Maternal and Child Health in Papua-Indonesia: 
The Epidemiology of Malaria and Strategies for its 
Treatment and Prevention

by

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Declaration

"I hereby declare that the work herein, now submitted as a thesis for the degree of Doctor of Philosophy of the Charles Darwin University, is the result of my own investigations, and all references to ideas and work of other researchers have been specifically acknowledged. I hereby certify that the work embodied in this thesis has not already been accepted in substance for any degree, and is not being currently submitted in candidature for any other degree".

Dr. Jeanne Rini Poespoprodjo
20th December 2010
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Abstract

Multidrug resistant *P. falciparum* and *P. vivax* infections are associated with high morbidity and mortality in Timika, Papua (Indonesia). This thesis sets out to define the burden of malaria in pregnant women and infants and determine the impact of better treatment protocols in reducing these. We established a systematic surveillance system at a local hospital. From April 2004 to June 2009, clinical and laboratory data on more than 6000 pregnant women and 1500 infants attending the hospital were collected.

We found that pregnant women and infants in this region are affected by the adverse outcomes of malaria, including those with *P. vivax* infections. Maternal peripheral parasitaemia at delivery is associated with maternal severe anaemia (OR=1.9), low birth weight babies (OR=1.9), preterm delivery (OR=1.5), stillbirths (OR=2.3) and congenital malaria (OR=4.5). The risk of malaria starts at birth and it is often without symptoms. The majority of symptomatic malaria in the first 6 months of life is associated with *P. vivax* infections. Severe anaemia is the main determinant of severe disease in both 93% of pregnant women and 77% of infants.

Malaria control programs in this region focus on the early detection and prompt treatment of malaria. In March 2006 malaria treatment policy in Timika was changed from chloroquine (CQ) + sulfadoxine-pyrimethamine (SP) to dihydroartemisinin-piperaquine (DHP). Our observation cohort documented that DHP in the second and third trimesters of pregnancy is well tolerated and safe. In addition, DHP has an excellent post treatment prophylaxis effect in pregnancy reducing the risk of malaria at delivery by 3 fold and congenital malaria by 16 fold.

After more than 3 years of DHP deployment, we observe an encouraging effect of treatment policy change in improving maternal and infant’s health outcomes. The results of this study suggests that an important next step will be identifying the most effective method of service delivery of ACT in order to scale up treatment and prevention programs. Universal coverage is likely to impact further in reducing malaria associated morbidity and mortality in pregnant women and infants in this region.
Chapter 1

Introduction
Chapter 1 – Introduction

Aims and scope of the thesis
The overall aim of this thesis is to define the epidemiology of maternal malaria towards developing malaria treatment strategies to reduce the risk of adverse outcomes in both mothers and infants in an area with a high prevalence of multidrug resistant *P. falciparum* and *P. vivax* malaria.

Malaria in pregnancy is associated with poor pregnancy outcomes (Desai et al., 2007). Severe anaemia, low birth weight, preterm delivery and perinatal deaths are recognized as the adverse outcomes of malaria in pregnancy and these contribute to the high maternal and infant mortality in malaria endemic areas (Desai et al., 2007, Guyatt and Snow, 2001, Luxemburger et al., 2001, van Geertruyden et al., 2004, Brabin et al., 2001a, Granja et al., 1998). In addition, malaria in pregnancy has adverse effects to the infant’s health from exposure in utero, as described above, and also from vertical malaria transmission (Brabin, 2007, Menendez and Mayor, 2007).

Early diagnosis and prompt treatment with an effective antimalarial drug would reduce the risk of poor outcomes (WHO/AFRO, 2004, Nosten et al., 2007). Following the declining efficacy of chloroquine (Cq) and sulfadoxine-pyrimethamine (SP) in curing malaria (Adam et al., 2004, Collignon, 1991, Fryauff et al., 1997, Fryauff et al., 1998, Garavelli and Corti, 1992, Molta et al., 2003, Ratcliff et al., 2007b, Ronn et al., 1996, Checchi et al., 2002, Wongsrichanalai et al., 2002), WHO has recommended locally effective artemisinin combination therapy (ACT) as the first line treatment for *falciparum* malaria in the second and third trimester of pregnancy and in infants (WHO, 2006a). ACT is also recommended to treat chloroquine resistant *P. vivax* infections (WHO, 2006a, Ratcliff et al., 2007a, Douglas et al., 2010).

Timika (Papua province, Indonesia) is a malarious area, which is unusual in harbouring highly drug resistant isolates to both *Plasmodium falciparum* and
*Plasmodium vivax*. The overall malaria incidence in this area is 876 per 1,000 per year (Range: 711-906) (Karyana et al., 2008), with the risk of treatment failure by day 28 following chloroquine monotherapy for *P. vivax* being 65% and 48% following chloroquine plus sulfadoxine-pyrimethamine for *P. falciparum* infections (Ratcliff et al., 2007b). Unsupervised oral quinine is associated with 80% treatment failure, likely due to poor adherence with a thrice daily dosing for 7 days (Ratcliff et al., 2007b). Maternal mortality ratio in this area is very high (1145 per 100,000 live births), infant mortality rates reaching 68 deaths per 1000 live births (Hidayat, 2001). Malaria contributes significantly to maternal and child morbidity and mortality in this region. Every year, more than 300 infants up to one year old of age are admitted with malaria to the local hospital with a total of 800 outpatient consultations (Karyana et al., 2008). Pregnant women exposed to multidrug resistant *P. falciparum* and *P. vivax* are at increased risk of adverse pregnancy outcomes even when the parasitaemia is asymptomatic (Ratcliff et al., 2007b, Poespoprodjo et al., 2008).

Therefore, it is important to ensure that pregnant women and infants with malaria in this region are diagnosed promptly and treated effectively. Local efficacy studies have shown that dihydroartemisinin-piperaquine (DHP) has high therapeutic efficacy (failure rates < 10%) in treating *P. falciparum* and *P. vivax* infection in non pregnant individuals (Ratcliff et al., 2007a, Ronny et al., 2006). In March 2006 the local health authority in Timika took the difficult decision to change policy to DHP in this region, as the first line treatment for uncomplicated malaria under a specific government agreement (NIHRD/CDCKS.02.012.1.3431). In view of the risk of infection, the poor local efficacy of established treatments regimens and the lack of viable alternatives, DHP was adopted as the first line treatment for pregnant women with malaria in the 2nd and 3rd trimester and in children weighing more than 5 kg (NIHRD/CDC KS.02.012.1.3431).

In view of the significant burden of malaria in mothers and infants in Papua Indonesia, the malaria studies presented in this thesis were designed to address the following aims and hypotheses:

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1 National Institute of Health Research and Disease/Communicable Disease Control – Ministry of Health Indonesia
Define the epidemiology of peripheral malaria and major risk factors for infection in pregnant women and their offspring in Timika, southern Papua.

- Hypothesis 1: Maternal malaria is associated with adverse pregnancy outcomes and high risk of congenital infections.
- Hypothesis 2: Malaria in the first year of life is associated with adverse complications.

Assess potential safety and toxicity of DHP on both mothers and their newborns as well as evaluate the impact of treatment policy change to the risk of malaria at delivery and adverse pregnancy outcomes.

- Hypothesis 3: DHP treatment during pregnancy is safe and effective for the treatment of uncomplicated *P. falciparum* and *P. vivax* malaria and reduces recurrent and adverse pregnancy outcome of maternal malaria.

The burden of malaria in pregnant women and infants in Timika, Papua-Indonesia is discussed in Chapters 3 to 6. Early diagnosis and prompt treatment with an effective antimalarial drugs has been the focus of malaria control program in this region. Chapter 7 reviews the safety and effectiveness of DHP exposures in pregnant women following the change in treatment policy in March 2006. The impact of improving malaria treatment in pregnancy to maternal and infant’s health is evaluated in Chapter 8. The overall findings and recommendations are summarized in Chapter 9.

### 1. Malaria

Currently, almost half of the population of the world (3.3 billion) is at risk of malaria (WHO, 2008b). In 2008 the World Health organization estimated that there were more than 200 million cases of malaria and 863,000 associated deaths per year (WHO, 2009), however other estimates suggest that the figure is considerably higher, in the order of 349-552 million clinical episodes of *P. falciparum* infections and 132-391 million *P. vivax* infections (Hay et al., 2010, Hay et al., 2004). The majority of *P. falciparum* malaria is in Africa (~60%) (Snow et al., 2005), whereas outside of Africa half of malaria cases are due to *P. vivax*, this figure rising to 50-65% in parts
of Southern Asia and Western Pacific (Mendis et al., 2001). Globally approximately 2.85 billion people are at risk of *P. vivax* infection (Guerra et al., 2010). Pregnant women and young children are particularly vulnerable to the adverse outcomes of malaria and will thus constitute the focus of this thesis.

Malaria is an infection caused by five species of *Plasmodium* (*P. falciparum*, *P. vivax*, *P. malariae*, *P. ovale* and *P. knowlesi*). These parasites are transmitted from man to man through female mosquito bite of the genus *anopheles* (Najera and Hempel, 1996). The number of infective mosquito bites per year (Entomological Inoculation Rate – IER) determines the level of malaria transmission. High transmission is defined as having >10 infective bites/year and low transmission is defined as having < 1 infective bites/year (Beier et al., 1999).

The level of endemicity determine the speed of acquisition of individual immunity to malaria and the resulting clinical outcomes (WHO, 2000a). In high malaria transmission areas, children under five years old are more susceptible to malaria and severe disease compared to older children and adults (Snow et al., 1999, Bloland et al., 1999). In Sub Saharan Africa, *P. falciparum* malaria accounts for 11-18% of underfives mortality and this age group has the highest rate of anaemia (WHO and UNICEF, 2005, Brabin et al., 2001b). However, this situation is now changing with an observed declining trend in malaria attributed child mortality in Africa. The risk of cerebral malaria in children decreases with increasing transmission intensity (Snow et al., 1999, Snow et al., 1997). If they survive, ongoing malaria exposures will further stimulate immunity development (Snow et al., 1997).

Outside Africa, malaria transmission is generally lower. In these areas the acquisition of immunity takes longer to develop and the level of protection may vary. Severe symptoms such as decreased consciousness, respiratory distress, severe anaemia, renal failure and severe jaundice are found in all age groups (WHO, 2000a). On the Thai Burmese border where endemicity is low, most malaria infections are symptomatic with clinical symptoms range from mild to severe (Luxemburger et al., 1996), the risk of severe malaria are declining with age (Luxemburger et al., 1997). Severe anaemia is more prevalent in children under five years old than in older
children and adults, on the other hand the risk of cerebral malaria increases with age (Luxemburger et al., 1997, Allen et al., 1996, Tjitra et al., 2008).

In areas where both species are prevalent, *P. vivax* malaria is more common in infants and young children, with immunity to this infection generally acquired by in the age of 15 (Cattani et al., 1986, Luxemburger et al., 1996, Genton et al., 1995). On the other hand susceptibility to *P. falciparum* increases until age 15-29 years old in low to moderate malaria transmission area and 5-9 years old in areas with higher malaria transmission (Luxemburger et al., 1996, Genton et al., 1995, Karyana et al., 2008). This suggests earlier acquisition of immunity to *P. vivax* malaria than to *P. falciparum* infections. In addition, immunity to *Plasmodium vivax* may protect the development of clinical and severe symptoms of *Plasmodium falciparum* malaria either from subsequent or concomitant infections (Maitland et al., 1996, Maitland et al., 1997, Luxemburger et al., 1997).

**Pathogenesis: host-parasite interactions**

The pathogenic mechanism of malaria starts with parasites invasion to host red blood cells (RBCs) (Najera and Hempel, 1996, Miller et al., 2002). *P. falciparum* can rapidly invade a large number of RBCs at any stage of erythrocytes’s age (Miller et al., 2002). On the other hand, *P. vivax* infection is mostly restricted to young erythrocytes with positive Duffy blood group (Miller et al., 2002, Anstey et al., 2009), although may also be able invade Duffy negative erythrocytes (Menard et al., 2010). *P. vivax* is better able to survive and maintain parasite transmission. The gametocytes are released earlier in *P. vivax* infections compared to *P. falciparum*, can be transmitted before symptoms appear and can transmit at much lower parasitaemias to mosquito hosts (Miller et al., 2002, Anstey et al., 2009). In addition, *P. vivax* hypnozoites have the ability to remain dormant for a long time in human liver and causes relapsing infection (Miller et al., 2002, Anstey et al., 2009).

Severe anaemia following malaria infection is associated with both infected and uninfected RBC destruction (ruptures and phagocytosis of RBCs) and reduced RBC production due to inflammatory effects (Menendez et al., 2000a, Lee et al., 1989). Although *P. falciparum* infections have the ability to invade a large number of RBCs, the risk of having severe anaemia is greater following *P. vivax* infections.
Repeated episodes of hemolysis resulting from *P. vivax* relapses could explain the higher risk of severe anaemia found in these patients (Price et al., 2007b, Collins et al., 2003). In addition, the number of uninfected erythrocyte destruction is much higher in *P. vivax* malaria (1 IRBC to 32 non infected RBC) (Collins et al., 2003) than those in *P. falciparum* infection (1 IRBC to 8 non infected RBC) (Jakeman et al., 1999, Price et al., 2001), a condition that can be associated with increased red cells fragility and higher cytokine mediated hemolysis found in *P. vivax* infection (Anstey et al., 2009). Untreated recurrent *P. vivax* infections is associated with higher TNF α production than in those of *P. falciparum* infections with similar parasite biomass (Karunaweera et al., 1992, Miller et al., 2002, Hemmer et al., 2006).

Organ dysfunction following malaria infection is associated with the impairment of tissue perfusion following infected RBCs (IRBC) sequestration in the small blood vessels, a condition that almost entirely attributed to *P. falciparum* infections (Miller et al., 2002). The parasite expresses PfEMP1² (*P. falciparum* erythrocytes membrane proteins-1) on the surface of RBC and this mechanism plays a central role in *P. falciparum* pathogenesis. PfEMP1 binds to various receptors in small blood vessels endothelium, notably Intracellular cell adhesion molecule-1 (ICAM-1) in the brain’s blood vessels and Chondroitin Sulphate A (CSA) in syncytiotrophoblast of the placenta, resulting in parasite sequestration and further circulation disruption (Miller et al., 2002). In the case of pregnant women, parasite sequestration in the placenta can impair maternal-fetal circulation and result in fetal wastage, low birth weight and stillbirth (Beeson et al., 2001).

*Plasmodium vivax* malaria has also been associated with severe manifestations. Severe anaemia, coma and respiratory insufficiency and liver dysfunction have all been described (Price et al., 2007b, Anstey et al., 2007, Tjitra et al., 2008, Genton et al., 2008, Poespoprodjo et al., 2009, Anstey et al., 2009). The pathogenesis of severe vivax malaria is less well described (Anstey et al., 2009). Recent in vitro findings show that *P. vivax* has the ability to induce partial sequestration via CSA and ICAM-1 receptor in the human lung and Saimiri brain endothelial cells (Carvalho et al.,

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² PfEMP1 is protein expressed by *Plasmodium falciparum* in the surface of RBC and is “encoded by the large and diverse var gene” (Miller et al, 2002)
A major pathogenic process in *P. vivax* is the formation of rosetting, which is binding of the uninfected erythrocytes (Miller *et al.*, 2002). Pathogenic mechanisms of other severe manifestations of *P. vivax* infections still warrant further studies (Anstey *et al.*, 2009).

