99.9) against *P. vivax* (Table 3.2). Corresponding figures for doxycycline were 96.3% (85.4-99.6) and 98% (88.0-99.9).
Table 3.2 Incidence rates of malaria and the prophylactic efficacies of azithromycin and doxycycline relative to placebo against both malaria species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Azithromycin (148, 38.88)*</th>
<th>Doxycycline (75, 22.15)</th>
<th>Placebo (77, 11.83)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>IR†</td>
<td>PEf‡</td>
</tr>
<tr>
<td>Both</td>
<td>28</td>
<td>0.72</td>
<td>84.7 (75.6-90.7)</td>
</tr>
<tr>
<td>Pf</td>
<td>27</td>
<td>0.69</td>
<td>71.6 (50.3-83.8)</td>
</tr>
<tr>
<td>Pv</td>
<td>1</td>
<td>0.03</td>
<td>98.9 (93.1-99.9)</td>
</tr>
</tbody>
</table>

* (number randomised, total follow-up time in person-years).
† IR=incidence rate: cases / person year.
‡ PEf=prophylactic efficacy (95% CIs).

3.1.4 Sub-group analysis of civilians and soldiers

At enrolment, four malarriometric indices (reported malaria, splenomegaly, prestudy slide positivity, and Hb) showed that prestudy malaria exposure was significantly higher in the civilians compared to the soldiers (Table 3.3). However, civilians and soldiers who received placebo did not have significantly different outcomes with respect to: (i) overall attack rate, (ii) occurrence of symptomatic parasitaemia, and (iii) levels of parasitaemia (Pf or Pv). The estimated protective efficacies of azithromycin against *P. falciparum* and *P. vivax* were higher in the civilians compared to the soldiers but neither of the differences (*P*=0.08 and 0.68, respectively) were statistically significant.
Table 3.3 Civilian/soldier subgroup analysis: (1) enrolment markers of prior malaria exposure, (2) placebo group outcomes, (3) azithromycin prophylactic efficacy.

<table>
<thead>
<tr>
<th>(1) Enrolment characteristics</th>
<th>Civilians n=75</th>
<th>Soldiers n=225</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>At least one, %</td>
<td>98.7 (74/75)</td>
<td>75.5 (170/225)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Median (range)</td>
<td>10 (0-20)</td>
<td>2 (0-16)</td>
<td></td>
</tr>
<tr>
<td>Splenomegaly, % (n)</td>
<td>32 (24)</td>
<td>4 (9)</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Positive malaria slides, % (n)</td>
<td>38.6 (29)</td>
<td>23.5 (53)</td>
<td>0.02†</td>
</tr>
<tr>
<td>Hb, g/dl, mean (SD)</td>
<td>13.5 (1.7)</td>
<td>14.5 (1.3)</td>
<td>&lt;0.001‡</td>
</tr>
</tbody>
</table>

(2) Placebo group outcomes

| Attack rate, %§               | 83.3 (15/18)   | 69.5 (41/59)   | 0.36†   |
| Symptomatic cases, %          | 86.6 (13/15)   | 78 (32/41)     | 0.70†   |
| Parasite count/µL ||                |               |               |         |
| Pf                           | 940 (80-20,640) | 1,600 (60-32,920) | 0.53¶   |
| Pv                           | 200 (80-500)   | 400 (100-1,880) | 0.10¶   |

(3) Prophylactic Efficacy**

| Pf                           | 88.4 (56.6-97.4) | 62.9 (29.5-80.4) | 0.08††  |
| Pv                           | 100 (83.9-100)   | 98.3 (89.4-99.9) | 0.68††  |

* Reported malaria illness, not microscopically confirmed, within one year of enrolment.
† Fisher’s exact test.
‡ Unpaired t-test.
§ Crude attack rate: % (number of cases/number randomised).
£ Median (range).
¶ Mann-Whitney test.
** Prophylactic efficacy (95% CIs), based on incidence rates relative to placebo.
†† Exact (conditional) test.

3.1.5 Drug compliance

Of the 26,822 intended doses, 25,181 (93.8%) were administered under direct observation by the research team, 860 (3.2%) were witnessed by a post commander, 781 (2.9%) were taken unwitnessed, and 35 (0.13%) doses were not taken.
3.1.6 Adverse events, study withdrawal and code breaking

There were eight adverse events (AEs) necessitating study withdrawal. Study code was broken in three of these subjects. One azithromycin recipient, a 26 years old soldier, developed a widespread, pruritic, erythematous, maculopapular rash with areas of crusting after four doses; giving a risk of 0.67% (95% CI 0.034 – 3.29). This moderately severe adverse event was considered azithromycin induced. The seven other AEs were all considered unrelated to study drugs: ureteric colic, dengue fever, motor cycle accident (azithromycin), acute bronchitis with hyperventilation, sub-arachnoid haemorrhage (doxycycline), headache with photophobia, and severe malaria (placebo).

3.1.7 Loading dose tolerance and monthly reported symptoms

Responding to the symptom questionnaire, the proportions of subjects reporting either a new symptom (not present on Day 0) or any symptom on Day 1 were similar between all three arms (Figure 3.3). After Day 1, several questionnaire-elicited symptoms appear to show a downward trend in the azithromycin and doxycycline arms. Itching appears to show an inconsistent trend in all three arms (Figure 3.3). Discerning trends for a number of symptoms in the placebo arm is problematic because of loss to follow up.

3.1.8 Self reported daily symptoms over 20 weeks

3.1.8.1 Proportional comparisons

By study end, the majority of all subjects had reported at least one symptom: A=96.6% (143/148), D=93.3% (70/75), P=96.1% (74/77). Subjective hearing loss was reported by small, roughly equal proportions of patients from all three arms [5.7% (17/300)], \( \chi^2 \) (df=2)=2.17; \( P=0.34 \). Of the azithromycin-doxycycline comparisons, more doxycycline subjects reported subjectively severe abdominal pain [6.7% (5/75) vs. 1.3% (2/148), \( P=0.031 \)], and more azithromycin subjects reported paraesthesiae [12.2% (18/148) vs. 4% (3/75), \( P=0.048 \)].

3.1.8.2 Rate comparisons

The incidence rates of any self reported symptom ranged from 0-9.05/p-y, a maximum of nine days over one year (Table 3.4). Compared to placebo, azithromycin recipients reported more heartburn, mild and severe itching, paraesthesiae, and 'Others'. Doxycycline recipients reported more heartburn, severe abdominal pain, 'pusing', difficulty sleeping, and 'Others'. Of the azithromycin-doxycycline comparisons, heartburn
[rate ratio=1.6 (95% CI 1.04-2.7)], paraesthesiae [8.8 (95% CI 3.6-27.9)], and severe itching [5.3 (95% CI 1.3-47.5)] were reported more often by the azithromycin recipients, and the doxycycline recipients reported severe abdominal pain [8.85 (1.89-83.03)], and difficulty sleeping [1.77 (1.17-2.68)] more frequently. Subjective hearing loss in any arm was reported with a frequency of < 0.5/p-yr.

3.1.9  **Self reported daily symptoms within the first four weeks**

3.1.9.1  **Proportional comparisons**

The proportions of subjects reporting any symptom at least once during the first four weeks were broadly similar in the three arms (Table 3.5): A=58.8% (87/148), P=66.2% (51/77), D=61.3% (46/77), [χ²(df=2) =1.19, P=0.55]. Heartburn was the only symptom occurring in excess compared to placebo, true for both drug arms. Subjective hearing loss was reported by nine (3%) participants distributed equally across the three arms [χ²(df=2)=1.1; P=0.57].

3.1.9.2  **Rate comparisons**

The incidence rates of any self reported symptoms from the three arms during the first four weeks ranged from 0-7.8/p-y (Table 3.6). Compared to placebo, heartburn and myalgia were reported more frequently by both drug arms, and difficulty sleeping only by the doxycycline arm. Of the azithromycin-doxycycline comparisons, difficulty sleeping [2.8 (1.64-4.87)] and sweats [3.34 (1.22-10.0)] were reported more frequently in the doxycycline arm. Subjective hearing loss in any arm was reported with a frequency of < 0.53/p-y.

3.1.10  **Health questionnaire and hearing at study end**

The health questionnaire was answered by 257/300 (85.6%) subjects. End points in these subjects were: (i) study completion [n=151 (58.7%)], (ii) prophylaxis failure [n=76 (29.6%)], (iii) serious AE [n=4 (1.5%)], and (iv) study withdrawal for other reasons [n=26 (10.1%)]. The majority of subjects [94.9% (244/257)] reported feeling healthier compared to study start. The proportions of subjects who recollected experiencing nausea and/or vomiting, diarrhoea, or hearing impairment at any time during the study were 25.3% (65/257), 23.3% (60/257), and 5.1% (13/257), respectively. None of these proportions were significantly different between the three arms (details not shown). Weber lateralisation was detected in 7 (2.7%) of 262 subjects, similarly
distributed between the three arms: A = 3/132 (2.3%), D = 2/64 (3.1%), and P = 2/70 (2.8%)
$\chi^2 (df=2) = 0.14; P = 0.57$]. All had normal Weber tests at enrolment.

3.1.11 Laboratory evaluations

Mean biochemical and haematological values at Day 0, Week 4, and study end were not significantly different both between and within each arm (details not shown). Mean changes in these laboratory values (study end – Day 0) were also not significantly different between each drug arm and placebo (A vs. D vs. P): (i) Creatinine (mg/dL): -0.02 vs. -0.03 vs. 0, P=0.86, (ii) AST: 3.4 vs. 6.86 vs. 10.8, P=0.15, (iii) total bilirubin (mg/dL): 0.079 vs. 0.17 vs. 0.11, P=0.54, (iv) Hb (g/dL): 1.2 vs. 0.76 vs. 1.08, P=0.14, (v) total white cell count ($/\mu$L): -0.520 vs. -0.240 vs. 0.530, P=0.23, and (vi) platelet count ($x10^3$/uL): 7.3 vs. 7.7 vs. 28.4, P=0.53.

At enrolment, the proportions of anaemic women (Hb <12 g/dL) and men (Hb <13.5 g/dL) were 5/14 (35.7%), and 64/286 (22.3%), respectively. At Week 4, 4/5 women were not anaemic (D=2, P=2). At Week 4 and study end, the proportions of anaemic males had declined in all three arms but these were not significantly different comparing either active drug with placebo (details not shown). During follow up, the total white blood cell count (WBC) of all azithromycin recipients did not fall below 4,500/$\mu$L, the lower limit of the normal range. Three doxycycline recipients with normal Day 0 WBCs [4,600/$\mu$L (n=1), 6,400/$\mu$L (n=2)] developed WBCs of 4,400/$\mu$L at study end.

Subjects from all arms with an elevated AST (>40 IU/L) at enrolment, one month later, and study end were 17/300 (5.6%), 23/265 (8.6%) and 14/154 (9.1%), respectively (P=0.28). These proportions were distributed similarly between each arm at each time interval (details not shown). Of the 283 subjects with a normal AST (≤ 40) at enrolment, 145 (51.2%) had AST levels done at study end. Nine of these 145 subjects (6.2%) had an elevated AST; 6 were azithromycin subjects (AST=41-50 IU/L), and three were doxycycline recipients (AST=41-63 IU/L).
Figure 3.3 Trends in selected symptoms reported by questionnaire on Days 0 and 1, and at monthly intervals during the study. These symptom data are different to those presented in Tables 3.4 and 3.6. Note: m=mild, s=severe.
Table 3.4 Incidence rates per person-year by drug arm (azithromycin, doxycycline, placebo) for all symptoms volunteered daily over 20 weeks of trial. Significant rate ratio comparisons only are shown.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Azithromycin 37.91 p-yr *</th>
<th>RR (95% CI) A vs P</th>
<th>Placebo 10.88 p-yr</th>
<th>RR (95% CI) D vs P</th>
<th>Doxycycline 21.43 p-yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>All symptoms</td>
<td>1,525 (40.22)</td>
<td>416 (38.22)</td>
<td>852 (39.75)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anorexia</td>
<td>104 (2.74)</td>
<td>39 (3.58)</td>
<td>0.56 (0.35-0.89)</td>
<td>43 (2.0)</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>53 (1.39)</td>
<td>19 (1.74)</td>
<td>35 (1.63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>11 (0.29)</td>
<td>6 (0.55)</td>
<td>5 (0.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heartburn§</td>
<td>73 (1.92)</td>
<td>10.48 (2.79-8.14)</td>
<td>2 (0.18)</td>
<td>6.35 (1.58-55.3)</td>
<td>25 (1.17)</td>
</tr>
<tr>
<td>Mild abdominal pain</td>
<td>43 (1.13)</td>
<td>18 (1.65)</td>
<td>25 (1.17)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe abdominal pain</td>
<td>2 (0.05)</td>
<td>0 (0)</td>
<td>10.7 (1.14-∞)</td>
<td>10 (0.47)</td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>34 (0.89)</td>
<td>18 (1.65)</td>
<td>18 (0.84)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhoea &gt;5/day</td>
<td>3 (0.08)</td>
<td>2 (0.18)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS symptoms</td>
<td>492 (12.98)</td>
<td>151 (13.88)</td>
<td>278 (12.97)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild headache</td>
<td>227 (5.98)</td>
<td>64 (5.88)</td>
<td>119 (5.55)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe headache</td>
<td>14 (0.37)</td>
<td>8 (0.73)</td>
<td>7 (0.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Pusing'§</td>
<td>100 (2.63)</td>
<td>19 (1.75)</td>
<td>2.06 (1.23-3.6)</td>
<td>77 (3.59)</td>
<td></td>
</tr>
<tr>
<td>Tinnitus</td>
<td>9 (0.24)</td>
<td>0.10 (0.04-0.21)</td>
<td>27 (2.48)</td>
<td>0.13 (0.05-0.31)</td>
<td>7 (0.33)</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>9 (0.24)</td>
<td>5 (0.46)</td>
<td>6 (0.28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blurred vision</td>
<td>6 (0.16)</td>
<td>4 (0.37)</td>
<td>7 (0.33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paraesthesia§</td>
<td>78 (2.06)</td>
<td>2.04 (1.08-4.24)</td>
<td>11 (1.01)</td>
<td>0.23 (0.06-0.72)</td>
<td>5 (0.23)</td>
</tr>
<tr>
<td>Difficulty sleeping</td>
<td>49 (1.3)</td>
<td>13 (1.2)</td>
<td>1.91 (1.02-3.84)</td>
<td>49 (2.29)</td>
<td></td>
</tr>
<tr>
<td>Hallucinations</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (0.05)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dermatological</td>
<td>160 (4.22)</td>
<td>1.84 (1.20-2.92)</td>
<td>25 (2.3)</td>
<td>1.65 (1.04-2.69)</td>
<td>81 (3.78)</td>
</tr>
<tr>
<td>Mild itching</td>
<td>135 (3.56)</td>
<td>1.55 (1.01-2.48)</td>
<td>25 (2.3)</td>
<td>75 (3.49)</td>
<td></td>
</tr>
<tr>
<td>Severe itching§</td>
<td>19 (0.5)</td>
<td>11.2 (1.34-∞)</td>
<td>0 (0)</td>
<td>2 (0.09)</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>6 (0.16)</td>
<td>0 (0)</td>
<td>4 (0.19)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>207 (5.46)</td>
<td>71 (6.52)</td>
<td>144 (6.72)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cough</td>
<td>143 (3.77)</td>
<td>43 (3.95)</td>
<td>94 (4.39)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>11 (0.29)</td>
<td>0.21 (0.09-0.49)</td>
<td>15 (1.38)</td>
<td>0.37 (0.15-0.87)</td>
<td>11 (0.51)</td>
</tr>
<tr>
<td>Chills</td>
<td>10 (0.26)</td>
<td>2 (0.18)</td>
<td>5 (0.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweats</td>
<td>10 (0.26)</td>
<td>4 (0.37)</td>
<td>13 (0.60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td>33 (0.87)</td>
<td>7 (0.64)</td>
<td>21 (0.98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Others'</td>
<td>343 (9.05)</td>
<td>1.51 (1.16-2.01)</td>
<td>65 (5.97)</td>
<td>1.47 (1.1-1.98)</td>
<td>188 (8.77)</td>
</tr>
</tbody>
</table>

* Person-years of follow-up
† Rate ratio (95% confidence interval)
‡ Total number of times symptom reported (incidence rate/p-yr)
§ A vs D: heartburn RR=1.6 (1.04-2.7), paraesthesia RR=8.8 (3.6-27.9), itching (s) RR=5.3 (1.3-47.5).
|| ½ added to the numerator counts only to estimate the rate ratio. 95% CIs calculated using the observed data.
¶ D vs A: severe abdominal pain RR=8.85 (1.89 – 83.03), difficulty sleeping RR=1.77 (1.17 – 2.68).
# 'Pusing' - dizziness/muzzy head
Table 3.5 Number (percent) of persons reporting symptoms at least once (daily reporting) within the first four weeks of trial.*

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Azithromycin n=148</th>
<th>Placebo n=77</th>
<th>Doxycycline n=75</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All symptoms</td>
<td>87 (58.8)†</td>
<td>51 (66.2)</td>
<td>46 (61.3)</td>
</tr>
<tr>
<td>GI symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All symptoms</td>
<td>49 (33.1)</td>
<td>32 (41.6)</td>
<td>27 (36.0)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>23 (15.5)</td>
<td>13 (16.9)</td>
<td>13 (17.3)</td>
</tr>
<tr>
<td>Nausea</td>
<td>15 (10.1)</td>
<td>10 (13.0)</td>
<td>11 (14.7)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3 (2.0)</td>
<td>2 (2.6)</td>
<td>2 (2.7)</td>
</tr>
<tr>
<td>Heartburn§</td>
<td>14 (9.5)§</td>
<td>0 (0)</td>
<td>7 (9.3)§</td>
</tr>
<tr>
<td>Mild abdominal pain</td>
<td>14 (9.5)</td>
<td>6 (7.8)</td>
<td>5 (6.7)</td>
</tr>
<tr>
<td>Severe abdominal pain</td>
<td>1 (0.7)</td>
<td>0 (0)</td>
<td>2 (2.7)</td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>12 (8.1)</td>
<td>8 (10.4)</td>
<td>9 (12.0)</td>
</tr>
<tr>
<td>Diarrhoea &gt;5/day</td>
<td>1 (0.7)</td>
<td>1 (1.3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>CNS symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All symptoms</td>
<td>62 (41.9)</td>
<td>29 (37.7)</td>
<td>31 (41.3)</td>
</tr>
<tr>
<td>Mild headache</td>
<td>28 (18.9)</td>
<td>19 (24.7)</td>
<td>15 (20.0)</td>
</tr>
<tr>
<td>Severe headache</td>
<td>3 (2.0)</td>
<td>3 (3.9)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>'Pusing'¶</td>
<td>29 (19.6)</td>
<td>10 (12.9)</td>
<td>14 (18.7)</td>
</tr>
<tr>
<td>Hearing loss</td>
<td>5 (3.4)</td>
<td>1 (1.3)</td>
<td>3 (4.0)</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>6 (4.1)</td>
<td>4 (5.2)</td>
<td>3 (4.0)</td>
</tr>
<tr>
<td>Parasthesiae</td>
<td>13 (8.8)</td>
<td>7 (9.1)</td>
<td>3 (4.0)</td>
</tr>
<tr>
<td>Blurred vision</td>
<td>5 (3.4)</td>
<td>2 (2.6)</td>
<td>6 (8.0)</td>
</tr>
<tr>
<td>Hallucinations</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Difficulty sleeping</td>
<td>15 (10.1)</td>
<td>7 (9.1)</td>
<td>10 (13.3)</td>
</tr>
<tr>
<td><strong>Dermatological</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All symptoms</td>
<td>22 (14.9)</td>
<td>6 (7.8)</td>
<td>6 (8.0)</td>
</tr>
<tr>
<td>Mild itching</td>
<td>19 (12.8)</td>
<td>6 (7.8)</td>
<td>5 (6.7)</td>
</tr>
<tr>
<td>Severe itching</td>
<td>3 (2.0)</td>
<td>0 (0)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Rash</td>
<td>2 (1.4)</td>
<td>0 (0)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All symptoms</td>
<td>51 (34.5)</td>
<td>26 (33.8)</td>
<td>29 (38.7)</td>
</tr>
<tr>
<td>Cough</td>
<td>26 (17.6)</td>
<td>11 (14.3)</td>
<td>11 (14.7)</td>
</tr>
<tr>
<td>Fever</td>
<td>3 (2.0)</td>
<td>4 (5.2)</td>
<td>5 (6.7)</td>
</tr>
<tr>
<td>Chills</td>
<td>2 (1.4)</td>
<td>1 (1.3)</td>
<td>1 (1.3)</td>
</tr>
<tr>
<td>Sweats</td>
<td>6 (4.1)</td>
<td>3 (3.9)</td>
<td>2 (2.7)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>6 (4.1)</td>
<td>1 (1.3)</td>
<td>5 (6.7)</td>
</tr>
<tr>
<td>'Others'</td>
<td>28 (18.9)</td>
<td>14 (18.2)</td>
<td>18 (24)</td>
</tr>
</tbody>
</table>