### 1.1. Maternal malaria

Globally, 82.6 million live births born from pregnant women are at risk of *P. falciparum* and/or *P. vivax* infection, with 54.4 million of those occurring in the Asia-Pacific region where both species are coendemic (Dellicour *et al.*, 2010). Pregnant women with malaria are at higher risk of developing anaemia (Singh *et al.*, 1999, Shulman *et al.*, 1996, Nosten *et al.*, 1991, McGregor, 1984, Steketee *et al.*, 2001) and dying (Brabin *et al.*, 2001a). Low birth weight (including preterm delivery) is an important risk factor for infant mortality in malarious area (Guyatt and Snow, 2001, Luxemburger *et al.*, 2001, Steketee *et al.*, 2001). In areas of unstable transmission, prematurity is associated with increased mortality in the first three months of life independent of other systemic manifestations of infection (Luxemburger *et al.*, 2001).

Physiological suppression of cell-mediated immunity during pregnancy helps to prevent the immune response against the fetus, but has the adverse effect of increasing pregnant women’s susceptibility to infections, including malaria (Brabin, 1983, Guilbert, 2001). This susceptibility occurs even if some degree of immunity was acquired before pregnancy (Brabin, 1983).

Immunity develops with subsequent pregnancies and increasing gestational age. First time mothers living in high malaria endemic areas are at the greatest risk of malaria (McGregor, 1984). With each infection in subsequent pregnancies, the specific immunity against malaria develops (Maubert *et al.*, 1999). In lower malaria transmission area, primigravidae are more susceptible to malaria, but multigravidae are also affected (Nosten *et al.*, 1991, Poespoprodjo *et al.*, 2008). This suggests that the lower exposure to malaria infections can promote the development of immunity, but with less effective premunition. Immunity developing in the second trimesters of
pregnancy may result in a lower risk of malaria in later pregnancy (Bray and Anderson, 1979, McGregor, 1984).

The degree of severe manifestations of malaria in pregnant women is influenced by the level of malaria transmission and associated immunity. In highly endemic areas, severe anaemia is the main manifestation of severity (Singh et al., 1999, Shulman et al., 1996, Nosten et al., 1991, McGregor, 1984, Steketee et al., 2001) whereas in low malaria transmission settings, severe systemic manifestations such as pulmonary edema and cerebral malaria can occur (Menon, 1972, Wickramasuriya, 1937).

In all malaria endemic communities maternal malaria is associated with low birth weight\textsuperscript{3} (LBW) (Menendez et al., 2000b, McGregor, 1984, Brabin et al., 1990, Nosten et al., 1991, Singh et al., 1999, Steketee et al., 2001, Akum et al., 2005), whereas preterm delivery and perinatal deaths\textsuperscript{4} associated with placental malaria are predominantly restricted to areas where \textit{P.falciparum} is highly prevalent (Sullivan et al., 1999, Menendez et al., 2000b, Rogerson et al., 2003a, van Geertruyden et al., 2004). In areas of mixed endemicity where both \textit{P.falciparum} and \textit{P.vivax} are prevalent, the effect of malaria on both adverse outcomes are thought to be a consequence of the systemic effect of malaria infection, such as fever and anaemia (Menon, 1972, Poespoprodjo et al., 2008, Wickramasuriya, 1937, Luxemburger et al., 2001, Newman et al., 2003, Allen et al., 1998).

Information on adverse outcomes of \textit{P. falciparum} infection in pregnancy is plentiful whereas data on \textit{vivax} malaria is less well described, although is starting to accrue. Globally, about 92 million pregnant women live in \textit{P. vivax} endemic area with the resulting 59 million live births that at risk of the adverse outcome of maternal \textit{vivax} malaria (Dellicour et al., 2010). Dogma previously held \textit{P. vivax} to be a benign infection, but it is clear that \textit{vivax} malaria in pregnancy is associated with anaemia

\textsuperscript{3} Newborn is classified as low birth weight if the weight is \textless{} 2500 gram regardless of gestational age. Low birth weight can be resulted either from prematurity or intra uterine growth retardation (UNICEF-WHO, 2004).

\textsuperscript{4} Perinatal death is defined as death in the perinatal period. This period starts from fetal gestational age of 22 weeks (when the birth weight is 500 g) to seven days after birth. (WHO, 1996).
and low birth weight (Nosten et al., 1999, Poespoprodjo et al., 2008, Singh et al., 1999).

1.1.1. The effect of systemic infections

**Maternal deaths**

Malaria can cause maternal deaths either directly or indirectly. Cerebral malaria is the most common direct cause of maternal deaths in non-immune populations or during epidemics (Wickramasuriya, 1937, Menon, 1972, Nosten et al., 1991, Singh et al., 1999). On the other hand, severe anaemia is recognized as an indirect cause of fatal malaria in pregnancy in high malaria transmission areas. In Africa, 18% of maternal deaths in primigravidae are associated with severe malarial anaemia (Brabin et al., 2001a), furthermore pregnant women with severe anaemia are more likely to suffer the consequences of post partum hemorrhage (McDermott et al., 1996, Piper et al., 2001).

Coinfections of drug resistant *P. falciparum* malaria and HIV infections are associated with an increased risk of maternal deaths as indicated by the striking increase of maternal mortality rate in Africa between 1988 and 1997 (from less than 20/100,000 live births prior to the late 1980s to more than 50/100,000 live births thereafter)(Brabin and Verhoeff, 2002).

**Abortion and stillbirths**

The role of maternal malaria in causing stillbirth varies. In low to moderate endemic areas, abortion and stillbirth are associated with uterine contraction induced by systemic effect of acute malaria infections such as high fever (Wickramasuriya, 1937, Menon, 1972, Poespoprodjo et al., 2008). On the other hand, placental malaria is associated with a two fold risk of fetal death in some highly malaria endemic areas (van Geertruyden et al., 2004) but not in others (McGregor et al., 1983, Newman et al., 2003).
1.1.2. The effect of parasitization

Low Birth Weight

Maternal malaria is associated with low birth weight, particularly in primigravidae (McGregor, 1984, Nosten et al., 1991, Brabin and Piper, 1997, Rogerson et al., 2003b, Akum et al., 2005). However in lower malaria transmission areas malaria also reduces birth weight in the second and third pregnancies (Nosten et al., 1991). The pathogenesis of low birth weight is attributed to placental insufficiency. This results from mechanical obstruction of maternal to fetal circulation by the binding of PfEMP-1 to Chondroitin Sulphate A (CSA) in the syncytiotrophoblast of the placenta (Fried, 2001). Massive parasite sequestration may lead to less oxygen and nutritional transport to the fetus (Brabin et al., 2004b).

Women with placental *P. falciparum* parasitaemia are at higher risk of having low birth weight babies (Sullivan et al., 1999, McGregor et al., 1983). In addition placental histological changes (monocytes pigmentations, massive monocytes intervillous infiltration (MMI) may also contribute to LBW (Rogerson et al., 2003b, Menendez et al., 2000b).

Apart from local placental changes, placental inflammatory responses have an effect on birth weight reduction. TNF (tumor necrosis factor) α, IFN (interferon) γ and IL (interleukin)-8 – the type 1 helper T cells pathways – are known to induce vasodilatation and the subsequent alteration of utero-placental haemodynamics may lead to maternal reduction in perfusion of fetal blood (Brabin et al., 2004b).

Primigravidae delivering low birth weight babies have significantly higher TNF α and IFN γ compared to those women having normal birth weight babies (Kabyemela et al., 2008, Fried et al., 1998). TNF α and IL-8 have also been shown to be associated with intra uterine growth retardation (Moormann et al., 1999). In addition to the effect of the maternal inflammatory response, reduced fetal feritin is also associated with low birth weight. (Kabyemela et al., 2008).

The effect of placental malaria on low birth weight is less apparent in malaria endemic areas where both *P. falciparum* and *P. vivax* are prevalent. In Papua New Guinea (PNG), maternal parity, rather than placental malaria was associated with
low birth weight (Desowitz and Alpers, 1992). On the other hand, on the Thai Burmese Border, following early detection and prompt treatment apparent birth weight reduction was found despite the absence of placental structural changes. (McGready et al., 2004). In Papua (Indonesia) and central India, peripheral parasitaemia of any parasites at delivery is associated with LBW (Poespoprodjo et al., 2008, Singh et al., 1999), suggesting that malaria attributed low birth weight in such area is likely to result from other mechanisms.

Maternal anaemia is likely a major cause of low birth weight, particularly when compounding other causes of anaemia such as nutritional deficiency, worm infestation and other chronic infections (Whitty et al., 2005). Indeed past and chronic placental malaria together with severe anaemia increase the risk of low birth weight in all gravidities (Shulman et al., 2001). However another study in a mesoendemic areas demonstrated that anaemia was not associated with low birth weight after controlling for the other effects of malaria (Nosten et al., 1991).

Both *P. falciparum* and *P. vivax* infection of the mother reduce birth weight but the reduction is higher with *P. falciparum* (Nosten et al., 1991, Poespoprodjo et al., 2008). In highly endemic areas, *P. falciparum* reduces birth weight by up to 168 g (Brabin, 1983) and 150-190 g in mesoendemic areaa whereas *P. vivax* malaria reduced birth weight by 107-108 g (Poespoprodjo et al., 2008, Nosten et al., 1999). Parasite sequestration and inflammatory response in the placenta has been hypothesized to contribute to the reduction of birth weight after *P. falciparum* infection (Brabin et al., 2004b, Rogerson et al., 2003b, Menendez et al., 2000b). In contrast the mechanism of birth weight reduction in *P. vivax* infection is not clearly understood, although placental inflammatory response may play a role (Nosten et al., 1991). In highly malaria endemic areas, malaria in the second trimester and the frequency of *P. falciparum* infections correlated with a greater risk of low birth weight (Kalilani et al., 2010). However the comparable reduction in mean birth weight between hyper endemic Africa and areas of low endemicity suggests that the impact of malaria on birth weight is not simply related to the frequency of infection.

Malaria in pregnancy contributes to a significant amount of infant mortality through low birth weight. In Africa, the risk of dying before the age of one year is three times
higher among babies with low birth weight compared to those with normal weights and malaria in pregnancy accounts for almost 6% of all causes of infant mortality or ~3700 infant deaths per year (Guyatt and Snow, 2001). Perinatal mortality in malarious areas tends to be significantly higher than in a non malarious area even after controlling for socioeconomic factors (van Geertruyden et al., 2004). In a follow up study carried out in low endemic area, low birth weight attributable to malaria (both preterm and full term) was associated with neonatal mortality and prematurity alone led to a rise in infant mortality in the first three months of life (Luxemburger et al., 2001).

**Preterm delivery**

The actual burden of preterm delivery in resource poor settings could be underestimated (UNICEF and WHO, 2004). Prenatal method of assessing gestational age (date of last menstrual period and fundal height) is sometime inaccurate whereas ultrasound examination is not always available in such setting. In addition, post-natal gestational age assessment by physical examination requires a certain skill and its use is mostly restricted to hospital (Verhoeff et al., 1997).

Acute placental malaria is associated with preterm delivery in highly malaria endemic areas in Africa, (Sullivan et al., 1999, Menendez et al., 2000b) whereas in lower malaria endemic regions, prematurity has a greater association with the systemic effects of malaria such as fever or anaemia (Wickramasuriya, 1937, Allen et al., 1998, Poespoprodjo et al., 2008).

In a malarious area in southern Papua, severe anaemia and primigravidae are significant risk factors for preterm delivery and the effect appears to interact with malaria (Poespoprodjo et al., 2008). Similar findings are described in malaria endemic regions of PNG where reduced haemoglobin and primigravidae were associated with preterm delivery (Allen et al., 1998).

**Maternal anaemia**

In highly endemic malaria regions in Sub Saharan Africa, where *P. falciparum* is the predominant parasite, first time mothers are at significantly greater risk of developing severe anaemia (McGregor, 1984, Shulman et al., 1996). In Africa,
mortality due to malaria associated severe anaemia was significant among primigravidae, with almost a fifth of maternal deaths in primigravidae attributed to malarial severe anaemia (Brabin et al., 2001a).

On the other hand, except in PNG (Brabin and Piper, 1997), multigravidae women living in regions where both *P.falciparum* and *P.vivax* are coendemic are more likely to have anaemia than primigravidae (Poespoprodjo et al., 2008, Nosten et al., 1991, Singh et al., 1999). Apart from the varying premunition status of pregnant women in such area, the interaction of malarial anaemia and iron deficiency anaemia in multigravidae in this region should be considered, since the intensity of iron deficiency anaemia increases with the number of parity (Isah et al., 1985, Brabin et al., 1990, Shulman et al., 1996).

The prevalence of moderate to severe anaemia in malaria positive pregnant women living in high malaria transmission areas ranges from 1-20% (Steketee et al., 2001), lower than the 35% described in pregnant women living in low to moderate malaria transmission areas (Nosten et al., 1991). Although this may reflect the lower malaria premunition (or other common causes of anaemia) among pregnant women in this area, the risk of anaemia among infected pregnant women is almost double that of non infected women in all endemic regions.

Anaemia is common in pregnant women with *P. vivax* malaria (Singh et al., 1999, Poespoprodjo et al., 2008, Nosten et al., 1999). Although *P. vivax* only infects young red cells (reticulocytes), the degree of anaemia is exacerbated by *P. vivax* relapses and repeated infections (Nosten et al., 1991, Mendis et al., 2001, Price et al., 2007b, Collins et al., 2003).

**Congenital malaria**

In order to exclude infection from mosquito bites, congenital malaria is defined as any parasite found in the peripheral blood in the first week of life (Adachi et al., 2000), although this definition is likely to exclude late presentations of occult infection. Mother to child malaria transmission is mostly thought to occur during delivery process as a result of placental barrier disruption (Brabin et al., 2004b). However, evidence on possible antenatal *P. falciparum* malaria transmission is
Cumulating (Malhotra et al., 2006, Tobian et al., 2000, Xi et al., 2003, Malhotra et al., 2009).

Congenital *falciparum* malaria was thought to be rare in malarious area (McGregor, 1984), however studies from Nigeria and Zambia described prevalences as high as 47% and 29% respectively (Larkin and Thuma, 1991, Obiajunwa et al., 2005). Lower congenital malaria prevalence has been reported in endemic areas where both *P. falciparum* and *P. vivax* prevalent. Congenital *P. vivax* malaria has been described in several case reports (Valecha et al., 2007, Vottier et al., 2008, Singh et al., 2003, De Silva et al., 1982, Pappas, 1984) and is not uncommon. In Thailand, 76% (28/37) of congenital malaria cases reported between 1981 and 1999 were due to *P. vivax* infections (Pengsaa, 2007) whereas in Timika this figure was only 17% (6/35) (Poespoprodjo et al., 2009). This may be a reflection of the overall proportion of *P. vivax* infections in the population, which is about 55% in Thailand and 35% in Timika (Karyana et al., 2008, Luxemburger et al., 1996).

Malaria infection in the newborn often appears without symptoms and diagnosis may be delayed to 2-20 weeks of age when fever, anaemia or feeding problems manifest (WHO, 2000a). This is likely due to the low parasite density, innate immunity and maternal immunity (Kitua et al., 1996, Wagner et al., 1998). In utero exposure to malarial antigens can result in immuno-tolerance in the foetus (Malhotra et al., 2009). This suggests a greater risk of having malaria infections in infants born to placental malaria positive mothers (Mutabingwa et al., 2005), emphasizing the need for malaria control programs to address congenital or neonatal malaria (Brabin, 2007, Menendez and Mayor, 2007).