* all A vs P, and D vs P) comparisons (except heartburn) were not significant (P<0.05)
† n (%)  
‡ ½ added to both numerators and sample sizes to estimate the relative risks.
§ A vs P RR=15.13 (1.97-4), P=0.005
  D vs P RR=15.4 (1.93-4), P=0.006.
¶ 'Pusing' - dizziness/muzzy head.
Table 3.6 Incidence rates per person-year by drug arm (azithromycin, doxycycline, placebo) for all symptom categories and selected symptoms volunteered daily within first four weeks of trial.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Azithromycin RR (95% CI)t</th>
<th>Placebo RR (95% CI)</th>
<th>Doxycycline RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Person-years of follow-up</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All symptoms</td>
<td>11.2 p-yr *</td>
<td>5.23 p-yr</td>
<td>5.75 p-yr</td>
</tr>
<tr>
<td>GI symptoms</td>
<td>123 (10.97)</td>
<td>63 (12.04)</td>
<td>69 (11.99)</td>
</tr>
<tr>
<td>Heartburn</td>
<td>27 (2.41)</td>
<td>0 (0)</td>
<td>24.6 (2.77-∞)§</td>
</tr>
<tr>
<td>CNS</td>
<td>157 (14.01)</td>
<td>85 (16.25)</td>
<td>99 (17.21)</td>
</tr>
<tr>
<td>Severe headache</td>
<td>5 (0.45)</td>
<td>7 (1.34)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Tinnitus</td>
<td>8 (0.71)</td>
<td>12 (2.29)</td>
<td>3 (0.52)</td>
</tr>
<tr>
<td>Difficulty</td>
<td>25 (2.23)</td>
<td>10 (1.91)</td>
<td>36 (6.26)</td>
</tr>
<tr>
<td>Dermatological</td>
<td>40 (3.57)</td>
<td>9 (1.72)</td>
<td>10 (1.74)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>81 (7.23)</td>
<td>39 (7.45)</td>
<td>45 (7.82)</td>
</tr>
<tr>
<td>Sweats</td>
<td>7 (0.62)</td>
<td>4 (0.76)</td>
<td>12 (2.1)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>19 (1.70)</td>
<td>1 (0.19)</td>
<td>9 (1.56)§</td>
</tr>
<tr>
<td>'Others'</td>
<td>74 (6.60)</td>
<td>26 (4.97)</td>
<td>45 (7.82)</td>
</tr>
</tbody>
</table>

* Person-years of follow-up
† Rate ratio (95% confidence interval)
‡ Total number of times symptom reported (incidence rate/p-yr)
§ ½ added to the numerator counts to estimate the rate ratio. No adjustment for the 95% CIs.
|| D vs A: RR=2.8 (1.64 – 4.87)
¶ D vs A: RR=3.34 (1.22 – 10.0)
3.2 Chloroquine-doxycycline therapeutic trial

3.2.1 Enrolment and withdrawals

Of 164 volunteers screened, 12 did not meet the entry criteria for the following reasons: negative malaria film (n=6), previous consumption of antimalarial drugs within 7 days (n=4), consent to participate not given (n=1), and one female with a positive urine pregnancy test (Figure 3.4). Of the 152 patients who entered the trial, 89 had *P. falciparum*, 63 had *P. vivax*. Twenty five did not complete the study to Day 28 because of malaria related, persistent vomiting during the first days of treatment (n=4), mixed infections during follow up (n=6), inadvertent primaquine administration before Day 28 days (n=7), and (iv) voluntary withdrawal (n=8). There were no withdrawals because of drug related toxicity.

Baseline characteristics of the patients were broadly similar, apart from a lower parasite count and residential time in the doxycycline group (Table 3.7). All patients had symptoms or signs of malaria. Of the 152 patients, 142 (93.4%) had measured total white cell counts: median 6,300/μL (range 2,100-13,600/μL)]. Using the measured white cell counts, and assuming a count of 8,000/μL for the 10 missing values, the Day 0 parasite counts ranged from 20-74,432/μL (median 2,373/μL) for falciparum patients, and 54-14,124/μL (median 2,567/μL) for vivax patients. Papuan and transmigrants had similar Day 0 median falciparum parasitaemias: 2,359.5/μL vs 2,856/μL, respectively, (*P*=0.7) but transmigrants had higher Day 0 median vivax parasitaemias: 3,168/μL vs 703.5/μL (*P*=0.05). Residential time in Irian Jaya ranged from one month to 40 years and was similar between CQ and CQD groups but significantly less in the doxycycline arm compared to the CQD arm for falciparum patients. All drug regimens were well tolerated.
164 volunteers considered

12 excluded

152 randomised

*P. falciparum* = 89
CQ=30, CQD=39, D=20

*P. vivax* = 63
CQ=23, CQD=24, D=16

19 exclusions from analysis:
clinical failure = 4
given primaquine = 5
new infections = 4
subject withdrawal = 6

6 exclusions from analysis:
clinical failure = 0
given primaquine = 2
new infections = 2
subject withdrawal = 2

70 evaluable by Day 28:
43 sensitive: CQ=5 CQD=26 D=12
27 resistant: CQ=18 CQD=3 D=6

57 evaluable by Day 28:
27 sensitive: CQ=6 CQD=15 D=6
30 resistant: CQ=15 CQD=7 D=8

Figure 3.4 Trial profile for the chloroquine-doxycycline study.
Table 3.7 Baseline characteristics at enrolment of patients with malaria randomised to the three drug regimens.

<table>
<thead>
<tr>
<th></th>
<th>P. falciparum patients</th>
<th>P. vivax patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CQ n=30</td>
<td>CQD n=39</td>
</tr>
<tr>
<td>% Male</td>
<td>93</td>
<td>85</td>
</tr>
<tr>
<td>Mean (SD) age in y</td>
<td>24.5 (5.0)</td>
<td>23.7 (5.2)</td>
</tr>
<tr>
<td>Median (range) residential time in Irian Jaya in months or years</td>
<td>17 (1m-40)</td>
<td>19 (4m-30)</td>
</tr>
<tr>
<td>Reported malaria*</td>
<td>2 (0-10)</td>
<td>3 (0-11)</td>
</tr>
<tr>
<td>Mean (SD) weight in kg</td>
<td>55 (6.9)</td>
<td>52 (7.1)</td>
</tr>
<tr>
<td>n (%) symptomatic</td>
<td>30 (100)</td>
<td>39 (100)</td>
</tr>
<tr>
<td>n (%) febrile</td>
<td>25 (83)</td>
<td>35 (90)</td>
</tr>
<tr>
<td>Mean (SD) temp in °C</td>
<td>38 (1)</td>
<td>38 (1)</td>
</tr>
<tr>
<td>Mean (SD) Hb in g/dl</td>
<td>13.2 (1.9)</td>
<td>12.3 (2.0)</td>
</tr>
<tr>
<td>Median parasite count /μL (range)</td>
<td>3,108 (20-74,432)</td>
<td>2,928 (20-64,998)</td>
</tr>
</tbody>
</table>

* median (range) of reported malaria attacks within the previous two years
3.2.2 Parasitological and clinical responses of *P. falciparum* patients

3.2.2.1 *In vivo test*

The cure rates for the three arms were: (i) CQ-5/23 [22% (95% CI 8-44)], (ii) CQD-26/29 [90% (73-98)], and (iii) D-12/18 [67% (41-87)] (Table 3.8). These rates were significantly higher in: (i) the CQD vs CQ arms: RR=4.12 [(95% CI 1.88-9.04), \( P < 0.001 \)], and (ii) the D vs CQ arms: RR=3.07 [(1.32-7.11, \( P = 0.0037 \)]. The CQD vs D rates were not significantly different: RR=1.34 [(0.95-1.91), \( P = 0.067 \)]. All three grades of resistance were present in the chloroquine arm, RII/RIII in the doxycycline alone arm, and only RIII resistance in the CQD arm. Overall, resistance rates (RI/RII/RIII) were 18/23 [78% (95% CI 56-93)] for chloroquine alone, 3/29 [10% (95% CI 2-27)] for chloroquine/doxycycline, and 4/18 [22% (95% CI 6-48)] for doxycycline. Paired PCR genotyping results were available for 4 of the 9 CQ recurrent cases; all genotype pairs had matching gel electrophoretic patterns, supporting a diagnosis of RI resistance. There was a trend (\( P = 0.1 \)) towards a higher cure rate in the Irianese [21/29 (72.4%)] compared to the other ethnic groups [22/41 (53.6%)].

3.2.2.2 Parasite clearance time

Parasite clearance times (PCTs) were measured in patients with either sensitive or RI resistance. In total 52 [CQ=14 (61%), CQD=26 (90%), D=12 (67%)] patients cleared their parasitaemias. The mean PCTs were 2.9 days (CQ), 3.3 days (CQD), and 3.9 days (D). None of these differences were significantly different.

3.2.2.3 Fever and symptom clearance

The fever clearance times (FCTs) ranged from 8 to 168 hours. Mean FCTs were not significantly different between study arms: 37.1h (CQ), 46.6h (CQD), and 56h (D). The proportions of symptomatic patients on Day 3 were similar in all arms: (i) CQ 11/18 (61%), (ii) CQD 15/26 (58%), and (iii) D 8/14 (57%), \( [\chi^2 \text{ (df}=2)=0.07, \ P=0.96] \). However, by Day 7 the proportion of symptomatic doxycycline recipients was significantly higher compared to the chloroquine arm: 6/13 (46%) vs 1/17 (6%), \( [\text{RR}=7.85 \ (95\% \ CI 1.07-57.4), \ P=0.02] \), but not compared to the CQD arm [6/26 (23%), \( P=0.16 \)].
3.2.3 Parasitological and clinical responses of *P. vivax*

### 3.2.3.1 *In vivo* test

The cure rates for the three arms were: (i) CQ-6/21 [28.6% (11.3-52.2)], (ii) CQD-15/22 [68.2% (45.1-86.1)], and (iii) D-6/14 [42.8% (17.7-71.1)], (Table 3.8). Only the CQD vs CQ cure rates were significantly different: RR=2.39 [(95% CI 1.15-4.97), *P*=0.01]. All the three grades of resistance were present in the chloroquine arm, RI/relapse in the CQD arm, and RI/relapse and RIII in the doxycycline arm. Overall resistance/relapse rates were 71% (CQ), 32% (CQD), and 57% (D). PCR results were available in 19 (CQ=11, CQD=6, D=2) of the 25 RI/relapse cases: 17 pairs matched, and two CQD patients had mismatching isolates. The cure rates in the Irianese [8/16 (50%)] and the other ethnic groups [19/41 (46.3%)] were similar (*P*=0.8).

### 3.2.3.2 Parasite clearance time

The PCTs were calculated for the 52 S/RI/relapse patients (CQ=19, CQD=22, D=11). The mean PCTs were significantly faster in the CQ and CQD arms compared to doxycycline alone: 2.4d (CQ) vs 5.4d (*P*<0.0001), and 2.9d (CQD) vs 5.4d (*P*=0.037). The CQD vs CQ comparison was unremarkable.

### 3.2.3.3 Fever and symptom clearance

Fever clearance times ranged from 8 to 144 hours. The mean FCTs were not significantly different between the arms: 34.1h (CQ), 47.3h (CQD), and 51.6h (D). Similarly, the respective proportions of symptomatic patients were not significantly different between the three arms on: (i) Day 3 [10/19 (53%) vs 11/22 (50%) vs 7/11 (64%), *χ²*(df=2)=0.57, *P*=0.75], and (ii) Day 7 [4/20 (20%) vs 2/20 (10%) vs 2/10 (20%), *χ²*(df=2)=0.89, *P*=0.63].
Table 3.8 The Day 28 parasitological cure rates of *P. falciparum* and *P. vivax* to chloroquine, chloroquine/doxycycline, and doxycycline.

<table>
<thead>
<tr>
<th></th>
<th><em>P. falciparum</em></th>
<th></th>
<th><em>P. vivax</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CQ  n=23</td>
<td>CQD n=29</td>
<td>D  n=18</td>
<td>CQ  n=21</td>
</tr>
<tr>
<td>Sensitive</td>
<td>5 (22)*</td>
<td>26 (90)†</td>
<td>12 (67)‡</td>
<td>6 (29)</td>
</tr>
<tr>
<td>RI</td>
<td>9 (39)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>13 (62)</td>
</tr>
<tr>
<td>RII</td>
<td>5 (22)</td>
<td>0 (0)</td>
<td>2 (11)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>RIII</td>
<td>4 (17)</td>
<td>3 (10)</td>
<td>4 (22)</td>
<td>1 (5)</td>
</tr>
</tbody>
</table>

* n (%)  
† CQD vs CQ: P<0.001  
‡ D vs CQ: P=0.004  
§ CQD vs CQ: P=0.01

### 3.2.4 Chloroquine concentrations and parasitological responses

Whole blood, Day 0 total chloroquine concentrations levels (TCQ) were available for a total of 119/152 (78.3%) patients of whom 38 (32%) had detectable chloroquine (Table 3.9). Only the Day 7-Day 3 TCQ concentrations (n=104) were positively correlated: $r=0.71$, $r^2=0.5$ (95% CI 0.34-0.64). The median Day 3 TCQs levels were similar between (i) falciparum and vivax patients, (ii) sensitive and resistant (RI/RII/RIII) patients (either species), and (iii) CQ (700 ng/ml) and CQD (716 ng/ml) arms ($P=0.45$). Insufficient TCQ data for the RIII (Pf=3, Pv=1) and RII (Pf=5, Pv=1) cases precluded further analyses based on the degree of resistance. However, there appears to be a trend of decreasing Day 3 TCQ concentrations and increasing resistance for both species. There was wide interindividual variation of absorption with Day 3 TCQ concentrations ranging from 315 to 1,504 ng/ml, a 4.7 fold difference.
Table 3.9 Whole blood, total chloroquine concentrations in patients treated with either chloroquine or chloroquine/doxycycline for falciparum or vivax malaria.

<table>
<thead>
<tr>
<th>Day</th>
<th>P. falciparum malaria</th>
<th>P. vivax malaria</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0-580)*</td>
<td>0 (0-272)</td>
</tr>
<tr>
<td>3</td>
<td>698 (315-1,545)</td>
<td>722 (316-1,405)</td>
</tr>
<tr>
<td>7</td>
<td>317 (0-769)</td>
<td>329 (162-956)</td>
</tr>
<tr>
<td>28</td>
<td>0 (0-460)</td>
<td>0 (0-769)</td>
</tr>
</tbody>
</table>

Day 3

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>686 (333-1,071)</td>
</tr>
<tr>
<td>Resistant</td>
<td>676.5 (315-1,355)</td>
</tr>
</tbody>
</table>

Day 3

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>766 (506-1,355)</td>
</tr>
<tr>
<td>RII</td>
<td>543 (461-1,179)</td>
</tr>
<tr>
<td>RIII</td>
<td>407 (315-435)</td>
</tr>
</tbody>
</table>

* median (range)
† one sample only

3.2.5 Haematological response

Day 0 haemoglobin data were available in 142 (85 males, 13 females) patients. There was no correlation between the Day 0 Hb and the Day 0 parasitaemia for either species (details not shown). At enrolment, the proportions of anaemic males (Hb < 13.5 g/d) were: (i) 38/72 (52.8%) for falciparum patients (CQ=11, CQD=18, D=9), and (ii) 29/57 (50.1%) for vivax patients (CQ=11, CQD=11, D=7). Anaemic females (Hb < 12 g/dl) numbered 11/13 [83.6% (CQ=3, CQD=5, D=3)]. Day 28 Hb data were available for 61 cured patients (males=53, females=8). The proportions of anaemic males for both species were 17/53 (32.1%): Pf=15/33 (45.5%), Pv=2/20 (10%). Only the vivax D0 vs D28 comparison was statistically significant: 50.1% vs 10% [RR=5.1 (1.3-19.5); P=0.001]. By study end, anaemic females numbered 6/8 (75%): Pf=3, Pv=3. Sample sizes for paired D28 D0 Hb values are small (Table 3.10). The mean change in haemoglobin (Day 28 relative to baseline) was significantly increased only in the CQD treated falciparum group.
Table 3.10 Haemoglobin data for falciparum and vivax patients at Day 0 and Day 28.

<table>
<thead>
<tr>
<th></th>
<th>P. falciparum</th>
<th></th>
<th>P. vivax</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CQ</td>
<td>CQD</td>
<td>D</td>
</tr>
<tr>
<td>Day 0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=27</td>
<td>13.1 (1.9)*</td>
<td>12.3 (1.9)</td>
<td>12.8 (2.3)</td>
</tr>
<tr>
<td>Day 28</td>
<td>n=4</td>
<td>n=24</td>
<td>n=14</td>
</tr>
<tr>
<td></td>
<td>12.3 (2.9)</td>
<td>13.5 (1.4)</td>
<td>13.8 (1.8)</td>
</tr>
<tr>
<td>Mean change</td>
<td>-0.6 (1.7)</td>
<td>1.2 (1.8) †</td>
<td>1.04 (2.5)</td>
</tr>
</tbody>
</table>

* mean (SD)
† P=0.003
‡ P=0.056
Chapter 4 - Discussion and conclusions

4.1 Azithromycin prophylaxis study.

4.1.1 Prophylactic efficacy

This study demonstrated excellent prophylactic efficacy of azithromycin against chloroquine resistant *P. vivax* but only modest efficacy against multidrug resistant *P. falciparum* in north-east Papua. Doxycycline achieved high protective efficacy against both species. The tolerability of both drugs was good.

This double blind, placebo-controlled trial followed a large number of clinically susceptible subjects with limited (6-15 months) malaria exposure who lived under basic field conditions, akin to those experienced by refugees, and backpackers. They suffered moderately intense malaria transmission, confirmed by the crude attack rate of just under 73% (placebo group) over 20 weeks. The high prophylactic efficacy of the doxycycline positive control excludes poor drug compliance and poor study conduct as causes of the low result of azithromycin against *P. falciparum*.