### 1.1.3. HIV infections and malaria in pregnancy

The immune suppression resulting from HIV infection has been known to have an impact on the susceptibility to other common infections in developing countries, including malaria. HIV-1 positive pregnant women from Sub-Saharan Africa are more likely to be parasitaemic compared to the HIV negative women – both during pregnancy and delivery (ter Kuile et al., 2004). Similarly, the risk of having placental malaria is 1.7 fold higher in HIV positive women compared to uninfected women. In
addition, placental malaria is higher among HIV positive multiparous women, suggesting impaired development of immunity in subsequent pregnancies (Steketee et al., 1996a).

HIV infected pregnant women with malaria are more likely to be anaemic (Steketee et al., 2001) and have a four fold increased risk of having stillbirth babies and preterm delivery compared to HIV negative mothers with malaria (Ticconi et al., 2003). Neonatal deaths are also significantly higher among babies born from HIV positive mothers with placental malaria and have a 4.5 fold risk of dying than those from mothers with placental malaria only (Bloland et al., 1995).

On the other hand, malaria has also been hypothesized to trigger viraemia in HIV infected patients. The TNF $\alpha$ production in malaria is thought to increase HIV replication and further disease progression (Rowland-Jones and Lohman, 2002). This mechanism partly explains the significant association of the increased mother-to-child HIV transmission risk with high malarial parasites density in the peripheral blood (Ayisi et al., 2004).

### 1.1.4 Helminth infections and malaria in pregnancy

Coinfection between malaria and intestinal helminths is well recognized in malaria endemic regions (de Silva et al., 2003, Snow et al., 2005, Petney and Andrews, 1998). Helminth infection is associated with T-helper 2 cytokine response that may alter immune responses to malaria infection (Geiger et al., 2002, Cooper et al., 2000). Nonetheless, the effect of helminth infection to malaria susceptibility is still inconclusive (Mwangi et al., 2006). Some studies show that worm infestation protects from clinical and severe malaria (Nacher et al., 2001, Nacher et al., 2002a) whereas others state otherwise (Le Hesran et al., 2004, Spiegel et al., 2003, Nacher et al., 2002b).

*P. falciparum* and intestinal helminth coinfection is also common in pregnancy (Yatich et al., 2009, van Eijk et al., 2009). Any helminthiasis in pregnant women is associated with an increased risk of having *falciparum malaria* (Yatich et al., 2009). On the other hand, the presence of *Ascaris lumbricoides* in pregnant women is
protective to *falciparum* malaria infection particularly in the 2\textsuperscript{nd} and 3\textsuperscript{rd} pregnancy (van Eijk et al., 2009). Less is known on the interaction between *P. vivax* malaria and worm infestation. On The Thai Burmese border, *Ascaris lumbricoides* infestation in pregnancy reduced the risk of having *vivax* malaria (Boel et al., 2010). School children with *P. vivax* and helminth infection are protected from anaemia (Melo et al., 2010).

Both malaria and helminth infection in pregnancy are associated with anaemia (Ndyomugyenyi et al., 2008, Brooker et al., 2008) and adverse pregnancy outcomes (Christian et al., 2004, de Silva et al., 1999, Desai et al., 2007). Pregnant women coinfected with both parasites have the lowest mean haemoglobin level (Brooker et al., 2007) and moderately higher rate of stillbirths (Yatich et al., 2010) compared with those having single infection. In view of this, the inclusion of deworming tablets to the antenatal care package would help improve pregnancy outcomes (Brooker et al., 2008).

### 1.2. Malaria in infancy

Malaria in pregnancy has adverse effects on the health of the infant (Brabin, 2007, Menendez and Mayor, 2007), from both exposure in utero and peripheral parasitaemia which can begin to manifest soon after birth. Congenital malaria is common in all malaria endemic regions and infants are also affected from severe symptoms. In this section the effect of malaria on infant health is discussed according to the level of malaria endemicity and the species of infection.

#### 1.2.1. High malaria transmission areas where *P. falciparum* infections predominate

In these regions, infants up to 4-6 months old are relatively protected from clinical malaria, particularly in the neonatal period (Snow et al., 1998, Kitua et al., 1996, Wagner et al., 1998). Interactions between innate immune mechanism and transfer of a degree of maternal immunity combine to reduce the risk of having malaria in the first year of life (Kitua et al., 1996, Riley et al., 2001).
Malaria in early life is usually associated with a low parasite density and may even be cleared within 4 weeks by the infant’s immune system (Wagner et al., 1998, Franks et al., 2001). Maternal IgG3 antibody to *P. falciparum* merozoit surface protein 2 (MSP2) may limit parasite growth and prevent clinical malaria in infants, but not prevent malaria infections entirely (Taylor et al., 1998, al-Yaman et al., 1994, Wagner et al., 1998, Riley et al., 2000). However this is not always the case since infants can remain asymptomatic in the absence of maternal antibody (Riley et al., 2000, Riley et al., 2001).

Innate immunity reduces the risk of malaria in early life. Foetal haemoglobin (HbF) is resistant to *P. falciparum* growth and persists in infant’s blood for several weeks after delivery (Pasvol et al., 1976). Parasite growth is halted by a lack of p-amino benzoic acid (pABA) in breast milk that is essential for parasite growth (Riley et al., 2001). In addition, cytokine transforming growth factors (TGF β) found in breast milk are associated with parasites clearance by upregulating FC-γR expressions on macrophages (Saito et al., 1993, Omer and Riley, 1998). Other forms of innate protections have also been proposed, such as sporozoite resistant neonatal liver cells and different glycosylation of ABO antigens and band 3 in neonates that inhibit erythrocyte invasion and cytoadherence (Riley et al., 2001).

Despite greater levels of host immunity in African highly endemic areas, malaria remains as an important cause of illness in young infants. The prevalence of infection can reach 50% and are associated with severe complications such as anaemia and respiratory distress (Ibhanesebhor, 1995, Afolabi et al., 2001, Kitua et al., 1996, Slutsker et al., 1996). The risk of malaria starts in the first week of life (Larkin and Thuma, 1991, Obiajunwa et al., 2005, Okafor et al., 2006), with up to 30% of newborns having persistent asymptomatic peripheral *P. falciparum* parasitaemia requiring treatment (Okafor et al., 2006). Untreated infants often present 2-20 weeks after birth with fever, anaemia or feeding problems (WHO, 2000a).

The impact of maternal falciparum malaria on the infant’s health goes well beyond the neonatal period (Le Hesran et al., 1997). Recent findings suggest that foetal exposure to maternal malaria antigens induces immuno-tolerance to malaria
infecteds in the first months of life (Malhotra et al., 2009). Infants born from placental malaria positive multigravidae women were more susceptible to malaria in the first six months of life compared with those of placental malaria negative women (Mutabingwa et al., 2005), the risk of clinical malaria almost doubling in the first 30 months of life (Schwarz et al., 2008).

1.2.2. Moderate malaria transmission areas with heterogenous plasmodium species
Less is known about infant malaria in regions where *P. falciparum* and *P. vivax* are both prevalent. Congenital malaria with both parasites has been reported in Papua New Guinea (PNG) (Lehner and Andrews, 1988), Thailand (Pengsaa, 2007) and Timika (Poespoprodjo et al., 2008). In these regions, *P. vivax* is the predominant malaria parasite in infants and young children. In PNG and Timika (Papua-Indonesia), *P. vivax* infections were more prevalent in young children aged 1-4 years old than in older children (Smith et al., 2001, Genton et al., 1995, Karyana et al., 2008). In Timika malaria was present in 31% of hospitalized infants younger than 1 year old with *P. vivax* accounting for more than half of the cases of malaria and severe symptoms commonly reported with both *P. falciparum* and *P. vivax* infections (Poespoprodjo et al., 2009).

*Plasmodium falciparum* prevalence peaks later in older children and adults (Genton et al., 1995, Karyana et al., 2008), consistent with immunity to *P. vivax* infections developing earlier than that against *P. falciparum* (Luxemburger et al., 1996, Genton et al., 1995, Karyana et al., 2008). Nonetheless, little is known on passive (maternal antibody) or active immune mechanisms in young infants with *P. vivax* infections or indeed the interactions of host immunity and mixed species infection (Pengsaa, 2007, Brabin, 2007).

The pathogenesis of *P. vivax* infection starts with the invasion of merozoite Duffy binding protein (DBP) to human erythrocytes with Duffy antigen/receptors for chemokines (DARC) (Barnwell and Galinski, 1995, Chitnis, 2001). Clinical studies suggest that young children are more susceptible to *P. vivax* malaria. Immunity acquired to *P. vivax* DBPII increases with age and provides some degree of
protection to \textit{P. vivax} infections by enhancing parasite clearance and/or limiting parasite growth (Cole-Tobian et al., 2002). Furthermore, cellular immune responses to \textit{P. vivax} malaria improve with age, as shown by an increasing level of IFN \(\gamma\) and IL-10 in older children and adults (Xainli et al., 2002).

Why \textit{P. vivax} is more prevalent in young children is not clearly understood. It is speculated that \textit{P. vivax} benefits from the high availability of young red blood cells (reticulocytes) in infancy, providing the main target of \textit{P. vivax} invasion. The competition between Plasmodium species will select the more transmissible parasites, which in this case is \textit{P. vivax} infection (Maitland et al., 1997). The higher risk of \textit{P. vivax} infections in these infants to some extent would provide protection benefit to the adverse complications of \textit{P. falciparum} malaria (Smith et al., 2001).

\section*{1.2.3. Symptoms and diagnosis}
Infants develop non-specific symptoms to malaria which are similar with those of other systemic infections. Fever, feeding problems, respiratory distress, pallor, jaundice and diarrhea are frequently found in infants with malaria (Ibhanesebhor, 1995, Poespoprodjo et al., 2009). Hepatomegaly and splenomegaly are also common in young infants with malaria (Ibhanesebhor, 1995, Afolabi et al., 2001). Therefore in malaria endemic areas, malaria should be included as a differential diagnosis in all symptomatic infants.

Severe anaemia and respiratory insufficiency are the two most common severe signs in infants with \textit{P. falciparum} malaria (Ibhanesebhor, 1995, Afolabi et al., 2001, Kitua et al., 1996, Slutsker et al., 1996) and also present in those with \textit{P. vivax} infections (Poespoprodjo et al., 2009, Genton et al., 2008). In coendemic areas severe anaemia was more prevalent in infants with \textit{P. vivax} malaria than in \textit{P. falciparum} and this was apparent in infants as young as 3 months old (Poespoprodjo et al., 2009). The relapsing nature of \textit{P. vivax} infections could explain the higher risk of severe anaemia found in these infants (Collins et al., 2003, Price et al., 2009). In addition, the relatively low number of red blood cells in young infants compared to older children might explain the higher risk of having anaemia in this age group (Dipchand et al., 1997).
Respiratory distress was more common in young children living in Papua New Guinea with severe *P. vivax* malaria than in those with *P. falciparum* infections, although the definitions of respiratory distress were considerably broader that that applied in other studies (Genton et al., 2008). The cause of respiratory distress is unknown and may reflect both an inflammatory response and sequestration by *P. vivax* infected erythrocytes in pulmonary microvasculature (Anstey et al., 2007).

### 2. Malaria Control in Pregnant Women and Infants

Providing treatment and intermittent prophylaxis therapy (IPT) with an effective antimalarials drug, together with insecticide treated nets (ITN) are currently the three main strategies in averting the burden of malaria in pregnancy and infants in moderate to high malaria endemic settings (WHO/AFRO, 2004, Muller et al., 2006, Aponte et al., 2009). The majority of evidence for malaria prevention programs in pregnant women and infants comes from combating *P. falciparum* malaria. The significance of *P. vivax* malaria in this vulnerable group has only recently been appreciated and therefore, the effectiveness of intermittent preventive treatment (IPT) or insecticide treated nets (ITN) for *P. vivax* prevention program has yet to be determined. Indeed, the different pathogenesis of this infection is likely to require a modified approach.

Although antimalarial drug efficacy trials have been conducted in *P. vivax* infections, pregnant women and young infants are usually excluded from these trials due to safety and ethical issues. As a result, present antimalarial drugs recommended by WHO for this particular group are based on limited evidences and expert opinion. Furthermore dosing regimens are often based on non pregnant adults and yet the pharmacokinetics of many drugs may vary considerably in pregnant women (Nosten et al., 2007, McGready et al., 2006b, McGready et al., 2006a, Karunajeewa et al., 2010, Lee et al., 2008). The need for more clinical and pharmacokinetics studies in pregnant women is paramount (Nosten et al., 2007). In the next sections, the main malaria control strategies are briefly discussed.
2.1. Early diagnosis and prompt treatment

Pregnant women and infants are vulnerable to the adverse effect of malaria. Early diagnosis and prompt treatment with an effective antimalarial drug would help to reduce any adverse outcomes (WHO, 2006a, Nosten et al., 2007).

Early Diagnosis

Microscopy is mostly used for malaria screening, but this requires skilled technician, therefore rapid diagnostic tests (RDT) has been used as an alternative method in resource constrained settings (WHO, 2010). However, using RDT for malaria screening in pregnancy should be interpreted with caution since in malaria endemic areas, *P. falciparum* can be sequestered wholly in the placenta without any peripheral parasitaemia (Mockenhaupt et al., 2002, Rogerson et al., 2003a). HRP-2 antigen detection test in peripheral blood is more sensitive in detecting placental malaria (80-89%) (Mockenhaupt et al., 2002, Leke et al., 1999) than parasite Lactate dehydrogenase (pLDH) antigen detection test (38% sensitivity) (Mankhambo et al., 2002).

In addition, Histidine Rich Protein 2 (HRP-2) antigen detection test has limitations in detecting low *P. falciparum* parasitaemia (<100 parasites/µL) and the sensitivity of this test as a screening tool is 88% (Ochola et al., 2006). The sensitivity of RDT (combined antigen detection) in detecting non-falciparum infection is considerably lower (sensitivity of 50-52%) (Ochola et al., 2006). The recent FIND report the sensitivity of RDTs for *P. vivax* has been improved, but remains lower than that for *P. falciparum*. A major issue continues to be the wide variability in the quality and reproducibility of the tests (WHO, 2008a).

Malaria treatment

Older antimalarial drugs such as CQ and SP have high failure rates (> 10%) in treating *P. falciparum* in most malarious area in Africa and Asia-Pacific regions (WHO, 2006a, Adam et al., 2004, Collignon, 1991, Frayaff et al., 1997, Frayaff et al., 1998, Garavelli and Corti, 1992, Molta et al., 2003, Ratcliff et al., 2007b, Ronn et al., 1996, Checchi et al., 2002, Wongsrichanalai et al., 2002). Similar drug resistance is emerging in the Asia-Pacific region for *P. vivax* malaria (Douglas et al., 2010, Baird et al., 2007). Although the level of resistance to quinine is lower, adherence to
an unsupervised 7-day course (3 times a day) is a major issue that leads to poor effectiveness (Ratcliff et al., 2007b). In addition, quinine is poorly tolerated with a bitter taste and tinnitus further reducing its adherence (Yeka et al., 2009).