Recommending a drug for antimalarial prophylaxis depends on its tolerability and prophylactic efficacy. The latter should be measured ideally in a non-immune population because travellers to and pregnant women living in malaria endemic areas are candidates for malaria prophylaxis (since this study was conducted, the WHO has recommended intermittent preventive treatment for pregnant African women living in areas of intense transmission). The tolerability and prophylactic efficacy of a new drug should be comparable or better than currently used first line prophylactic drugs. However, there is no generally accepted minimum protective efficacy. This trial adopted *a priori* a minimum protective efficacy of 70%, in other words the lower 95% CI of the protective efficacy had to be greater or equal to 70%. Azithromycin had a lower 95% CI of only 50% against falciparum malaria whilst that of doxycycline was 85%. Direct comparisons with other studies gives some perspective but the lower 95% CI is critically dependent on the sample size. The lower 95% CIs recorded in Papua have been 93% for mefloquine 88% for doxycycline, and 48% and 57% for primaquine.136 370 380 Atovaquone/proguanil in relatively non immune transmigrasi adults had a protective efficacy of 96% with a lower CI of 72%.207 This is lower than those achieved in malaria immune Africans (≥ 92%) but these data are not applicable for non immune populations.204 205 206
The Indonesian soldiers with only six months of malaria exposure resembled more closely malaria naïve individuals compared to civilians who had 15 months of exposure. The prophylactic efficacy in the soldiers against *P. falciparum* was lower than in the civilians, 63% vs 88%. Although the difference was not statistically significant, this result suggests that a comparably low figure would be obtained in non immune travellers to the same area. At this level of protective efficacy, 250 mg of daily azithromycin cannot be recommended as a first line, prophylactic drug against falciparum malaria for non-immune populations.

Another azithromycin prophylaxis study has been conducted in malaria immune Kenyan adults. Daily administered azithromycin (250 mg), without a loading dose, had a prophylactic efficacy of 83% (68-91) against falciparum malaria; the efficacy of 1 gm administered weekly was 64% (47-77). All of the Kenyan prophylactic failures were asymptomatic consistent with a high degree of malaria acquired immunity. By contrast, the majority of the Indonesian soldiers and civilians were symptomatic, indicating a lesser degree of clinical immunity. The similarity of the efficacy results between the Indonesian civilians and the Kenyans suggests that the civilians had acquired sufficient immunity after only 15 months of malaria exposure to enhance the antimalarial effect of azithromycin. This suggests that the results of malaria prophylactic studies that are conducted in populations with even limited immunity to malaria may not be reliably applied to non immunes.

The marked difference in the protective efficacy between falciparum and vivax malaria was an unexpected result and is probably due to different mechanisms of action of azithromycin within vivax and falciparum parasites. Our knowledge of how azithromycin acts in malaria parasites is limited to *in vitro* evidence of protein synthesis inhibition necessary to support DNA production; the site of action is probably the parasite mitochondrion. There are no comparable *in vitro* data for *P. vivax*.

4.1.2 Tolerability

Azithromycin was well tolerated by these Indonesian adults over 20 weeks and did not appreciably affect routine haematological or biochemical measurements. Tolerability was broadly similar to that of doxycycline.

Assessing drug related side effects in patients in randomised, placebo-controlled trials is different than in clinical practice. Clinicians assess presenting symptoms in the
context of the patients' past medical history, the drug history, the local disease epidemiology, physical signs, and pertinent investigations. Clinical trials focus primarily on broad assessments involving group comparisons, and benefit from blinding. In this phase III trial, a lot of data have been analysed in detail. Some of the issues inherent in such an analysis are raised in this discussion.

The symptom data were reported as proportions and rates. The former is an indication of how many subjects reported a given symptom whilst rates are an indication of how often these symptoms were reported. The proportional analysis was done for the first four weeks of follow-up when all subjects were approximately at the same risk. The differential attrition of the placebo group because of malaria precluded proportional comparisons of risk, relative risk, and symptom trends over the whole period of follow up. Analysis of symptom rates takes into account the unequal follow-up time and calculates the symptom frequency per reported days of all subjects. This analysis does not, however, distinguish recurrent symptoms from those reported on consecutive days, or whether a few symptoms were reported by many subjects and vice versa. Another issue involves the interpretation of results obtained from multiple statistical comparisons. Determining which symptoms are important or whether differences exist based on simple hypothesis testing is challenging. The problems of multiple comparisons e.g. the over reporting (type I error) or failing to detect (type II error due to adjusting $P$ values) significant symptoms is well recognised but there are no completely satisfactory solutions. Cognisant of these issues, and with the view that the analysis of complex symptom data is largely exploratory, it was decided to flag comparisons where the $P$ value was 0.05.

The most important adverse event was an azithromycin induced maculopapular rash in one subject that was classified as a moderately severe drug reaction. The reported risk of developing any rash with azithromycin from large clinical series is 1.1%. Several symptoms in the both drug arms were noteworthy. Heartburn, a well recognised effect of doxycycline, was also reported by our doxycycline recipients but, interestingly, at a lower frequency than the azithromycin recipients. In published reports of azithromycin, heartburn is not often reported separately from abdominal pain, making it difficult to put these heartburn data into context. At four weeks, itching, paraesthesiae, and severe abdominal pain were unremarkable symptoms reported by the
strag arms but over 20 weeks, itching and paraesthesiae were reported more frequently by the azithromycin arm (vs. placebo), and severe abdominal pain by the doxycycline recipients (vs. placebo), suggesting these symptoms emerged gradually over time. By contrast, myalgia, reported more frequently by both drug arms at four weeks, might be a symptom that develops acutely.

Hearing loss, a significant symptom in some clinical reports, was reported by a minority of all our subjects with a correspondingly low frequency, approximately one complaint every two years. At study end, clinical signs consistent with a sensineural hearing loss (Weber lateralisation and a normal Rinné test) were detected in a small number (2.6%) of subjects across all three arms. Certainly, tuning fork examination tests only hearing at the frequency of the tuning fork and is subject to inter examiner variation. Audiometry would have strengthened considerably this aspect of safety monitoring but is impractical under field conditions and requires expertise in performing and interpreting the test. Never the less, we doubt these Rinné and Weber signs represent appreciable auditory nerve pathology. Azithromycin-induced, sensineural hearing loss has been reported in individuals treated for MAC disease and is usually transient, dose-related, and improves with drug discontinuation or dose reduction. Nevertheless, clinicians should be aware that azithromycin can cause rarely irreversible hearing loss. It would be prudent to monitor hearing in individuals on long term azithromycin. Hearing impairment is also a rare feature of chloroquine and mefloquine use and has recently been described with artemether/lumefantrine. This underlies the importance of continual safety monitoring after drug registration.

All azithromycin recipients had total white blood cell counts within the normal range when tested at four weeks and study end. This schedule would have missed early, transient leukopenia which has been reported, without adverse clinical effect, following standard and high dose azithromycin. A small proportion of azithromycin recipients developed mild elevation of AST by study end; the peak value was 50 IU/L. This modest increase is of doubtful clinical significance but a mild, drug-induced hepatitis cannot be excluded. Azithromycin usually causes mild and reversible elevations in liver enzymes in a small proportion (< 2%) of treated patients. Clinically significant liver toxicity is rare e.g. hepatitis as part of a systemic hypersensitivity reaction, and cholestatic jaundice. This is similar to erythromycin which may produce an allergic mediated, mixed
hepatic injury that is predominantly cholestatic; eosinophilia is present in ~ 50% of cases.\textsuperscript{390}

One azithromycin recipient was withdrawn from the study for drug related toxicity, giving a rate of 0.7% with an upper 95% CI of 3.7%. These figures are consistent with rates of withdrawal for azithromycin, for other indications, and the main prophylactic drugs (< 1% - < 7%).\textsuperscript{217,337}

To conclude, azithromycin had good tolerability in this study when used for up to 20 weeks. These data support the use of long term azithromycin for other clinical indications.

4.1.3 Limitations of the azithromycin trial

This trial was conducted in a comparatively small number of predominantly healthy and fit young soldiers. Few women were included. Two important vulnerable groups for malaria were excluded - pregnant women and young children. The findings of both efficacy and tolerability are primarily applicable to men and cannot be extended with confidence to children, pregnant women, and patients with AIDS. The small sample size precluded the detection of rare and, possibly, serious drug side effects.

4.1.4 Conclusions of the azithromycin trial and future directions

The prophylactic efficacy of azithromycin against \textit{P. falciparum} malaria was low (~ 72%) and appeared to be lower in the lesser immune soldiers (~ 62%). These levels of protective efficacy are insufficient to recommend azithromycin as a first choice for non immune travellers or pregnant women who are at risk of contracting \textit{P. falciparum} malaria. Azithromycin might be useful for patients with absolute contraindications or unacceptable toxicity to the mefloquine, doxycycline, chloroquine/proguanil, and atovaquone/proguanil. Such numbers of travellers are likely to be very small. Irrespective of the prophylactic regimen employed, clinicians should always remind travellers that chemoprophylaxis does not confer absolute protection against malaria and that full compliance is essential.

Azithromycin has demonstrated favourable tolerability and reasonable antimalarial activity that could be exploited in drug combinations for malaria treatment or prophylaxis. If azithromycin were safe in pregnant women, it would be a useful addition for this vulnerable group. This role may be of heightened importance in the HIV era where a sizeable minority (~ 25%) of African women are HIV positive.\textsuperscript{391} With its broad
antimicrobial spectrum, azithromycin might be useful against several HIV associated pathogens e.g. *Pneumocystis jirovecii* and *Toxoplasma gondii*. Azithromycin as malaria and streptococcal prophylaxis has not been assessed in children with sickle cell anaemia living in malaria endemic countries and is another avenue of research. The mechanisms of action and resistance to azithromycin in the two main malaria species also warrant further research.

### 4.2 Chloroquine-doxycycline study

#### 4.2.1 Efficacy

The combination of chloroquine and doxycycline improved markedly the cure rate of uncomplicated, drug resistant falciparum malaria; the increase against *P. vivax* was modest. Doxycycline alone showed some antimalarial activity that was either additive or synergistic when combined with chloroquine against both species.

Previous work with tetracycline or doxycycline has been limited to a few small studies in patients and volunteers with experimentally induced malaria. Both drugs produced slow clinical and parasitological responses. Mean parasite and fever clearance times against *P. falciparum* were 5 and 3-5 days, respectively, and 7 and 6 days, respectively, against *P. vivax*. This study has redocumented the slow parasite clearance times of falciparum (mean=4d), and vivax malaria (mean=5d), but the mean fever clearance times were faster, just over 2 days for both species.

Chloroquine alone in this study was clearly inadequate against *P. falciparum*; the cure rate was only 22%, and there was a substantial proportion (40%) of high grade RII/RIII infections. The cure rate against *P. vivax* was marginally better (29%) but the proportion of high grade resistance was lower, 10%. These cure rates probably indicate a further decline in CQ efficacy over time. In 1984, 9 (56.2%) of 16 patients from Jayapura with falciparum malaria had S/RI responses to chloroquine. There were 4 cases (25%) of high grade (RII/RIII) resistance. Other pertinent data from Papua have documented high failure rates with chloroquine in Arso e.g. 54% by Day 7, 83% by Day 14, and Nabire (89% by Day 28). Chloroquine resistant *P. vivax* has also emerged in Papua and is also associated with high rates of treatment failure. These were 22 and 58% (Day 14), and 78 and 83% % (Day 28) in Arso, and 64% (Day 28) at Nabire.

Chloroquine/doxycycline cured the majority (90%) of patients with *P. falciparum* malaria but this result is tempered by the occurrence of three patients (10%) with RIII
resistance, indicating a failure to kill the most resistant parasites. These patients remained symptomatic and had rising parasite counts at 48 hours but had no features of severe malaria. This contrasts to a trial of chloroquine and tetracycline to treat falciparum malaria in which two patients with RIII resistance became seriously ill. The investigators concluded that chloroquine/tetracycline was a potentially dangerous combination. Clinicians should be aware that RIII resistance is the most serious form of resistance because treatment has little or no effect in lowering the parasite count and patients are at risk of developing severe malaria.

RIII chloroquine-resistant *P. falciparum* is well documented in Papua and was 12.5% in Jayapura, 16.2% in Nabire, and 22.8% in Arso. Figures from this study were consistent - 17%. The 10% rate for CQD suggests the combination might have had a small beneficial effect against RIII resistant parasites. However, overcoming RIII chloroquine resistance in this area over the long term will require more efficacious antimalarial drugs or drug combinations that can also be used in young children and pregnant women. It is time to move away from chloroquine.

Chloroquine/doxycycline had a higher cure rate against *P. falciparum* compared to chloroquine-resistant *P. vivax*. Intuitively, one would have expected the opposite result. This finding underscores the importance of studies aimed at understanding the mechanisms of action and resistance of chloroquine and doxycycline in *P. vivax* and *P. falciparum*. Data from this study suggest different mechanisms of drug resistance may be operating between *P. falciparum* and *P. vivax*.

4.2.2 Haemoglobin response

Haematological recovery is a clinically important outcome for patients who are treated for malaria but this has received less attention than parasite clearance. The current WHO *in vivo* guidelines now place more emphasis on this issue. In low and moderate transmission areas, like some parta of north-eastern Papua, anaemia is closely related to the acute infection and improves with disease resolution. In this study, those patients who had sustained parasite clearance to Day 28 had higher mean Hb levels compared to Day 0 for both malaria species but only the mean change within the CQD group was statistically significant. Small sample sizes are the probable cause of the lack of statistical significance for the two other arms.
4.2.3 Chloroquine concentration and in vivo parasite response

In the assessment of drug resistant malaria, excluding inadequate drug absorption as the cause of treatment failure is important.\textsuperscript{75} This is complicated by the lack of a therapeutic range for chloroquine and the wide interindividual absorption of CQ.\textsuperscript{83} This study found an almost five fold difference in CQ absorption measured on Day 3. The PK data from this study do not contribute to defining a therapeutic range for chloroquine. Certainly, there was a trend in declining chloroquine concentrations and increasing degrees of resistance for both species, a finding consistent with a PK PD study in Tanzania.\textsuperscript{147} Understanding better the pharmacokinetic - pharmacodynamic relationship of CQ as treatment in both malaria species is one area of future research.

Chloroquine resistant \textit{P. vivax} warrants comment. There is now a WHO recommended in vivo test for vivax and falciparum malaria that does not mandate the measurement of chloroquine drug levels.\textsuperscript{123} Failure to clear vivax parasitaemia despite administered CQ is evidence of resistance despite the absence of CQ drug levels. Recurrent parasitaemia presents a challenge because this may be due to a new or resistant infection, or a relapse of the current or antecedent infection. All of these possibilities are considered as treatment failures by the new WHO criteria.

The classification used in this study was an adaptation of the parasitological response (S/RI/RII/RIII) for \textit{P. falciparum} and classified recurrent parasitaemia as RI/relapse. Another approach, developed by Baird, has been to classify a recurrent parasitaemia as resistant if (i) it occurred on or before Day 14, or (ii) if the measured whole blood CQ concentration was $\geq 100\text{ng/ml}$ at the time of recurrence.\textsuperscript{153} This cut off value is the MIC for fully sensitive vivax parasites that was derived from dose ranging studies that were conducted in 1948.\textsuperscript{151} Indeed fully sensitive vivax parasites do not usually reappear during 28 days of follow up.\textsuperscript{124} This system is a good early marker of 'resistant' parasites but does not in itself predict resistance to either another therapeutic course of chloroquine or to chloroquine prophylaxis. The mean, total whole blood levels achieved by CQ prophylaxis (300 mg/week) range from 710-263 ng/ml over 7 days, concentrations appreciably higher than the 100 ng/ml cut off.\textsuperscript{393} A greater understanding of the PK-PD relationship between chloroquine and the different strains of \textit{P. vivax} is needed to define a therapeutic range and enable a distinction to be made between sensitive and resistant \textit{P. vivax}. 

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4.2.4 The use of PCR to distinguish recurrent parasitaemias

This study used PCR to help differentiate recurrent parasitaemia of both species. Such data are useful to support the clinical diagnosis of resistance. In the case of the chloroquine treated falciparum patients, only four of nine had paired samples. The results for all four were consistent with RI infections. For the vivax patients with recurrent parasitaemia, most of the paired isolates matched and were consistent with relapse or resistance of the primary infection; two mismatched pairs were consistent with relapses of antecedent infections or new infections. The effectiveness of PCR is critically dependent on the collection of good quality specimens, and unfailing laboratory techniques. In this study, there was a high proportion (~ 40%) of recurrent parasitaemia that was unable to be classified mainly because samples were not collected. As this technology evolves, it would be important to standardise techniques so that results can be compared between laboratories.

4.2.5 Limitations of the chloroquine/doxycycline study

There were several study limitations in this study. This was an open label study with small sample sizes in which at the beginning drug allocation was not concealed adequately. The study enrolled mostly male patients. Although chloroquine/doxycycline was effective against P. falciparum, doxycycline had to be administered for seven days - a distinct drawback for patient compliance outside the research setting. This regimen cannot be used in pregnant women and children under eight years because doxycycline is contraindicated in these two groups.

4.2.6 Conclusions of the chloroquine-doxycline trial and future directions

This study has demonstrated that drug resistant falciparum malaria in north eastern Irian Jaya can be cured with a combination of chloroquine and doxycycline. These two drugs are cheap, currently licensed, and widely available in Indonesia. Whilst this combination will not be the definitive answer to the dual problem of drug resistant vivax and falciparum malaria in this area, it is certainly a better option than chloroquine alone, the current standard of care. Seven days of generic doxycycline would increase the cost of chloroquine alone from approximately 7 US cents to 51 cents; this compares to 13 cents an adult course of sulfadoxine/pyrimethamine, and US$2.35 for an adult course (15
mg/kg) of generic mefloquine (Mephaquine, Switzerland). The latter is about the same price as the recently WHO negotiated price of artemether/lumefantrine for adults.

More drug trials are needed to assess other drugs and drug combinations in all groups susceptible to malaria in NE Papua. The WHO is now advocating the use of artemisinin based combinations as a means to reduce the development of drug resistance, improve cure rates, and reduce transmission. One small trial of artesunate and S/P has already been conducted in NE Papua with a good result; the treatment failure rate by Day 28 was 4.4% but these two cases were determined to be new infections by PCR.

4.3 Retrospective critique of both studies

4.3.1 Azithromycin study

4.3.1.1 Study design

This study enrolled civilians and soldiers on the assumption that both groups had sufficiently low levels of immunity so as not to synergise with the drug. Indeed had the study started as planned, this assumption was very reasonable. Epidemiological data from Arso suggested that about two years of malaria exposure was necessary for newly arrived malaria naïve individuals (older children and adults) to establish a reasonable amount of malaria immunity against *P. falciparum*. However, by the time the study started, the civilians had 18 months of malaria exposure and epidemiological markers of higher malaria exposure compared to the soldiers. Not surprisingly, they also reported more episodes of self-diagnosed clinical malaria. Although the outcomes in the placebo group of the civilians and soldiers were not significantly different in terms of crude attack rate, levels of parasitaemia, and proportions of symptomatic breakthrough infections, the prophylactic efficacy against *P. falciparum* in the civilians was higher than that in the soldiers. Although the confidence intervals overlap and the *P* value was 0.08, one can conclude that these data are suggestive of an immune enhancing effect in the civilians. In retrospect, recruiting both soldiers and civilians proved to be an error because the two groups were not comparable. However, an important conclusion has been brought to light that even a small amount of immunity enhances drug action when used as prophylaxis.

4.3.1.2 Ethical aspects

4.3.1.2.1 Use of a placebo
This was a placebo controlled trial that was conducted in order to register azithromycin as a prophylactic with the US FDA. Because this was a pivotal phase III trial, the FDA required a placebo arm.

The views on the use of placebos have changed in the past 10 years because of the question of risk to study participants and the view that an experimental arm should be compared with the best alternative. However, in 2001, the World Medical Association [WMA (http://www.wma.net/e/ accessed December 2005)] issued a clarification note that stated that placebo may be used for 'compelling and sound methodological reasons'. It is widely accepted that randomised blinded studies generate the best quality data for assessing an intervention. In this trial, a placebo allowed the prophylactic efficacy to be calculated, attack rates to be measured, and the adequate assessment of the tolerability of azithromycin.