In view of the limitations of these regimens, the WHO recommends the use of ACT as the first line treatment for *P. falciparum* malaria and CQ resistant *P. vivax* malaria, including pregnant women in the second and third trimesters of pregnancy and infants weighing >=5 kgs (WHO, 2006a). However pregnant women and young infants are usually excluded from antimalarial treatment trials and therefore the knowledge of efficacy, safety, pharmacokientics and toxicity information on ACT use in this vulnerable group is limited (Dellicour et al., 2008, Dellicour et al., 2007). Continuing efforts are needed to improve these knowledge gaps and optimize their use (Ward et al., 2007, Greenwood et al., 2007, WHO, 2003). Artemisinin combination therapy and its use in pregnant women and young children are further discussed in section 3 and 4 of this chapter.

### 2.2. Insecticide Treated Nets (ITN)

**Pregnant women**

Gamble *et al.* (2006) reviewed the use of ITNs in Africa and found that they were effective in reducing placental malaria and peripheral parasitaemia prevalence at the time of delivery in all pregnancies when compared with no nets, but did not significantly reduce anaemia or severe anaemia. In contrast in Asia a study by Dolan *et al.* 1993 showed that ITNs, when compared with untreated nets, reduced anaemia prevalence but had no effect on parasitaemia reduction (Gamble et al., 2006, Dolan et al., 1993). Data is still lacking on the efficacy of ITNs in Asia, where the malaria transmission was generally low and *Plasmodium vivax* accounts for half of infections. In PNG, ITN use reduces *falciparum* malaria but not *P. vivax* infections prevalence among children aged 0-4 years (Graves et al., 1987). This could be associated with the tendency of *P. vivax* infected mosquito to bite during daytime when people are not protected by ITN (Bockarie and Dagoro, 2006, Douglas et al., 2010).
A review of ITN use in Africa highlights an association with a lower risk of low birth weight, stillbirths and abortion in the 1st to 4th pregnancies only (Gamble et al., 2006, ter Kuile et al., 2003b), while in Asia ITN use has no apparent effect to pregnancy outcomes (Dolan et al., 1993).

Despite the benefits of ITN in preventing malaria in pregnant women, there is a major issue with the net coverage and usage. In The Gambia ITN usage was only taken up by half of all primigravidae (D'Alessandro et al., 1996) and in Kenya the community usage was only 10% (Njagi et al., 2003). Recent study in Sub Saharan Africa has demonstrated the low coverage of ITN in pregnant women (van Eijk et al., 2011). Although another study in Kenya showed higher usage (85% of all pregnant women), the self reporting methods used in this study were less reliable and the exact number could have been significantly lower (ter Kuile et al., 2003b). In general individual knowledge on malaria appears to improve ITN usage (Nganda et al., 2004).

**Infants**

ITN use is associated with a reduction of malaria morbidity and mortality in infants as well as young children (Lengeler, 2000, Lindblade et al., 2004). The use of ITN in the first few days of life is not associated with an increase risk of malaria susceptibility and mortality in either late infancy or childhood (Muller et al., 2006, Lindblade et al., 2004). Lower maternal IgG antibody levels found in the cord blood of infants using ITN than in those without an ITN, did not correlate with a higher risk of poor health outcomes (Kariuki et al., 2003). In the first 24 months of life ITN use also resulted with fewer clinical malaria episodes, delayed the first malaria infection and was associated with better growth (ter Kuile et al., 2003a).

**2.3. Intermittent Preventive Treatment (IPT)**

Intermittent preventive treatment is defined as giving a curative treatment dose of an effective antimalarial drug irrespective of malaria diagnosis at a certain predefined intervals (White, 2005).
Pregnant women (IPTp and chemoprophylaxis)

Malaria prevention in pregnant women is a crucial component of any malaria control program. On the Thai Burmese Border, weekly malaria prophylaxis using mefloquine started from 20th weeks of pregnancy is effective in preventing *P. falciparum* and *P. vivax* malaria (Nosten et al., 1994). Study in Kenya showed that both intermittent preventive treatment (IPT) and monthly prophylaxis using SP were effective in reducing placental malaria (Parise et al., 1998). Currently, The World Health Organization (WHO) recommends one dose of SP in the second trimester followed by one second dose early in the third trimester to women of all parities (WHO, 2000b).

In the last 10 years, IPTp with SP has been implemented and studied in several countries as part of malaria control programs in pregnancy. Some studies showed the effectiveness of IPTp in reducing the risk of placental malaria and/or low birth weight (Parise et al., 1998, Schultz et al., 1994, Shulman et al., 1999, Rogerson et al., 2000, van Eijk et al., 2004, Garner and Gulmezoglu, 2003). However, the role of IPTp remains contentious and the emergence of parasites resistant to SP is likely to undermine the effectiveness of this approach (ter Kuile et al., 2007). A replacement antimalarial drug for IPT and optimal dosing strategy must therefore be identified urgently (White, 2005).

Another important confounder in the effectiveness of IPTp is the high prevalence of HIV infections which makes pregnant women more susceptible to malaria and reduces the effect of antimalarial drug used for IPTp (Stekete et al., 1996a, Filler et al., 2006). In addition, the current dosing interval of SP might not be optimum for malaria prevention since the half life of SP in the 2nd trimester of pregnancy is halved than those of non pregnant individuals (Green et al., 2007).

Infants (IPTi)

Preventive treatment strategies in infants are still hotly debated (Greenwood, 2004). The majority of preventive treatment starts at 3-4 months old of age when malaria risk is high (Chandramohan et al., 2007).
Currently, two interval methods are applied in the fields. Those are intermittent (e.g. at 3, 4 and 9 months old) and monthly (for 3 consecutive months) preventive treatment (Schellenberg et al., 2001, Cisse et al., 2006). In a resource constrained setting, 47-76% coverage of IPTi with SP attached to immunization program at 2, 3 and 9 months results in less malaria (31% vs 38% in comparison group) and anaemia (Hb<11 g/dl: 80% vs 88% in comparison group) in infants compared to those only receiving immunization (Armstrong Schellenberg et al., 2010). The maximum protective effect of IPT from malaria and anaemia in infants was achieved during 4-6 weeks following each IPT dose (Cairns et al., 2008), suggesting that prevention treatment strategies should be tailored according to the timing of infections and level of malaria transmission (Kobbe et al., 2007, White, 2005).

Preventive treatment strategies in infants have not been shown to increase the risk of morbidity and mortality from malaria in older children. IPTi appears not to interfere with the development of natural immunity to malaria in this age group. Although the level of IgG antibody to *P. falciparum* malaria is lower in infants receiving IPT, this was not associated with an increased risk of malaria in the following months (Schreiber et al., 2007). Malaria morbidity remained lower in the first two years of life in infants receiving IPT with SP in early lives compared with those in the placebo group (Protective effect of 36% [95%CI 11-53] (Schellenberg et al., 2005). Furthermore, giving IPTi together with immunization was not associated with lower IgG/IgG1 antibody activities against *P. falciparum* antigen (MSP1, AMA1 and EBA 175) (Quelhas et al., 2008, Otoo et al., 1988).

Until a suitable antimalarial drug for prevention is identified, the use of SP for IPTi remains the WHO’s choice for IPTi, even in areas where SP resistance is increasingly prevalent. A review on IPTi with SP in Africa showed acceptable results with good protective efficacy against clinical malaria, anaemia, hospital admission with parasitaemia and all cause of hospital admissions, that ranged from 20-40% (Aponte et al., 2009). However, in areas with high levels of SP resistant parasites, the use of this drug was not effective in reducing clinical malaria in the first year of life (Gosling et al., 2009). Therefore, a replacement long acting effective antimalarial drug is urgently required.
A major issue constraining the impact of IPTi and IPTp on malaria in mothers and babies arises from the proportion of these people who routinely attend for antenatal care or vaccination in early and thus will be successfully targeted by these interventions. These issues are particularly relevant in Papua where less than half of a target population have ready access to healthcare support.

3. Artemisinin Combination Therapy
Artemisinin combination therapy (ACT) combines the rapid parasite clearance of artemisinin or its derivatives with a long acting partner drug that kills the remaining peripheral parasites (WHO, 2010). Three-day course of ACT provides the most effective treatment duration for parasite clearance that not only provides high cure rate but also prevents the development of resistance by significantly reducing the number of parasite exposed to each drug (White, 2004). In addition, this regimen will also reduce malaria transmission by reducing gametocytes carriage (Price et al., 1996).

In order to ensure effective treatment, the choice of ACT must take into account the adherence to a complete course of treatment and the efficacy of each component of the combination. Adherence has huge implications for effectiveness, related to a number of factors including the duration of treatment, complexity of dosing regimen, drug tolerability profile and the level of information imparted to the patient. Fixed dose combination improve adherence to a complete course of treatment, and also reduce the chance that drugs will be used separately (WHO, 2010). Currently, there are 5 WHO recommended ACTs: Artemeter-lumefantrine, Artesunate-amodiaquine, Artesunate-mefloquine, Artesunate-Sulfadoxine pyrimethamine and Dihydroartemisinin-piperaquine (WHO, 2010). This thesis will focus on reviewing Dihydroartemisinin piperaquine.

3.1. Artemisinin And Its Derivatives
Artemisinin (qinghaosu), an endoperoxide sesquiterpene lactone, is characterized by 1,2,4 trioxane ring which is essential for antimalarial activity (Webster and Lehnert, 1994). The derivatives artesunate and artemether have more rapid parasite clearance activity than the parent drug (Webster and Lehnert, 1994). Both artemisinin and its
derivatives are rapidly metabolized to dihydroartemisinin (DHA) its active metabolite. Although the exact mechanism of action remains unclear current opinion suggests that the endoperoxide-bridge in artemisinin selectively interacts with iron in intraparasitic heme resulting in toxic free radicals productions that kill malaria parasites (Meshnick, 2002, Creek et al., 2009).

Artemisinin is effective against asexual parasite stage (early trophozoit, late rings and schizont) and also against stage I-IV gametocytes (Meshnick, 2002, Dutta et al., 1989, Kumar and Zheng, 1990). In China, dihydroartemisinin given 60 mg once daily for 7 days (first dose: 120 mg) was associated with a low parasite recrudescence rate (2%) (Li et al., 1994). Clinical trials show that the artemisinins are associated with rapid parasite clearance and reduction of immature gametocytes reduction (Adjuik et al., 2004, Price et al., 1996). Both of these mechanisms contribute to the reduction of malaria transmission.

Neurotoxicity

Neurotoxicity has been demonstrated in dogs and rats injected intramuscularly with high cumulative doses of the lipophilic derivatives arteether and artemether (Brewer et al., 1994). The effect is associated with brain stem (reticular formation, the vestibular system nuclei and the trapezoid nucleus) function disturbance. In animal studies this results in gait disturbance, loss of spinal, brain stem and pain responses, cardiorespiratory depression and death (Brewer et al., 1994, Kamchonwongpaisan et al., 1997). Neurotoxicity is related to prolonged central nervous system exposures to high doses of artemisinin derivatives rather than to intermittent short exposures. This makes toxicity in human is very unlikely, as also further demonstrated in clinical studies (Price et al., 1999, Gordi and Lepist, 2004). Artemether lumefantrine exposure is not associated with hearing loss and brainstem toxicity as measured by auditory evoked potentials (Kissinger et al., 2000, van Vugt et al., 2000). Neurotoxicity was not found in patients with severe malaria treated with artemether or artesunate (Davis et al., 1997, Hien et al., 2003). Artemisinin regimens exposure in pregnancy is not associated with neurotoxicity (Dellicour et al., 2007).
Reproductive toxicity

Early observations in animal models have raised concerns of potential fetal toxicity. Rats and rabbits, given artesunate compounds or dihydroartemisinin (DHA) in early pregnancy resulted in fetal wastage, cardiovascular and bone formation defects (Greenwood et al., 2007, WHO, 2003).

The mechanism is postulated to be via artesunate inducing embryonic erythroblast destruction in early foetal development leading to fetal hypoxia, malformations and foetal deaths (White et al., 2006). Unknown actual drug concentration that affects the embryo in human makes it difficult to directly conclude the animal study findings to human. However, since reduction of reticulocyte counts (young nucleated red cells) has been found in healthy adults exposed to oral artesunate and dihydroartemisinin (Kongpatanakul et al., 2009), it is possible that the drug may also affect embryonic erythroblasts formation in early pregnancy and that this can lead to fetal loss and malformation (Clark, 2009).

Antimalarials exposure in the second and third trimesters of pregnancy are less likely to be teratogenic compared to drug exposure in the first trimester. Predicted from animal studies, the most sensitive period of artesunate embryotoxicity in humans is between 4th to 10th weeks of pregnancy (White and Clark, 2008, Clark et al., 2008). In the absence of maternal toxicity, artesunate exposures in rats, rabbits and monkeys in early pregnancy were more likely to cause foetal deaths than congenital malformation, although foetal deaths can also result from severe malformation (Clark et al., 2004, Clark et al., 2008). The toxic effect was observed with dose increment and duration of exposure (Clark et al., 2004, Clark et al., 2008).

A low incidence of congenital malformation was found in animal studies receiving lower dose of artesunate and dihydroartemisinin. The defect was mainly cardiac (ventricular septal defects and vascular defects) and skeletal development (Clark et al., 2008, Clark et al., 2004, Longo, 2006 #422, Longo et al., 2006). Nonetheless, a review on artemisinin antimalarial use in pregnancy suggests that the regimens are efficacious and appear not to compromise the pregnancy outcomes (Dellicour et al., 2007).
3.2. Piperaquine

The partner drug, piperaquine, is a bisquinoline antimalarial drug\(^5\) that is highly lipid soluble (Davis et al., 2005). The parasite killing effect is thought to result through an inhibition of heme digestive pathway of parasite’s food vacuola of intraerythrocytic throphozoites and gametocytes (Davis et al., 2005). Piperaquine has excellent activity against chloroquine resistant plasmodium strains and was used in China since 1979 as the first line treatment of CQ resistant *P. falciparum* malaria (Davis et al., 2005). Combined with DHA, piperaquine had an additive effect in clearing murine malaria in mice making it a good partner drug (Moore et al., 2008).

Piperaquine has a long elimination half life, of approximately 19-28 days (Hung et al., 2004, Davis et al., 2005) making it a good candidate for ACT and preventive treatment (White, 2005). In Senegal, where SP resistance malaria was low, monthly piperaquine combination (with SP or DHA) preventive treatments in 3-59 months old children were equally effective in reducing malaria parasitaemia at the end of malaria season (Cisse et al., 2009). Three doses of DHP monthly treatment for 3 months in this age group reduced malaria parasitaemia to 4.8\% and was well tolerated (Cisse et al., 2009). The protective efficacy from clinical malaria of intermittent preventive treatment in children with DHP is 87\% (95\%CI, 71-94) (Bojang et al., 2010).

Piperaquine has longer terminal elimination half-life in children but its shorter distribution half-life results in rapid plasma drug clearance (Davis et al., 2005, Hung et al., 2004, Tarning et al., 2008, Karunajeewa et al., 2008a). More recently an analysis of piperaquine drug concentrations after the treatment of uncomplicated malaria in Timika demonstrated lower plasma concentrations and higher failure rates in young children suggesting that a revision of dosing maybe warranted in this age group (Price et al., 2007a). In order to achieve good malaria prophylactic efficacy, higher weight adjusted PQ doses may be required (Tarning et al., 2008, Karunajeewa et al., 2008a). On the other hand, there are currently no published studies on the pharmacokinetics of piperaquine in pregnancy (Ward et al., 2007, D'Alessandro, 2009).

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\(^5\) Piperaquine: a bisquinoline compound (1,3-bis[1-(7-chloro 4 quinolyl)-4 piperazinyl] – propane), is a 4 aminoquinolines
**Toxicity**
High PQ dose (50 times human mg/kg dose) in mice was associated with mild to moderate toxic effect to the liver, kidney and some haematological indices (low platelet counts, low albumin and increased ALT) (Batty et al., 2008). Although this has been rarely reported in humans exposed to much lower doses.