An assessment of the potential impact of using a placebo is warranted. For the transmigrants, the government policy was to take chloroquine for three months, thereafter, malaria diagnosis and treatment with chloroquine became the policy. Similarly, the soldiers were issued with prophylaxis for the first few months of their deployment (Several colleagues are unable to remember the exact duration). Prophylaxis for the whole duration of their deployment was considered impractical because compliance could not be assured. As with the civilians, prophylaxis was replaced by a policy of diagnosis and treatment with chloroquine. SP was used by some soldiers but this is a suboptimal choice because of the well recognised risk of severe cutaneous reactions; SP prophylaxis is no longer recommended by many authorities. Before the study started, the research team provided the soldiers with doxycycline that was taken unsupervised and limited in time to when they would have been taken off prophylaxis. Once the study started, some of these soldiers would have been randomised to the placebo arm.

In terms of risk of acquiring malaria, the civilians and the soldiers were not at an increased of malaria except for a group of soldiers who would have taken doxycycline up until the time it would have stopped, as per Indonesian Army policy. There was a good system of follow up in place. Daily visits were made to all subjects by the research team health workers who were also trained to take a malaria slide if a participant reported fever. In PIR V, all villagers had access to good quality medical care if they fell ill, and
the soldiers were visited several times per week by the mobile medical teams. Therefore, there was a robust system to pick up malaria early and provide prompt and effective rescue treatment.

A reasonable question to ask is whether a placebo controlled prophylactic study could be conducted in US soldiers? The answer is yes but with two caveats: (i) the soldiers are on peace keeping duties (like the Indonesian soldiers in this study), and (ii) the system of follow is robust, as was the case in this study. The latter point is crucial.

**4.3.1.2.2 Post research responsibility**

This issue is first mentioned in the 2000 Declaration of Helsinki of the WMA and encompasses providing the intervention to the wider community for a specified length if time. Regarding azithromycin, it is unlikely that the community as a whole from Arso would have benefited from azithromycin had it been successful for two reasons. Long term antimalarial prophylaxis is not the policy of the Indonesian MoH and the cost of such a policy is likely to be prohibitive. However, short term prophylaxis could have been an option in all age groups, and with future research, azithromycin use in pregnancy would was an area of keen interest of the Indonesian MoH. Azithromycin is due to come off patent in 2007 (C. Ohrt, personal communication) so the price may fall to a level that the MoH finds attractive. With the increased global interest in malaria and the need to have good antimalarial drugs in use by those who need them, a system of dual pricing has been introduced for artemether/lumefantrine which is marketed as Riamet in the North and CoArtemether in the malaria endemic countries. Such ideas were not circulating in the mod to late 90s but there is no reason to suppose that Pfizer, the producer of azithromycin, could not have considered this option. Indeed, Pfizer is involved in a humanitarian capacity in the trachoma control.

For the Indonesian Army, azithromycin would have certainly been an option because cost is less of an issue. Also with its close toes to the US military, obtaining drug supplies would be easy. In addition, the need for prophylaxis of soldiers would be short either because of short stays in endemic areas or because the policy is to only provide short term prophylaxis.

In view of the need to consider what to do after the research, then this should be discussed at the start of any proposed research. It is reasonable that sponsors should give something back to communities, especially if the intervention might be difficult to implement locally because of high cost. In the case of azithromycin, had it been
successful, then more research could have been done in collaboration with the Indonesian MoH to exploit its antimalarial activities, focusing primarily on the local community. The sponsor could agree to support local clinics in a sustainable way for a given period of time. Again, such ideas should be discussed and negotiated with the MoH.

4.3.2 Chloroquine doxycycline study

4.3.2.1 Choice of drugs to study

This study chose to examine the efficacy of chloroquine combined with doxycycline and have a doxycycline alone arm. The rationale for the latter was to see if any improvement in the combination arm could be ascribed to the doxycycline alone.

Ideally, conducting clinical trials for policy should be done in the context of a comprehensive assessment of current drug policy. This means that a series of clinical trials should be planned to evaluate different options for treatment. In this study, chloroquine and doxycycline was evaluated. There is no reason why other inexpensive antimalarial drugs could not have been assessed in follow up studies against chloroquine, the current standard of care, or SP, the second line drug. A number of possibilities spring to mind: (i) amodiaquine alone, (ii) chloroquine plus SP, (iii) amodiaquine plus SP, (iv) chloroquine plus primaquine, and (v) quinine alone or with doxycycline. In terms of the more expensive options, then mefloquine alone and atovaquone / proguanil. With the recent thrust of the WHO to encourage the use of artemisinin based combinations, options include CoArtemether, artesunate plus amodiaquine, or SP, or mefloquine, and Artekin (dihydroartemisinin and piperaquine). Indeed, since the year 2000, a number of trials have been conducted in Papua assessing SP vs. artesunate plus SP, Artekin vs artesunate plus amodiaquine, and Artekin vs CoArtemether.

A retrospective critique of the chloroquine / doxycycline study is certainly a useful exercise but at the same time, one should not be naïve as to think that that by generating a long list of potential alternative drugs to study that such studies can be done easily. The conduct of large clinical trials is an undertaking that requires considerable human and financial resources which are a challenge for the Ministry of Health. Much of the research is done in collaboration with the US Navy, the Menzies School of Health Research, and the Wellcome Trust.
4.3.2.2 Study design

This was a relatively small study that was conducted in hospital. Many potential patients did not like the idea of remaining an inpatient for one month. Consequently, the recruitment of patients was slow. Had the study been conducted in the field, patient numbers would have been higher and this could have accommodated an additional study arm or arms so that several drugs would have been studied in parallel. However, more PCR sampling would have been required and more logistical support would have been needed.

4.4 Future research in Indonesia and implications for public health

Malaria ranks as one of Indonesia’s principal public health challenges. The size of the country and the scale of the challenge cannot be underestimated. The malaria epidemiology varies widely with the eastern islands experiencing the greatest malaria burden. Within Papua the malaria epidemiology also varies, being stable in the lowlands and unstable in the highlands. Epidemics in both settings have caused much morbidity and mortality. In Java and Bali, malaria control has been highly effective but the potential for hypoendemic malaria to cause epidemics has been graphically brought home by the epidemic in central Java in 1997.\textsuperscript{34} Chloroquine resistant \textit{P. falciparum} is widespread and there are also focal areas of S/P resistance. Chloroquine resistant \textit{P. vivax} is well established and S/P resistance is emerging. \textit{P. malariae} resistant to chloroquine has also been described in a focus in southern Sumatra.\textsuperscript{56} Clearly, drug resistance is a major challenge for Indonesia. Clinical trials of efficacious drugs and drug combinations will be needed to determine the optimal treatment for children, adults, and pregnant women.

The results from this thesis have shown that azithromycin would be unsuitable as malaria prophylaxis, and that the combination of chloroquine and doxycycline would at best be an interim measure for treating drug resistant \textit{P. falciparum} whilst awaiting better and affordable alternatives. The response of chloroquine resistant \textit{P. vivax} was modest; CRPV still awaits suitable treatment.

The goals of research should be to inform policy makers, deepen our understanding of unresolved scientific issues, and validate research methodologies. There are two areas of future research that stem directly from this thesis; namely, clinical trails.
to determine optimal drug treatment and prophylactic regimens, and more scientific work on the mechanisms of drug resistant falciparum and vivax malaria.

The current thrust of the WHO of combining standard antimalarial drugs with an artemisinin derivative (AD) for treating drug resistant falciparum malaria also applies to Indonesia. Options include artesunate combined with mefloquine, amodiaquine, or S/P, and artemether/lumefantrine. Dihydroartemisinin combined with piperaquine is emerging as another promising combination but this drug has not yet been registered to international standards. These drugs are suitable for all age groups. Experience with the artemisinins in pregnancy is limited but has shown favourable outcomes for both mother and child. The question of the optimal treatment of chloroquine resistant P. vivax remains unanswered. A major policy decision needs to be taken as to whether CRPV should also be treated with an AD. This question has not yet been addressed by the WHO (K. Mendis, personal communication). The problem of the optimal treatment of vivax hypnozoites also poses a challenge because of different sensitivities to primaquine. Radical cure of vivax malaria is considered a reasonable public health goal in areas of low transmission. Clearly, more research on P. vivax is needed.

There are several interesting and important questions that can be addressed in therapeutic efficacy trials. The end points of the current WHO in vivo tests for both malaria species could be validated and / or refined for an Asian setting. Added to this could be the relationship between molecular markers (dhfr, dhps, and pfcrt) and the in vivo response. The number of such studies from Indonesia has been small. More work is needed to determine the predictive value of these markers and to assess their role in resistance surveillance e.g. molecular data could be used for targeted in vivo tests. The long term tracking of resistance markers in sentinel sites could be a useful public health tool. The PK-PD relationship of antimalarial drugs needs to be better understood for the established and newer antimalarial drugs. Ideally, PK data should be collected in all efficacy drug trials.

As well as clinical trials, a further step would be to conduct deployment studies of efficacious artemisinin based combinations to assess their impact on morbidity, mortality, and disease transmission as well as the economic costs associated with deployment. Such research could underpin sound drug policies. In the low transmission areas of western Thailand, the systematic deployment of artesunate/mefloquine improved falciparum cure
rates and reduced transmission of *P. falciparum*. Such a favourable outcome is possible in many parts of Indonesia.

Prophylaxis studies would also have a role to play in Indonesia because many Indonesians travel to other parts of the archipelago and because of the transmigration programme. Transmigrasi are given three months of chloroquine prophylaxis when they first arrive in Papua. A more efficacious alternative is certainly needed. Malaria prophylaxis during pregnancy has not been addressed through clinical trials in Indonesia. Indeed, there are no published data on the epidemiology of malaria in pregnancy.

Broader areas of research not addressed in this thesis were the social, economic, and entomological aspects of malaria control. Another critical area is the relationship between malaria policy and research and how research findings are channelled to and evaluated by policy makers in order for timely decisions to be made.