Piperaquine exposure in pregnant mice was not associated with any severe congenital malformation, abortion or stillbirths but caused modest blood leucocytes and serum alanine aminotransferase (ALT) elevation in mice pups (Batty et al., 2009).

### 4. Dihydroartemisinin-Piperaquine use in pregnant women and children
Several chemotherapeutic drug trials study in nonpregnant adults and children aged one year old or older have highlighted DHP’s excellent tolerability and efficacy (Denis et al., 2002, Ashley et al., 2004, Ashley et al., 2005, Karema et al., 2006, Smithuis et al., 2006, Ratcliff et al., 2007a, Hasugian et al., 2007). The long terminal elimination tail of piperaquine prevents recrudescence and also decreases *P. falciparum* re-infection and *P. vivax* relapse (post treatment prophylaxis) (Ratcliff et al., 2007a). However, little is known on DHP safety and efficacy in children less than 5kg in weight or in pregnant women.

Pregnancy reduces the efficacy of several antimalarial drugs (Nosten et al., 2007). Despite being treated with more than twice the oral artesunate dose, the DHA plasma levels in pregnant women were significantly lower than that in the non pregnant patients and this may in part explain the relatively high treatment failures in pregnant women (McGready et al., 2006b). Lower plasma concentration of arthemeter, DHA and lumenfantrine are also found in pregnant women compared with non pregnant adults (McGready et al., 2006a, Tarning et al., 2009).

Preliminary data from the Thai Burmese border shows that DHP has a relatively good safety and efficacy as a rescue treatment in 45 pregnant women with *P.*
*P. falciparum* malaria in the second and third trimesters (day 63 cure rate of 92%) (Rijken et al., 2008). This could be due to these women had recrudescence after previous treatment (McGready et al., 2008). With limited information available, until dihydroartemisinin safety and toxicity data in pregnancy is available its use in the first trimester of pregnancy should be restricted.

Compared to older children, drug metabolism in infants is highly influenced by the maturation process of organ functions, which leads to slower drug absorption and metabolism and lower plasma drug concentration (Kearns et al., 2003). Recently this has been highlighted in a study of the use of sulfadoxine-pyrimethamine, which suggests that this widely deployed drug has systematically been under-dosed in children aged 2-5 years old for over 20 years (Barnes et al., 2006). The need of modified dose, particularly in young infants, has been emphasized by a number of clinical trials for DHP (Denis et al., 2002, Arinaitwe et al., 2009, Zwang et al., 2009, Karunajeewa et al., 2008b) and piperaquine (Price et al., 2007a, Tarning et al., 2008).

DHP is more efficacious than artemether-lumefantrine (AL) for the treatment of *P. falciparum* in infants aged 4-12 months (day 28 failure rates 11% in DHP group vs 34% in AL group), however after one year of follow up the overall malaria incidence was similarly low between the two treatment groups (4.6-4.8 treatments per person per year) (Arinaitwe et al., 2009). This suggests that post-treatment prophylactic effect of DHP is limited in areas with high malaria transmission. In addition, the relatively lower day 28 cure rate in infants receiving modified weight-base adult dose compared to older children and adults may have reduced DHP prophylactic effect. These findings further emphasize the need of dose adjustment in this age group in order to achieve high treatment efficacy.

There were no severe adverse outcomes reported on DHP use in infants. Cough, diarrhea and vomiting are commonly found following DHP exposures but did not lead to treatment termination (Arinaitwe et al., 2009, Katrak et al., 2009). Recurrent DHP treatment within 17-28 days increased the risk of vomiting (Katrak et al., 2009).
5. Reducing the burden of malaria on mother and child in Timika

Malaria is a major threat to maternal and infant survival in southern Papua. There is thus an urgent need to tailor a malaria control program specifically for these high risk populations. The key elements of such a program include early diagnosis, administration of effective antimalarial treatment and prevention of infection. By targeting mothers in early pregnancy, the aim is to reduce the immediate adverse effect of malaria on the unborn child, and potentially decrease and delay infection of the baby during the first year of life.

In other malaria endemic areas Insecticide Treated Nets (ITN) and Intermittent Preventive Treatment (IPT) are central strategies to prevent infection (WHO/AFRO, 2004, Muller et al., 2006, Aponte et al., 2009), although their efficacy in Papua is largely unknown. However accessibility and quality health care are still major issues with less than half of pregnant women currently attending antenatal care. Hence although the whole approach to maternal child health needs to be improved, the provision of early detection and good case management for mother and infants remains the most important priority, in the short term, for realistically reducing the burden of disease. The evaluation of this strategy in reducing the burden of disease in Timika is the central theme of this thesis.
Chapter 2

Research Methods
Chapter 2 – Research Methods

Study area
The work for my thesis was carried out at Rumah Sakit Mitra Masyarakat (RSMM) hospital, Timika, (Papua, Indonesia) which until November 2008 was the only hospital in the district servicing an area of 21,522 square kilometres with a population of 200,000 people living in 85 villages within 12 sub-districts. The area is largely forested with both coastal and mountainous areas. Malaria transmission is restricted to the lowland area where it is associated with three mosquito vectors: *Anopheles koliensis*, *An. farauti* and *An. punctulatus*. The annual incidence of malaria in the region is 876 per 1000 person years, divided 61:39 between *P. falciparum* and *P. vivax* infections (Karyana et al., 2008).

![Figure 2-1. Map of Papua - Indonesia](image)
Clinical drug trials conducted in this region in 2005 revealed the efficacy of standard regimens of chloroquine, sulfadoxine-pyrimethamine and unsupervised quinine were unacceptable with the risk of failure within 28 days reaching 65% after chloroquine monotherapy for *P. vivax* and 48% after CQ plus SP for *P. falciparum* (Ratcliff et al., 2007b). High grade drug resistance is evidenced by 2% of patients having early treatment failure and 16% of patients still being parasitaemic by day 7 (Ratcliff et al., 2007b).

Data on host genetic polymorphisms in the Timika population known to be related to host immunity are limited. The only estimates of haemoglobinopathies genetic polymorphisms in this area are from clinical trials in which Glucose-6-phosphate dehydrogenase (G6PD) deficiency was present in 15% of the patient enrolled (7% severe and 8% intermediate) (Hasugian et al., 2007, Ratcliff et al., 2007a). In a cross sectional survey, South East Asian ovalocytosis was present in 0.9% (1/114) and Gerbich in 1.7% (2/114) of highland Papuans (unpublished).

**Study population**

The maternal mortality ratio in this region is 1,145/100,000 live births with infant mortality rate reaching 68/1,000 live births (Hidayat, 2001). Of about 3000 pregnant women each year in Timika, less than 40% attend antenatal care clinic and approximately 40% deliver at the RSMM hospital (Mimika DHO, 2005). Between 2004 and 2008, infants accounted for 7.4% of all hospital outpatient consultations with malaria diagnosed in 14.4% of cases (unpublished).

By the end of 2007, Insecticide Treated Nets (ITN) had been distributed to ~52% of pregnant women and underfives in the area (Usman, 2009), however Intermittent Presumptive Treatment (IPT for infants and pregnant women) for malaria is not part of local policy and HIV testing is not routinely done.

Due to economic migration the ethnic origin of the local population is diverse, with highland Papuans, lowland Papuans and non-Papuans all resident in the region. In view of the high number of infections in non-immune patients, local protocols recommend that all patients with parasitaemia at any level are given anti-malarial therapy.
Data collection

Pregnant women and newborns

Clinical data

Since April 2004, all pregnant women admitted to the RSMM hospital were eligible for inclusion in this observational study, providing that they gave informed consent (Appendix 1). All women received standard care according to hospital protocols, irrespective of whether they were included in the study or not. Data collected until December 2006 was analysed to define the malaria burden in pregnant women and its effect to pregnancy outcomes (presented in Chapter 3). DHP safety and toxicity analysis included pregnant women enrolled until June 2009 (presented in Chapter 7).

Pregnant women were interviewed regardless of delivery status by a research nurse using a standardized questionnaire (Appendix 2). Examinations on mothers and babies were performed by the attending clinician or research clinician. Pregnancy gestation was determined by ultrasound. History of febrile illness and treatment received during the current pregnancy was collected by interview and review of clinical notes. Although routine antenatal surveillance was not available, those women who gave a history of a febrile illness during this pregnancy were regarded as having a history of possible malaria, and where possible, the details of this were extracted from hospital records. On admission to hospital women were defined as having a fever if they gave a history of fever within the preceding 24 hours or had an axillary temperature greater than 37.5°C.

Newborn/pregnancy outcomes were documented. Infants were weighed by the attending nurse/midwife soon after delivery to the nearest 100 grams by using standard infant spring scale. A trained research nurse performed newborn physical examination to assess clinical signs and gestational age (New Ballard Score) (Ballard et al., 1991) (Appendix 3). Since May 2007 the examination also included systematic evaluation on external congenital anomalies at birth (Appendix 4) (Gomella et al., 1994).
Neonatal adverse outcomes were defined according to WHO criteria (WHO, 1996). Babies weighing less than 2500g were defined as being low birth weight and babies with gestational age of less than 37 weeks according to the New Ballard Score as premature (Ballard et al., 1991). Any baby born dead with a weight of 500 grams (approximately 22 weeks of gestational age) or more was termed a still birth (Woods et al., 2004). Perinatal death was defined as death from foetal gestational age of 22 weeks to seven days after birth (WHO, 1996), thus including stillbirths and neonatal deaths.

**Laboratory data**

As per routine hospital protocol, venous blood samples (5 ml) were drawn from pregnant women for complete blood counts and malaria smears. All newborns were also screened for malaria by taking heel prick blood. Positive patients were treated according to RSMM hospital protocol.

Parasite counts were determined from the number of parasites per 200 white blood cells (WBC) on Giemsa-stained thick films, and peripheral parasitaemia calculated from the recorded white cell count. Slides were considered negative after review of 200 high-power fields. A thin smear was also examined to confirm parasite species and used for quantification if parasitaemia was greater than 200 per 200 WBC.

Haemoglobin concentration was determined by electronic counter (Coulter JT™). Malaria was defined as the presence of peripheral asexual parasitaemia irrespective of clinical signs or species with maternal anaemia categorized as either moderate (haemoglobin Hb >7 - <11 g/dl) (WHO, 1996) or severe (Hb ≤ 7 g/dl) (Shulman et al., 1996).

**Malaria treatment profile**

Any patients with parasitaemia were treated according to the standard protocol, which until March 2006 were SP, CQ or quinine. In March 2006, Ministry of Health treatment guidelines were changed in the Mimika District to reflect rising antimalarial drug resistance to both *P. falciparum* and *P. vivax*. DHP became the recommended first line treatment for uncomplicated malaria from any species of infection in the second and third trimester of pregnancy. Quinine and clindamycin
were given in the first trimester of pregnancy. Following severe malaria treatment multicentre trial in RSMM between April 2004 and May 2005, intravenous artesunate is recommended for the initial treatment of pregnant women with severe malaria from any species of infection in the second and third trimester of pregnancy followed with DHP as soon as the patient can take oral drugs (Dondorp et al., 2005). DHP is prepared as a fixed dose combination (40 mg dihydroartemisinin and 320 mg piperaquine) produced by Holly Pharmaceuticals (Artekin™) and each dose was given according to the body weight (dosage: 2-4 mg/kg of dihydroartemisinin and 16-32 mg/kg of piperaquine) once a day for three days.

Table 2-1. Dosage of dihydroartemisinin-piperaquine

<table>
<thead>
<tr>
<th>Body Weight (kg)</th>
<th>Number of Tablet(s)</th>
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<tbody>
<tr>
<td>= 5</td>
<td>0.25</td>
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<tr>
<td>6-10</td>
<td>0.5</td>
</tr>
<tr>
<td>11-17</td>
<td>1</td>
</tr>
<tr>
<td>18-30</td>
<td>1.5</td>
</tr>
<tr>
<td>31-40</td>
<td>2</td>
</tr>
<tr>
<td>41-60</td>
<td>3</td>
</tr>
<tr>
<td>61-80</td>
<td>4</td>
</tr>
<tr>
<td>81-100</td>
<td>5</td>
</tr>
</tbody>
</table>

Infants (birth – 12 months)

Clinical data

Data were collected from all infants admitted to the wards of RSMM Hospital from April 2004 through April 2008. Demographic details and diagnosis were recorded for all inpatients using a computerized hospital records system (Q-Pro™). In addition patients diagnosed with malaria were reviewed by a medically trained member of an onsite research team and further details of their admission recorded on a standardized data form as reported previously (Tjitra et al., 2008) (Appendix 5). Standard care was provided according to hospital guidelines by the attending physician.
The guardians of any baby less than 3 months old admitted with Plasmodium parasitaemia, were invited to have their baby enrolled in an additional study of young infants in which a research nurse completed a more detailed questionnaire documenting the history of illness in the baby (Appendix 6). All young infants were examined by either the attending physician or research clinician and their clinical findings documented. The presence of splenomegaly or hepatomegaly was determined by abdominal examination. Fever was defined as an axillary temperature greater than 37.5°C. Respiratory distress was defined by an age-stratified increased respiratory rate (>50x/minute in children aged 2 months to 5 years and >60x/minute in babies less than 2 months) (Tjitra et al., 2008, WHO, 2000a). Low weight for age was defined as weight-for-age 2 standard deviations (SD) below the median value of the reference (healthy) population and severely underweight as 3 SD below the reference population (WHO, 2006b).

Malaria was defined as a symptomatic illness associated with any peripheral parasitaemia. The diagnosis of severe disease made according to the WHO criteria for severe *P. falciparum* malaria (WHO, 2000a, Tjitra et al., 2008). Other co-morbidities (eg diarrhea, sepsis, pneumonia and meningitis) were diagnosed according to clinical, laboratory and radiological findings by attending physician.

**Laboratory data**

Malaria was diagnosed at the hospital laboratory by microscopy of Giemsa-stained blood films and parasite density recorded as 1+ to 4+. Slides were considered negative after review of 100 high-power fields. Infants enrolled in the more detailed survey had a repeat blood film which was read by an expert microscopist and parasite counts determined from the number of parasites per 200 white blood cells (WBC) on Giemsa-stained thick films. The smear reading results of the expert microscopist were considered as final. Peripheral parasitaemia was calculated from the recorded white cell count. Slides were considered negative after review of 200 high-power fields. A thin smear was also examined to confirm parasite species and used for quantification if parasitaemia was greater than 200 per 200 WBC. The hospital laboratory participates in ongoing training and quality control, with more than 90% of slide recordings confirmed on cross checking by an independent expert microscopist.
Venous blood samples (1-5 ml) were drawn from infants admitted with malaria to the pediatric wards for complete blood counts and hemoglobin concentration (using electronic counter- Coulter JT).

Malaria treatment profile
All infants with peripheral parasitaemia received standard antimalarial therapy and supportive care as per hospital protocol. Before March 2006, the local protocol for newborn infants recommended seven days of quinine for both *P. falciparum* and *P. vivax* infection. After March 2006, infants weighing more than 5 kg or as indicated by the attending paediatrician were treated with dihydroartemisinin-piperaquine for uncomplicated malaria. Oral/intravenous Quinine was given to infants weighing less than 5 kg.