Much work remains so that sound evidence can be used to roll back malaria.
Chapter 5 - the research effort

Both research projects were clinical trials that required much planning and work by a number of individuals. Funding for the azithromycin trial came from US Army Medical Material Development Activity and the US Naval Medical Research Command. The latter also funded the chloroquine doxycycline study.

5.1 Azithromycin study

The following contributed to the research effort in a significant way and are coauthors on the azithromycin prophylaxis and tolerability papers: Richie TL, Fryauff DJ, Picarima H, Ohrt C, Tang D, Braitman D, Murphy GS, Widjaja H, Tjitra E, Ganjar A, Jones TR, Basri H, Berman J. Their role/s in the study is/are listed below:

Protocol development: Richie T, Ohrt C, Tang D, Braitman D, Berman J.
Study execution: Richie TL, Fryauff DJ, Picarima H, Murphy GS, Widjaja H, Tjitra E, Ganjar A, Jones TR, and Basri H.
Database design & data management: Richie TL, Fryauff DJ, Picarima H.
Data analysis: Tang D.
Manuscript writing: Richie TL, Tang D and Berman J.
Critical review of manuscript: Fryauff DJ, Ohrt C, Braitman D, Murphy GS, Widjaja H, Tjitra E, Ganjar A, Jones TR, and Basri H.
Liason with Pfizer: Braitman D, Ohrt C, and Berman J.
Trial monitoring: Braitman D.

In this study, I was the overall manager and had to coordinate all aspects of the study and run the study in the field. The protocol for this study was written by me and Drs. Richie, Berman, and Ohrt. Dr. Tang provided the statistical expertise for the sample size calculation. An earlier azithromycin prophylactic study had been carried out in Kenya and this protocol was used as a guide. The case record forms and SOPs for the conduct of the study in the field were written by me and were reviewed by Drs. Richie, Berman, and Ohrt.

In the field, I managed a team of some 30 people comprising local health workers, microscopists, doctors, PhD scientists, and logisticians. The study was conducted to GCP and was monitored externally by the US Army: Drs. Braitman and Wesche. There was also a rigorous internal system of checks of work done by the health workers and team physicians done by myself and other senior team members. The study participants were
seen daily by the health workers. They were supervised and accompanied by team physicians (Drs. Murphy, Widjaja, Tjitra, Ganjar A, and Basri). I also participated occasionally. These physicians also ran the mobile medical service that visited the Indonesian Army posts. My involvement in this activity was small. The laboratory work was done by a small laboratory that was set up specifically for this trial. It also operated to GCP standards. All malarial slides were read and reread in a blinded fashion by a team of experienced microscopists in the field. Slide QC was organised by either me or a designee.

The databases (Epi Info) were designed by myself and reviewed by Drs Richie and Ohrt. Data were double entered and validated in the field by data entry clerks. Data validation against source documents, cleaning and cross checking between different databases was supervised by myself and senior team members, notably, Drs. Richie, Fryauff, and Murphy. Simple data analyses were done by myself with the help of Dr. Murphy. However, for the publication, the definitive analyses and Survival curves were done by Dr. Tang who also provided much critical review of the tolerability paper.

I wrote both azithromycin publications. For the prophylactic study, I received a lot of help from Drs. Berman and Richie in putting together the first draft. This was the first major paper I had written. Thereafter, critical input was received from the other co authors but in particular form Drs. Ohrt and Fryauff. Dr. Tang helped substantially with the discussion of the tolerability paper.

5.2 Chloroquine doxycycline study

The following authors on the paper: Taylor WR, Widjaja H, Richie TL, Basri H, Ohrt C, Tjitra E, Taufik, Jones TR, Kain KC, and Hoffman SL. Their roles are listed below.

Protocol development: Ohrt C, Richie T, Tjitra E, and Hoffman S.
Study execution & patient care: Widjaja H, Basri H, Taufik, and Tjitra E.
Data management: Ferry and Nurjaya.
Sample size calculation & data analysis: Jones TR.
Manuscript writing: Richie TL, and Ohrt C.
Critical review of manuscript: Widjaja H, Basri H, Tjitra E, Kain KC, and Hoffman SL.
Trial monitoring: Tjitra E.
The original idea for this comparative clinical trial came from Drs. Hoffman and Richie. Together with Dr. C. Ohrt, the process of protocol development started and a first draft was produced. Protocol development was subsequently taken over by myself and was completed with the help of Drs. Ohrt and Richie. Dr. T. Jones did the final sample size calculation. Thereafter all other research activities were my responsibility and I consulted my supervisor Dr. Richie as required. My work included writing the informed consent, designing the case record form and electronic database, overseeing ethical review and making amendments, supervision of team physicians for study execution and clinical care of patients (Dr. Widjaja assumed these responsibilities in my absence), overseeing data entry, cleaning, and source documentation checking, data analysis, and manuscript writing.

The malaria slides were read by team microscopists and checked by senior slide readers. Routine haematology was done by the hospital laboratories. The Parasight F tests were done by a team technician. Oversight was by me and Dr. Widjaja. The chloroquine concentrations were analysed by HPLC in Jakarta. The filter paper analyses for parasite genotyping were done in Canada in Dr. Kain's laboratory. I was not involved in PK or PCR analyses.
Appendix 1 The global distribution of malaria transmission, represented by the darker shaded areas (Source: WHO 2002).
Appendix 2 Map of Indonesia.
Appendix 3 Malaria burden in Indonesia presented as annual prevalence rates per 1,000 population (Source: Indonesian MoH, 1997).
Appendix 4 Map of Irian Jaya.
Appendix 5 Graph illustrating the parasite reduction ratios (PRR) of several antimalarial drugs. The artemisinins have the highest and antibiotics the lowest PRRs.
Appendix 6 Graph showing the sigmoid relationship between drug concentration (A) and it's antimalarial effect. As parasite resistance increases, the IC\textsubscript{50} increases but the E\textsubscript{max} is unchanged (B). With further parasite resistance, the IC\textsubscript{50} increases and the E\textsubscript{max} falls.

**Antimalarial effect/parasite killing**

![Image of a graph showing the sigmoid relationship between drug concentration and antimalarial effect.]
Appendix 7 The 1996 WHO in vivo test for *Plasmodium falciparum*.

**Early Treatment Failure (ETF) for all areas of transmission**

*Early Clinical Failure*: (i) development of danger signs or severe malaria on Day 1, Day 2 or Day 3, and parasitaemia, (ii) parasitaemia on Day 3 with fever (axillary temperature $\geq 37.5^\circ$C).

*Early Parasitological Failure*: (i) parasitaemia on Day 2 > Day 0 count, (ii) parasitaemia on Day 3 $\geq 25\%$ of count on Day 0.

**Late treatment failure for areas of intense transmission**

*Late Clinical Failure*: (i) development of danger signs or severe malaria after Day 3 in the presence of parasitaemia (same species as on Day 0), (ii) presence of parasitaemia and fever on any day from Day 4 to Day 14/28, without previously meeting the criteria of ETF, (iii) parasitaemia and significant decrease of haematocrit or haemoglobin.

**Late treatment failure for areas of moderate/low transmission**

*Late Clinical Failure*: (i) development of danger signs or severe malaria after Day 3 in the presence of parasitaemia (same species as on Day 0), (ii) presence of parasitaemia and fever on any day from Day 4 to Day 14/28, and not meeting the criteria of ETF.

*Late Parasitological Failure*: presence of parasitaemia on any day from Day 4 to Day 14/28 in the absence of fever (axillary temperature $<37.5^\circ$C) and without previously the criteria of ETF.

**Adequate clinical response (ACR) for areas of intense transmission**

*ACR and parasitaemia*: presence of parasitaemia without fever without previously meeting any criteria of ETF or LCF.

*ACR without parasitaemia*: absence of parasitaemia irrespective of the axillary temperature without previously meeting any criteria of ETF or LCF.

**Adequate clinical response for areas of moderate/low transmission**

ACR: absence of parasitaemia irrespective of the axillary temperature without previously meeting the criteria of ETF or LCF or LPF.
Appendix 8 The 2003 WHO in vivo test for *Plasmodium falciparum*.

**Early Treatment Failure (ETF) for all areas of transmission**

(i) development of danger signs or severe malaria on Day 1, Day 2 or Day 3, in the presence of parasitaemia, (ii) parasitaemia on Day 2 > Day 0 count, (iii) parasitaemia on Day 3 ≥ 25% of count on Day 0, (iv) parasitaemia on Day 3 with fever (axillary temperature ≥ 37.5°C).

**Late treatment failure for areas of intense transmission**

*Late Clinical Failure:* (i) development of danger signs or severe malaria after Day 3 in the presence of parasitaemia, (ii) presence of parasitaemia and fever on any day from Day 4 to Day 14, without previously meeting the criteria of ETF.

*Late parasitological failure:* (i) presence of parasitaemia on Day 14 and axillary temperature < 37.5°C, without previously meeting the criteria of ETF or LCF.

**Late treatment failure for areas of low to moderate transmission**

*Late Clinical Failure:* (i) development of danger signs or severe malaria after Day 3 in the presence of parasitaemia, (ii) presence of parasitaemia and axillary temperature ≥ 37.5°C (or history of fever) on any day from Day 4 to Day 28, without previously meeting the criteria of ETF.

*Late Parasitological Failure:* presence of parasitaemia on any day from Day 7 to Day 28 and axillary temperature < 37.5°C, without previously the criteria of ETF or LCF.

**Adequate clinical and parasitological response (ACPR)**

*High transmission areas:* absence of parasitaemia on Day 14 irrespective of axillary temperature without previously meeting any of the criteria of ETF or LCF or LPF.

*Low to moderate transmission areas:* absence of parasitaemia on Day 28 irrespective of the axillary temperature without previously meeting the criteria of ETF or LCF or LPF.
Appendix 9. Map of the world showing the risk of acquiring malaria (Source WHO).

A - no or low risk
B - low risk
C - high risk - transmission / resistance pattern
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