**Ethical approval and confidentiality**
Ethical approval for this study was obtained from the ethics committees of the National Institute of Health Research and Development, Ministry of Health, Indonesia and the Menzies School of Health Research, Darwin, Australia.

DHP has been recommended as the standard treatment for treating uncomplicated malaria in the 2nd and 3rd trimester of pregnancy since March 2006 (NIHRD/CDC6 KS.02.01.2.1.3431). Pregnant women in this study were seen by experienced physician, obstetrician and paediatrician. Any adverse reactions or severe disease were taken care according to hospital protocol. RSMM hospital has many experiences and expertise in managing severe malaria (Dondorp et al., 2005). Blood sample collections for study purposes did not compromise the well being of study subjects as it complied strictly with the study and hospital protocol or as clinically indicated. The manufacturers of the drugs are NOT involved in either the funding or conduct of the study.

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6 National Institute of Health Research and Disease/Communicable Disease Control – Ministry of Health Indonesia
**Statistical analysis**

Data from the questionnaire and the laboratory were entered into EpiData 3.02 software (EpiData Association, Odense, Denmark). Statistical analysis was done using SPSS vs 13.0 and later with vs 15.0 for windows software (SPSS Inc, Chicago, Illinois). Normally distributed data were compared by Student’s t test or one-way analysis of variance. Data not conforming to a normal distribution were compared by the Mann-Whitney U test or Kruskal-Wallis method. Categorical data were compared by calculating the $\chi^2$ with Yates’ correction or by Fisher’s exact test and the odds ratio (OR) with 95% confidence intervals. Specific statistical analysis was applied according to the study aims as stated below.

**Epidemiology of infant and maternal malaria**

Parasitaemia and fever were assessed using a receiver operator curve, and the pyrogenic threshold defined from the maximum value of Youden’s Index (sensitivity plus specificity minus 1). A multiple logistic regression model was used to determine adjusted odds ratios (AOR) for risk factors for adverse outcomes. Any variables found to be associated significantly with the dependent variable in a univariate analysis were entered into the equation and the model constructed using a forward stepwise analysis of the Wald statistic using $p<0.05$ as a cut off for significance and inclusion of the predictor variable. In the infant malaria study, healthy deliveries and malaria diagnosed from active screening were excluded from the analysis.

**Epidemiology of congenital malaria**

Multiple logistic regression was used to analyse independent risk factors for congenital malaria by entering all significant risk factors in univariate analysis. Population attributable risks (PARs) were calculated for all significant risk factors in univariate and multivariate analysis (see table 6.2) according to the following equation:

$$\text{PAR: } \frac{\text{Proportion of cases exposed to factor x (OR-1)}}{\text{OR}}$$

The PAR of the model was derived from AOR using the following formula:

$$\text{Combined PAR}= 1 - [(1-PAR1)*(1-PAR2)*(1-PAR3)]…$$
DHP safety and toxicity analysis
Multiple logistic regression to calculate adjusted odds ratios (AOR) of risk factors for adverse pregnancy outcomes. History of malaria treatment was entered into the model by using history of DHP exposures as the reference category and comparing the effect to other previous treatments. Survival analysis with the Kaplan Meier method was used to examine the effect of malaria treatment history during pregnancy to cumulative risk of malaria at delivery.

Impact of treatment policy change
The relative risk (RR) was defined as the ratio of risk of adverse outcomes in pregnant women with history of malaria treatment compared to the risk of women without prior malaria during pregnancy. Population attributable risk (PAR) was defined as the proportion of adverse outcomes in the population attributable to history of antimalarials exposures during pregnancy obtained by interview and when data is available, confirmed by hospital records. We use the following formula to calculate PAR:

\[
\text{PAR: } \frac{\text{proportion of population exposed} \times (\text{RR}-1)}{1 + [\text{proportion of population exposed} \times (\text{RR}-1)]}
\]

Both RR and population attributable risk were calculated before and after DHP deployment. The significance of differences in relative risks was calculated according to the method of Altman and Bland (Altman and Bland, 2003). The 95% confidence interval for PAR was calculated according to the delta method (Hildebrandt et al., 2006). A multiple logistic regression model was used to examine the odds of the occurrence of adverse pregnancy outcomes associated with the change in treatment policy. All potential confounding factors, including a dichomotous variable denoting pre and post DHP introduction, were entered into the analysis.
Chapter 3

Adverse pregnancy outcomes in an area endemic for multidrug resistant *P. vivax* and *P. falciparum*
Chapter 3 – Adverse pregnancy outcomes in an area endemic for multidrug resistant *P. vivax* and *P. falciparum*

**Abstract**

**Background.** *Plasmodium falciparum* infection exerts a considerable burden on pregnant women, but less is known about the adverse consequences of *P. vivax* infection.

**Methods.** In Papua, Indonesia, where multiple drug resistance has emerged to both species, we conducted a cross sectional hospital-based study to quantify the risks and consequences of maternal malaria.

**Results.** From April 2004 through December 2006, 3046 pregnant women were enrolled in the study. The prevalence of parasitaemia at delivery was 16.8% (432 of 2570 women had infections), with 152 (35.2%) of these 432 infections being associated with fever. The majority of infections were attributable to *P. falciparum* (250 [57.9%]); 146 (33.8%) of the infections were attributable to *P. vivax* and 36 (8.3%) were coinfections with both species. At delivery, *P. falciparum* was associated with severe anaemia (hemoglobin concentration <7 g/dl; odds ratio [OR], 2.8; 95% confidence interval [95% CI], 2.0-4.0) and a 192 g (95% CI, 119-265) reduction in mean birth weight; p< 0.001. *P. vivax* infection was associated with an increased risk of moderate anaemia (hemoglobin concentration, 7-11 g/dL; OR, 1.8; 95% CI, 1.2-2.9; p=0.01) and a 108 g (95% CI, 17.5-199) reduction in mean birth weight (p< 0.019). Parasitaemia was associated with preterm delivery (OR, 1.5; 95% CI, 1.1-2.0; p=0.02) and stillbirth (OR, 2.3; 95% CI, 1.3-4.1; p=0.007), but was not associated with these outcomes after controlling for the presence of fever and severe anaemia, suggesting malaria increases the risk of preterm delivery and stillbirth through fever and contribution to severe anaemia, rather than parasitaemia per se.

**Conclusions.** These observations highlight the need for novel safe and effective treatment and prevention strategies against both multidrug resistant *P. falciparum* and *P. vivax* in pregnant women in areas of mixed endemicity.

**Published in Clinical Infectious Diseases 2008; 46:1374-81**
Results

From April 2004 through December 2006, 3744 pregnant women were admitted to the maternity ward at RSMM hospital; 3046 (81.4%) of these women were enrolled in the study. Of these 3046 women, 2518 (82.7%) were admitted to the hospital for delivery, 307 (10.1%) were admitted to the hospital for the treatment of malaria, and 221 (7.3%) were admitted to the hospital for other medical reasons. Seventy-five pregnant women (2.5%) were admitted to the hospital more than once during the observation period, the majority of whom (69 [92%] of 75 women) were admitted to the hospital twice. Of the 3046 women, 331 (10.9%) were discharged from the hospital with an ongoing pregnancy, 2601 women (85.4%) delivered, and pregnancy was aborted in 114 women (3.7%) (figure 3-1). The remainder of the analysis was restricted to women at the time of delivery, with baseline characteristics and outcomes shown in table 3-1.

Maternal malaria

Malaria smears results were recorded for 2570 (98.9%) of 2601 pregnant women at delivery. The overall parasite prevalence was 16.8% (432 of 2570 women had parasites found), with P. falciparum accounting for 250 infections (57.9%) and P. vivax accounting for 146 infections (33.8%); 15 (3.8%) infections were mixed infections (P. falciparum and P. vivax), 19 (4.4%) were caused by P. malariae and 2 (0.5%) were caused by P. ovale. A history of possible malaria infection was reported by 388 (14.9%) of 2598) women, 76 (19.6%) of whom experienced >1 febrile episode and 356 (91.8%) of whom received empirical antimalarial drugs.

Of the 432 women with peripheral parasitaemia who delivered a neonate, 152 (35.2%) were febrile or had a fever in the preceding 24 hours. This proportion was significantly higher in women with P. falciparum infections (105 [42%] of 250 women) than among women with P. vivax infections (35 [23.9%] of 14; OR, 2.3; 95%CI, 1.4-3.7; p<0.001). Women with P. falciparum infections also presented with higher parasite loads (geometric mean parasite load, 1622 parasites/µL; 95%CI, 1187-2215 parasites/µL) than did those infected with P. vivax (geometric mean parasite load, 602 parasites/µL; 95%CI, 414-875 parasites/µL; p<0.001) (table 3-2).
Figure 3-1. Flow of pregnant women with or without *Plasmodium* parasitaemia (due to *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and/or *Plasmodium malariae*) throughout the study.

Pregnant Women admitted to Hospital

3744

698 Unable to enrol or patients refused

3046 Enrolled in Study

2518 Admitted for delivery
307 Admitted for malaria
221 Admitted for other reasons

2,601 Women with delivery

2505 Live Births
55 Still Births
41 Neonatal Death

114 Women with miscarriage

432 with maternal malaria:
- *P. falciparum* 250
- *P. vivax* 146
- *P. ovale* 2
- *P. malariae* 19
- Mixed Infection 15
- Smear Negative 2138
- No slide done 31

331 Women with ongoing pregnancy

241 with maternal malaria:
- *P. falciparum* 172
- *P. vivax* 37
- *P. ovale* 0
- *P. malariae* 3
- Mixed Infection 29
- Smear Negative 90

20 with maternal malaria:
- *P. falciparum* 12
- *P. vivax* 6
- Mixed Infection 2
- Smear Negative 94
Table 3-1. Maternal characteristic at delivery and Pregnancy Outcomes

<table>
<thead>
<tr>
<th>Maternal characteristic at delivery</th>
<th>Maternal malaria at delivery</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive n=432</td>
<td>Negative n=2138</td>
</tr>
<tr>
<td><strong>Maternal characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, mean years [95%CI]</td>
<td>24.5 (23.9-25.1)</td>
<td>25.9 (25.7-26.1)</td>
</tr>
<tr>
<td>Gravidity, median no. of</td>
<td>2 (1-3)</td>
<td>2 (1-4)</td>
</tr>
<tr>
<td>pregnancies [IQR]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primigravid</td>
<td>160/432 (37.0)</td>
<td>638/2135 (29.9)</td>
</tr>
<tr>
<td>Multigravid</td>
<td>255/432 (59.0)</td>
<td>1415/2135 (66.3)</td>
</tr>
<tr>
<td>Grand Multigravid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17/432 (3.9%)</td>
<td>82/2135 (3.8)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Papuan</td>
<td>342 (79.2)</td>
<td>1360 (63.6)</td>
</tr>
<tr>
<td>Non-Papuan</td>
<td>89 (20.6)</td>
<td>774 (36.2)</td>
</tr>
<tr>
<td>Hemoglobin concentration, mean g/dl±SD</td>
<td>8.9±2.0</td>
<td>9.8±1.9</td>
</tr>
<tr>
<td>Severe anaemia</td>
<td>67/429 (15.6)</td>
<td>178/2127 (8.4)</td>
</tr>
<tr>
<td><strong>Pregnancy Outcome</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivered</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singleton</td>
<td>418 (96.7)</td>
<td>2077 (97.1)</td>
</tr>
<tr>
<td>Twins</td>
<td>13 (3.0)</td>
<td>61 (2.9)</td>
</tr>
<tr>
<td>Gestational age, median weeks (range)</td>
<td>38 (28-43)</td>
<td>38.6 (28-43)</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>60/396 (15.1)</td>
<td>217/2000 (10.8)</td>
</tr>
<tr>
<td>Birth weight&lt;sup&gt;c&lt;/sup&gt;, mean g±SD</td>
<td>2896±567.9</td>
<td>3060±509.2</td>
</tr>
<tr>
<td>Low Birth Weight&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70/387 (18.1)</td>
<td>212/2015 (10.5)</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>17 (3.9)</td>
<td>38 (1.8)</td>
</tr>
<tr>
<td>Early neonatal deaths</td>
<td>12 (2.8)</td>
<td>27 (1.3)</td>
</tr>
</tbody>
</table>

Note. Data are no. (%) or proportion (%) of women, unless otherwise indicated. IQR, interquartile range; NS, not statistically significant.

<sup>a</sup> Determined by Mann-Whitney U test.
<sup>b</sup> Fifth or subsequent pregnancy.
<sup>c</sup> Live singleton births.
Using Youden’s index the pyrogenic density for *P. falciparum* was 2953 parasites/µL and that for *P. vivax* was 632 parasites/µL. At delivery, 15 (0.6%) of 2601 women presented with ≥ 1 modified World Health Organization criteria of severe malaria, and 14 (93.3%) of these 15 women had severe anaemia.

Three pregnant women died. One multigravid woman (at 38 weeks of gestation with fetal death in utero) had *P. vivax* infection and clinical features of severe bacterial sepsis. Two women (1 of whom was primigravid and 1 of whom was multigravid) both in their first trimester, had *P. falciparum* infection with multi organ failure.

A total of 348 women received documented treatment for malaria at the time of delivery; 194 (55.7%) of these women received quinine with or without clindamycin, 92 (26.4%) received dihydroartemisinin-piperaquine, 12 (3.4%) received intravenous artesunate plus dihydroartemisinin-piperaquine, 43 (12.4%) received chloroquine ± sulfadoxine-pyrimethamine, and 7 (2.0%) received oral artesunate only. An additional 84 (19.4%) of the 432 women with peripheral parasitaemia discharged themselves from the hospital before the administration of antimalarial medication.

### Table 3-2. Geometric mean parasite load in mothers with *P. falciparum* or *P. vivax* infection

<table>
<thead>
<tr>
<th>Mothers</th>
<th>Parasite load, geometric mean parasites/µL (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. falciparum</em></td>
</tr>
<tr>
<td>All Women</td>
<td>1622 (1187–2215)</td>
</tr>
<tr>
<td>Afebrile women</td>
<td>956 (653–1399)</td>
</tr>
<tr>
<td>Febrile women</td>
<td>3339 (2032–5487)</td>
</tr>
<tr>
<td>Primigravidae</td>
<td>1998 (8-3327)</td>
</tr>
<tr>
<td>Multigravidae</td>
<td>1436 (992-2208)</td>
</tr>
</tbody>
</table>
Risk Factors for Maternal Malaria
Parasitaemia at hospital admission was present in 160 (20%) of 798 primigravidae women, compared with 272 (15.4%) of 1770 multigravidae women ($P=0.004$). The magnitude of the risk was similar for both $P. falciparum$ and $P. vivax$ infections (table 3-3). Other univariate risk factors are shown in table 3-3. In a multivariate model, the following 4 factors were found to be independently associated with parasitaemia: primigravidity (OR, 1.3; 95% CI, 1.03-1.7), Papuan ethnicity (OR, 2.3; 95% CI, 1.8-2.9), fever or history of fever during the 24 h before hospital admission (OR, 10.8; 95% CI, 7.8-15.0)) and a history of possible malaria infection during the current pregnancy (OR, 2.6; 95% CI, 2.0-3.5).

Maternal anaemia
The mean Hb concentration at delivery was 9.7 g/dl (95% CI, 9.6-9.8 g/dL) with moderate anaemia noted in 1691 (66.1%) of 2558) women and severe anaemia noted in 245 (9.6%) of 2,558 women. Mothers infected with $P. falciparum$ had a mean Hb concentration that was 1.1 g/dl (95% CI, 1.0-1.4 g/dL) lower than the mean Hb concentration in mothers who did not have parasitaemia, and they had an increased risk of severe anaemia compared with women who did not have parasitaemia (OR, 2.8; 95% CI, 2.0-4.0; $p<0.001$). The difference in Hb concentration between women who did and did not have $P. vivax$ infection was more modest (0.4 g/dL; 95% CI, 0.1-0.7 g/dL), and there was an increased risk of moderate anaemia (but not of severe anaemia) among women with $P. vivax$ parasitaemia compared with women who did not have $P. vivax$ parasitaemia (OR, 1.8; 95% CI, 1.2-2.9; $p=0.01$).

Severe anaemia (Hb concentration <7 g/dL) was more prevalent among multigravid women than among primigravid women (prevalence of severe anaemia, 10.5% [185 of 1764 multigravid women] vs 7.6% [60 of 792 primigravid women]; OR, 1.4; 95% CI, 1.04-1.96; $p=0.02$). Other risk factors are presented in table 3-4. The risk of anaemia was also apparent in women with asymptomatic malaria (OR, 1.7; 95% CI, 1.2-2.5; $p=0.004$).
Table 3-3. Univariate Risk factors (Odds Ratio with [95% CI]) for parasitaemia on admission to hospital

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Parasitaemia, OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Any</td>
</tr>
<tr>
<td>Primigravid</td>
<td>1.4 [1.1-1.7]</td>
</tr>
<tr>
<td>Age ≤ 16 years old</td>
<td>2.4 [1.4-4.0]</td>
</tr>
<tr>
<td>Papuan ethnicity</td>
<td>2.2 [1.7-2.8]</td>
</tr>
<tr>
<td>Fever or history of fever in previous 24 h</td>
<td>14.3 [10.6-19.3]</td>
</tr>
<tr>
<td>History of possible malaria infection during pregnancy</td>
<td>4.7 [3.7-6.0]</td>
</tr>
</tbody>
</table>

*a* Risks compared to those women without parasitaemia present

b \( p<0.005 \)
c \( p<0.05 \)
d \( p<0.001 \)
e \( p=0.37 \)

**Pregnancy outcomes**

Of the 2,601 women who reached the end of pregnancy during the current admission 2505 (96.3%) had live births (including 67 twins), 55 (2.1%) had stillbirths, and 41 (1.6%) delivered neonates who died.

**Preterm delivery**

Gestational age was assessed for 2419 (93%) of 2601 women who delivered, with prevalence of preterm delivery of 11.5% (279 women). The risk of preterm delivery (< 37 weeks gestation) was 15.2% (60 of 396 women) among women with maternal malaria, compared with 10.9% (217 of 2,000) among women who did not have malaria (OR, 1.5; 95% CI, 1.1-2.0; \( p=0.02 \)). However the risk of preterm delivery while infected with malaria was not apparent after controlling for fever (adjusted OR, 2.0; 95% CI, 1.3-2.8; \( p=0.001 \)), severe anaemia (adjusted OR, 2.0; 95% CI, 1.3-2.8; \( p<0.001 \)), and primigravidity (adjusted OR, 1.5; 95% CI, 1.2-2.0; \( p=0.002 \)).
### Table 3-4. Risk factors for severe maternal anaemia and low birth weight

<table>
<thead>
<tr>
<th>Maternal Risk Factor</th>
<th>Univariate analysis, OR (95% CI)</th>
<th>Multivariate analysis, adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe anaemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>2.8 [1.9-4.0]*</td>
<td>1.9 [1.3-2.9]*</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>0.6 [0.3-1.4]*</td>
<td>NS</td>
</tr>
<tr>
<td>Papuan ethnicity</td>
<td>3.4 [2.4-4.9]*</td>
<td>2.7 [1.9-3.8]*</td>
</tr>
<tr>
<td>Fever or history of fever in previous 24 h</td>
<td>2.4 [1.7-3.4]*</td>
<td>1.7 [1.1-2.7]*</td>
</tr>
<tr>
<td>Age ≤ 16 years</td>
<td>2.0 [1.1-3.9]*</td>
<td>2.0 [1.0-4.0]*</td>
</tr>
<tr>
<td>Multigravid</td>
<td>1.4 [1.0-2.0]*</td>
<td>1.6 [1.2-2.3]*</td>
</tr>
<tr>
<td>Low birth weight</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malaria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Any parasitaemia</td>
<td>1.9 [1.4-2.6]*</td>
<td>1.5 [1.04-2.2]*</td>
</tr>
<tr>
<td><em>P. falciparum</em></td>
<td>1.9 [1.2-2.7]*</td>
<td>-</td>
</tr>
<tr>
<td><em>P. vivax</em></td>
<td>1.9 [1.2-3.1]*</td>
<td>-</td>
</tr>
<tr>
<td>Severe anaemia</td>
<td>3.3 [2.4-4.5]*</td>
<td>3.5 [2.3-5.3]*</td>
</tr>
<tr>
<td>Papuan ethnicity</td>
<td>1.5 [1.2-2.0]*</td>
<td>NS</td>
</tr>
<tr>
<td>Primigravid</td>
<td>1.7 [1.3-2.2]*</td>
<td>1.7 [1.3-2.4]*</td>
</tr>
<tr>
<td>Prematurity</td>
<td>22.8 [16.7-31.2]*</td>
<td>22.0 [15.9-30.5]*</td>
</tr>
</tbody>
</table>

**Note:** *a* p< 0.001; *b* p=0.01; *c* p<0.05; *d* p=0.005; NS not statistically significant

---

**Low birth weight**

The mean birth weight (±SD) of live singleton neonates in this study was 3024 ± 537 g compared with 2196 ± 648 g for twin neonates (p<0.001). Subsequent analysis is restricted to live singleton births. Low birth weight was observed in 212 (10.5%) of 2015 neonates of mothers who did not have malaria, compared with 70 (18.1%) of 387 neonates of mothers with malaria (OR, 1.9; 95% CI, 1.4-2.6; p<0.001) resulting in a population-attributable risk of 9%. Maternal malaria was a significant independent risk factor for low birth weight (adjusted OR, 1.5; 95% CI, 1.04-2.2; p=0.03) in multivariate analysis (table 3-4). The risk of low birth weight associated was also apparent in women with asymptomatic malaria (OR, 1.5; 95%: 1.1-2.1; p=0.008).
The mean birth weight of neonates delivered from mothers with *P. falciparum* infections at delivery was 192 g (95% CI, 119-265 g) lower than that of neonates of mothers who did not have such infection. Although the decrease in birth weight associated with *P. vivax* infections was more modest (108g; 95% CI, 17.5-199 g; p=0.019), both *P. vivax* and *P. falciparum* infections were associated with a similar risk of low birth weight (17.9% [39 of 218 neonates] vs 18.5% [25 of 135 neonates] respectively) (table 3-4).

**Perinatal Deaths**

Stillbirths occurred in 55 (2.1%) of 2,601 women, and early neonatal deaths (death of a neonate in the first 3 days of life) occurred for 41 (1.6%) of 2,601 women. Mothers with *P. falciparum* parasitaemia had an increased risk of having a stillbirth (OR, 2.3; 95% CI, 1.2-4.2; p=0.008) and early neonatal death, compared with women who were aparasitemic (OR, 2.2; 95% CI, 1.1-4.6; p=0.03). After controlling for fever (adjusted OR, 2.0; 95% CI, 1.3-2.8; p<0.001) and severe anaemia (adjusted OR, 2.0; 95% CI, 1.4-2.8; p<0.001), the difference in such risk was no longer statistically significant.

**Discussion**

In Asia *P. falciparum* and *P. vivax* are equally prevalent, although the latter is usually assumed to be benign and its associated morbidity often ignored (Baird et al., 2007, Price et al., 2007b). To investigate the relative impact of both species on maternal malaria we conducted a study in an area in Papua, Indonesia, where health services are limited and high levels of multi-drug resistance exist in both *P. falciparum* and *P. vivax* (Ratcliff et al., 2007b). Our results revealed that more than one-half of all women receiving empirical antimalarial therapy during pregnancy had subsequent patent parasitaemia at delivery. Because rates of reinfection were low (1-3 infective bites per year) many of these infections reflect recrudescence of drug-resistant malaria and relapses of *P. vivax* infection. We were unable to observe women throughout their pregnancies; thus, our analysis focused on the adverse effects associated with peripheral parasitaemia at delivery. At delivery, 17% of women had peripheral parasitaemia. *P. vivax* infection accounted for 34% of infections in our study, compared with 17% in a study from Thailand (Nosten et al.,
1991) and 16% in a study from Papua New Guinea in the 1980s (Desowitz and Alpers, 1992); a likely consequence of the high prevalence of drug resistant strains of *P. vivax* in Papua (Ratcliff et al., 2007b) and the paucity of antenatal health care in Papua. The higher transmission rate found in our study, compared with the transmission rate found in the study in Thailand, may provide enough immunity to suppress both symptoms and parasitaemia and result in persistent infections going undetected and untreated. Although *P. vivax* had a lower pyrogenic density (632 parasites/µL) than *P. falciparum* (2953 parasites/µL), only 24% of women infected with *P. vivax* presented with symptoms compared to 42% of women with *P. falciparum* infection who presented with symptoms, making *P. vivax*-infected women less likely to seek treatment. When either species was associated with fever the risk of anaemia and premature delivery almost doubled. Asymptomatic infections were also associated with poor outcome, but this was only apparent for low birth weight and severe maternal anaemia.

In Papua, both *P. falciparum* and *P. vivax* malaria susceptibilities were higher in first time mothers than in multigravid women. The modest difference in susceptibility contrasts with the marked effect of parity in areas of high *P. falciparum* transmission (McGregor, 1984). Similar to studies from Thailand and India (Nosten et al., 1991, Singh et al., 1999), but not Africa or Papua New Guinea (McGregor, 1984, Shulman et al., 1996, Brabin and Piper, 1997), multigravid women were more anaemic than primigravid women in our study. Less exposure to malaria and failure to develop protective immunity during the first pregnancy provide a plausible explanation, with iron deficiency, micronutrient deficiency, helminth infection and HIV likely to be significant co-factors (Isah et al., 1985, Brabin et al., 1990, Shulman et al., 1996, Rasmussen, 2001, Steketee, 2003).

In areas of high transmission, the effect of *P. falciparum* infection on birth weight is associated with chronic placental infection and inflammation rather than peripheral parasitaemia (Sullivan et al., 1999, Menendez et al., 2000b, Rogerson et al., 2003a). However, in areas of low transmission, placental changes are less common and reduction in birth weight can occur even with a single episode of malaria (Nosten et al., 1991, Nosten et al., 1999, McGready et al., 2004). In our study the most important determinant of low birth weight was premature delivery, with 22% of
premature deliveries being associated with malaria. In full term deliveries, both *P. falciparum* and *P. vivax* parasitaemia were associated with a similar risk of low birth weight. Low birth weight of the neonate after full term delivery was unlikely to have been a result of infection at the time of delivery but, instead may have been a result of repeated episodes or sustained parasitaemia during late pregnancy. We did not continue to follow babies after discharge from hospital and therefore could not quantify the overall burden of maternal malaria on infant mortality. However, because birth weight is inversely correlated with the risk of death during the first year of life (Guyatt and Snow, 2001, Luxemburger et al., 2001), the low birth weight observed among neonates of mothers with *P. falciparum* and *P. vivax* infections in Papua is likely to make a significant contribution to the high infant mortality present in the region.

Our study also demonstrated an association between maternal malaria and both prematurity and stillbirth, the major determinants of which were concomitant fever and severe anaemia. After controlling for these factors the effect of parasitaemia was no longer apparent. Fever from any cause has been shown to be independently associated with premature labour (Luxemburger et al., 2001), suggesting that malaria exerts an adverse effect on the outcome of pregnancy through an inflammatory response and exacerbation of anaemia, rather than through peripheral parasitaemia per se. Indeed the success of intense serial antenatal malaria screening and prompt antimalarial treatment on the Thai-Burmese border is likely to have resulted both from an absolute reduction in the number of parasitemic episodes and from the detection of parasitaemia before the onset of fever (Nosten et al., 1991).

Pregnant women with malaria were treated according to the local guidelines which until March 2006 were quinine and clindamycin for the first trimester of pregnancy and chloroquine ± sulphadoxine pyrimethamine for the second and third trimesters of pregnancy. After March 2006, dihydroartemisinin piperaquine was recommended for use in the second and third trimester of pregnancy.

A limitation of this study is that the data were collected from hospital. In this setting, hospital deliveries represented 30-40% of local delivers. The rests of women delivered at home by themselves, attended by other female relatives or traditional
birth attendants. Access to health facilities was difficult and the services provided to pregnant women were generally poor. In this case, the study results are very likely to underestimate and bias the analysis of the true burden of malaria in pregnancy in the population.

Another limitation is that the history of possible malaria infection could not be ascertained. Malaria screening is not part of ANC program in this region therefore data on malaria during pregnancy is very limited. Of whom malaria treatment could be ascertained the concordance between interview result and medical record data is 48%. Since asymptomatic malaria is common in this region, this may result in underestimation of the true burden of disease.

In conclusion, our study demonstrated a significant burden of maternal malaria in Papua that was associated with both *P. vivax* and *P. falciparum* infection. In areas where intense antenatal screening cannot be sustained, a strategy of both opportunistic and routine screening of pregnant women presenting to clinics, irrespective of the presence of symptoms, and the prompt administration of effective antimalarial therapy will be a key element of any successful antenatal intervention. The additional efficacy of insecticide-treated bed nets and intermittent presumptive therapy in regions of mixed endemicity is largely unknown, and the investigation of these infection-control measures warrants priority.
Chapter 4

Vivax malaria: a major cause of morbidity in early infancy
Chapter 4 - Vivax malaria: a major cause of morbidity in early infancy

Abstract

Background. In areas where malaria is endemic, infants aged <3 months appear to be relatively protected from symptomatic and severe Plasmodium falciparum malaria, but less is known about the effect of Plasmodium vivax infection in this age group.

Methods. To define malaria morbidity in the first year of life in an area where both multidrug-resistant P. falciparum and P. vivax are highly prevalent, data were gathered on all infants attending a referral hospital in Papua, Indonesia, using systematic data forms and hospital computerized records. Additional clinical and laboratory data were prospectively collected from inpatients aged < 3 months.

Results. From April 2004 through April 2008, 4976 infants were admitted to hospital of whom 1560 (31%) had malaria, with infections equally attributable to P. falciparum and P. vivax. The case fatality rate was similar for inpatients with P. falciparum malaria (13 [2.2%] of 599 inpatients died) and P. vivax malaria (6 [1.0%] of 603 died; p=0.161), whereas severe malarial anaemia was more prevalent among those with P. vivax malaria (193 [32%] of 605 vs. 144 [24%] of 601; p=0.025). Of the 187 infants aged <3 months, 102 (56%) had P. vivax malaria, and 55 (30%) had P. falciparum malaria. In these young infants, infection with P. vivax was associated with a greater risk of severe anaemia (odds ratio, 2.4; 95% confidence interval, 1.03-5.91; p=0.041) and severe thrombocytopenia (odds ratio, 3.3; 95% confidence interval, 1.07-10.6; p=0.036) compared with those who have P. falciparum infection.

Conclusions. P. vivax malaria is a major cause of morbidity in early infancy. Preventative strategies, early diagnosis and prompt treatment should be initiated in the perinatal period.

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Results

Inpatient Malaria in first year of life
During the period from April 2004 through April 2008, 4976 (12.4%) of 40,035 patients admitted to the hospitals were infants, and 1560 (10%) of 15,582 patients admitted to the hospital had malaria. In total 1560 (31.4%) of 4976 infants admitted to the hospital had malaria, with infection attributable to *P. falciparum* in 662 infants (42.4%), *P. vivax* in 668 (42.8%), and mixed species in 222 infants (14.2%). Eight infants were infected with *P. malariae*. Patients of Papuan origin were more likely to have malaria than non Papuans (OR, 3.1; 95% CI, 2.5-3.9; p<0.001) and this was apparent for all species of infection. Although there was an overall predominance of all cause-admissions for male infants throughout infancy (54.6%; 95% CI, 53.3% – 56.0%), female infants were at greater risk of *P. vivax* infection (OR, 1.25; 95% CI, 1.06-1.48; p=0.009), but not for any other species of infection. Infants admitted under one month old had a 9.0% (87 of 967) prevalence of malaria, which increased throughout the first year of life to 54% (792 of 1476) in the last quarter. *P. vivax* was the predominant species from birth to 8 months of age (Figure 4-1 and table 4-1).

![Figure 4-1](image)

**Figure 4-1.** Age-specific risk of malaria in patients admitted to hospital with *Plasmodium falciparum* (bold line), *Plasmodium vivax* (hatched line), and mixed (dotted line) infections.

Lines represent predicted values from a logistic regression model in which age was entered as a linear effect.
Table 4-1. Characteristics of infants admitted to the Rumah Sakit Mitra Masyarakat (Timika, southern Papua, Indonesia) with malaria

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient age, months</th>
<th>Total</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 – 1</td>
<td>&gt;1 - 3</td>
<td>&gt;3-6</td>
</tr>
<tr>
<td>Proportion of infants with malaria (%)</td>
<td>87/967 (9.0)</td>
<td>147/693 (21.2)</td>
<td>254/933 (27.2)</td>
</tr>
<tr>
<td>Etiology of malaria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( P. falciparum )</td>
<td>34 (39.1)</td>
<td>48 (32.7)</td>
<td>81 (31.9)</td>
</tr>
<tr>
<td>( P. vivax )</td>
<td>42 (48.3)</td>
<td>85 (57.8)</td>
<td>132 (52.0)</td>
</tr>
<tr>
<td>( P. malariae )</td>
<td>0 (0)</td>
<td>2 (1.4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mixed infection</td>
<td>11 (12.6)</td>
<td>12 (8.2)</td>
<td>41 (16.1)</td>
</tr>
<tr>
<td>Hemoglobin concentration, mean g/dL (95%CI) ( c )</td>
<td>7.9 (7.2-8.6)</td>
<td>6.5 (6.2-6.9)</td>
<td>6.4 (6.1-6.7)</td>
</tr>
<tr>
<td>Signs of severity ( c )</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe anaemia ( e )</td>
<td>16 (14.2)</td>
<td>41 (29)</td>
<td>75 (33)</td>
</tr>
<tr>
<td>Respiratory Distress</td>
<td>31 (14.2)</td>
<td>61 (44)</td>
<td>22 (9.7)</td>
</tr>
<tr>
<td>Coma</td>
<td>1 (1.2)</td>
<td>6 (4.3)</td>
<td>5 (2.2)</td>
</tr>
<tr>
<td>Mortality ( e )</td>
<td>2 (2.4)</td>
<td>4 (4.3)</td>
<td>3 (1.3)</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of infants, unless otherwise indicated.

\( a \) Determined by \( \chi^2 \) for trend

\( b \) Determined by overall \( \chi^2 \) test

\( c \) Data restricted to 1424 infants (91%) for whom further details were available.

\( d \) Determined by 1-way analysis of variance.

\( e \) Hemoglobin concentration, <5 g/dL.
Additional clinical details were available in 1424 (91.2%) of the 1560 infants with malaria, with severe disease (defined as severe anaemia, coma or respiratory distress) present in 547 (38.4%) of 1424. The overall risk of severe malaria decreased with age (p<0.001), with the risk of respiratory distress significantly greater in infants < 3 months, compared with older infants (OR, 9.2; 95% CI, 4.5-7.5; p<0.001) (table 4-1). The mean hemoglobin concentration in infants who were admitted to the hospital with malaria was 6.7g/dL (95% CI, 6.6-6.9), compared with 9.7g/dL (95% CI, 9.6-9.9 g/dL) for 1389 infants who were admitted to hospital without malaria during the same period (p<0.0001). The corresponding rates of severe anaemia were 30% (420 of 1424 infants) and 3.9% (54 of 1389 infants; OR, 13.7; 95% CI, 10.1-18.6; p<0.001). Infants with pure \( P. \text{vivax} \) infection were at greater risk of severe anaemia (193 [31.9%] of 6605) than were those with \( P. \text{falciparum} \) malaria (144 [24%] of 601; OR, 1.3; 95% CI, 1.04-1.7; p=0.025) and this risk remained after controlling for age (adjusted OR, 1.5; 95% CI, 1.16-1.95; p=0.002). The risk of severe anaemia in infants was 38.6% (81 of 210) with mixed infections, significantly greater than that with pure \( P. \text{falciparum} \) (p<0.001), but not \( P. \text{vivax} \) infection (p=0.093).

During the study period 173 deaths in infants > 1 week were reported at the hospital of which 23 (13.9%) were associated with malaria. The risk of death was significantly greater in those without malaria (150 [4.4%] of 3416) than for those with malaria (23 [1.6%] of 1474; OR, 2.9; 95% CI, 1.8-4.6; p<0.001). The overall infant mortality associated with \( P. \text{falciparum} \) was 2.2% (13 of 599 infants), which is similar to the rate for \( P. \text{vivax} \) malaria (6 [1.0%] of 603) and mixed species malaria (4 [1.9%] of 210; p=0.258).

Substudy of malaria in young infants (0-3 months old)
Of the 234 infants aged 3 months or younger who were admitted to the hospital with malaria, 187 (76%) were enrolled in the additional study with more detailed data collection (Figure 4-2). No significant difference was found in the age and sex distribution of infants enrolled compared with those not enrolled in the nested study. Microscopic reexamination of the admission blood film revealed concordance between first and second readings of 88% (134/152), with 6 patients (3.2%) found to be blood smear negative. Of the 181 young infants with confirmed malaria, \( P. \)
*falciparum* was present in 55 (30%) patients, *P. vivax* in 102 (56%), mixed infections in 22 (12%) and *P. malariae* in 2 (1%).

**Figure 4-2.** Profile of a study of Plasmodium vivax malaria in early infancy.

Most infants were treated with intravenous quinine alone (93 [51%] of 181 patients), with the remaining receiving intravenous artesunate followed by oral dihydroartemisinin-piperaquine (46 [25%]), oral dihydroartemisinin-piperaquine alone (7 [4%]), oral artesunate (18 [10%]) and oral artesunate-amodiaquine (13
Antimalarial treatment was not documented in 4 young infants. Almost one half (73 [40%] of 181 patients) of the young infants also received antibiotic treatment.

At hospital admission, 152 (84%) of 181 young infants had fever or a history of fever, with a median duration of symptoms before admission of 3 days (interquartile range 2-7 days). Severe disease was present in 128 (70%) of the 181 young infants, splenomegaly in 86 (48%) and malnutrition in 31 (20%) of 154 patients. No significant difference was found in the prevalence of signs and symptoms, nutritional status, or markers of severity according to the species of infection (Table 4-2) or between neonates and infants 1-3 months old.

**Laboratory Investigations**

The geometric mean parasite count was moderately high in both patients infected with *P. falciparum* (8046 per µL\(^{-1}\); 95% CI, 3103-20,827 per µL\(^{-1}\)) and in those infected with *P. vivax* (5814 per µL\(^{-1}\); 95% CI, 4,179-8,425 per µL\(^{-1}\)) (Table 4-2). The mean hemoglobin concentration was 6.8 g/dL (95% CI, 6.4-7.2), with severe anaemia present in 52 (29%) of 176 infants and severe thrombocytopenia (thrombocyte count, < 50,000 cells/mm\(^3\)) in 33 (21%) of 159 infants. Compared with infants who had *P. falciparum* malaria, young infants with *P. vivax* malaria were at greater risk of severe anaemia (OR, 2.4; 95% CI, 1.03-5.9; p=0.041) and severe thrombocytopenia (OR, 3.3; 95% CI, 1.07-10.6, p=0.036) (Table 4-2); both ORs remained statistically significant after we controlled for age (Adjusted OR, 2.2 and 3.1 respectively; p=0.04). The median white blood cell count was 8,500 (Range, 1,300 – 62,400 cells/mm\(^3\)) and did not differ according to the species of infection.

**Outcome**

The median duration of hospitalization for young infants with malaria was 4 days (range, 1-38 days). In total 7 infants (4%) died, 5 of whom had *P. falciparum* malaria, and 2 of whom had *P. vivax* malaria. Three of these infants died ≤ 24 hours after admission to hospital. Additional details are provided in table 4-3.
Table 4-2. Admission characteristics and laboratory investigation of young infants (age less than 3 months of age) admitted with malaria

<table>
<thead>
<tr>
<th></th>
<th>P. falciparum infection (n=55)</th>
<th>P. vivax infection (n=102)</th>
<th>Mixed infection (n=22)</th>
<th>P&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>30/55 (55)</td>
<td>58/102 (57)</td>
<td>14/22 (67)</td>
<td>0.77</td>
</tr>
<tr>
<td>Papuan ethnicity</td>
<td>41/51 (75)</td>
<td>85/99 (86)</td>
<td>19/22 (86)</td>
<td>0.32</td>
</tr>
<tr>
<td>Fever and/or history of fever</td>
<td>46/55 (84)</td>
<td>85/102 (83)</td>
<td>19/22 (86)</td>
<td>0.94</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>26/55 (47)</td>
<td>45/101 (45)</td>
<td>14/22 (64)</td>
<td>0.27</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>16/55 (29)</td>
<td>29/101 (29)</td>
<td>8/22 (36)</td>
<td>0.77</td>
</tr>
<tr>
<td>Symptoms and signs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever and/or history of fever</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenomegaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sign of severity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin concentration, &lt;5 g/dl</td>
<td>10/54 (18)</td>
<td>35/98 (36)</td>
<td>7/22 (32)</td>
<td>0.08&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Respiratory distress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coma</td>
<td>1/54 (2)</td>
<td>2/101 (2)</td>
<td>0/22 (0)</td>
<td>0.81</td>
</tr>
<tr>
<td>Underweight&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13/44 (29)</td>
<td>16/90 (18)</td>
<td>2/18 (11)</td>
<td>0.16</td>
</tr>
<tr>
<td>Geometric mean parasite count, µL&lt;sup&gt;-1&lt;/sup&gt; (95% CI)</td>
<td>8046 (3103-20,827)</td>
<td>5814 (4,179-8425)</td>
<td>1990 (619-6393)</td>
<td>0.10</td>
</tr>
<tr>
<td>Hemoglobin Concentration, mean g/dl (95% CI)</td>
<td>7.8 (7.1-8.5)</td>
<td>6.5 (5.9-6.9)</td>
<td>6.3 (5.2-7.4)</td>
<td>0.005&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Anaemia&lt;sup&gt;d&lt;/sup&gt;</td>
<td>49/54 (91)</td>
<td>94/98 (96)</td>
<td>21/22 (95)</td>
<td>0.41</td>
</tr>
<tr>
<td>Platelet Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median platelets/mm&lt;sup&gt;3&lt;/sup&gt; (range)</td>
<td>113,500 (32,000-685,000)</td>
<td>72,500 (23,000-962,000)</td>
<td>63,000 (35,000-116,000)</td>
<td>0.002&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>&lt;100,000 cells/mm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>24/51 (47)</td>
<td>57/88 (65)</td>
<td>13/18 (72)</td>
<td>0.06</td>
</tr>
<tr>
<td>&lt;50,000 cells/mm&lt;sup&gt;3&lt;/sup&gt;</td>
<td>5/51 (10)</td>
<td>23/88 (26)</td>
<td>5/18 (28)</td>
<td>0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>White Cell Count, median cells/mm&lt;sup&gt;3&lt;/sup&gt; (range)</td>
<td>9,850 (2,500-60,000)</td>
<td>8,050 (1,300-62,400)</td>
<td>7,600 (4,200-12,700)</td>
<td>0.06&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leukopenia&lt;sup&gt;f&lt;/sup&gt;</td>
<td>6/44 (14)</td>
<td>5/74 (7)</td>
<td>4/18 (22)</td>
<td>0.14</td>
</tr>
<tr>
<td>Leukocytosis&lt;sup&gt;g&lt;/sup&gt;</td>
<td>10/44 (23)</td>
<td>7/74 (9)</td>
<td>0/18 (0)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Notes: Data are proportion of infants (%), unless otherwise indicated.

<sup>a</sup> p<0.05 for P. vivax versus P. falciparum.
<sup>b</sup> Weight-for-age 2 SDs below the median value of the reference (healthy) population (WHO, 2006b).
<sup>c</sup> p< 0.001 for P. vivax versus P. falciparum.
<sup>d</sup> Hemoglobin concentration<11 g/dL.
<sup>e</sup> p= 0.003 for P. vivax versus P. falciparum.
<sup>f</sup> White blood cells count <5000 cells/mm<sup>3</sup>.
<sup>g</sup> White blood cells count >25,000 cells/mm<sup>3</sup> in neonates and >15,000 cells/mm<sup>3</sup> in children aged 1-3 months.
Discussion

In Papua severe disease and hospitalization are observed after infection with multidrug-resistant strains of both *P. falciparum* and *P. vivax* (Tjitra et al., 2008). Previous studies in this region have demonstrated that, although the prevalence of *P. falciparum* infection peaks in young adults aged 15-25 years, the burden of *P. vivax* is predominantly in children aged <5 years (Tjitra et al., 2008, Karyana et al., 2008). In the present study, we highlight that the risk of admission to the hospital with malaria with either species starts in early infancy and is associated with hospitalization, severe disease and death. At the Rumah Sakit Mitra Masyarakat hospital, one third of all infant admissions are associated with malaria, with *P. vivax* the predominant species up to 8 months of age (figure 4-1). These findings mirror those of an associated community-based survey in which *P. vivax* infection accounted for 67% of malaria infections in infancy (Karyana et al., 2008). Prospective studies from both Papua New Guinea and Vanuatu have previously described the importance of *P. vivax* in young children with symptomatic disease uncommon in adults (Smith et al., 2001, Maitland et al., 1996, Genton et al., 1995), suggesting the rapid development of immunity (Michon et al., 2007).

In regions of high *P. falciparum* endemicity the risk and morbidity from malaria in early infancy (the first 3 months of life) are relatively low (Wagner et al., 1998, Kitua et al., 1996). In contrast, in Papua where *P. vivax* is highly prevalent we found that malaria was present in almost one fifth of all-cause hospital admissions in young infants, with a risk of severe disease similar to older infants. Our study was restricted to infants who presented to hospital and is thus likely to have preferentially selected those with clinical complications, nonetheless, the degree of morbidity was striking and suggests a significant risk of infection and susceptibility to severe disease. The low prevalence of severe malaria in infants has been attributed to passive immunity from maternal antibodies and a degree of innate immunity (Kitua et al., 1996, Riley et al., 2000). However despite the high prevalence of maternal malaria in this region (17%) (Poespoprodjo et al., 2008) and the high prevalence of antibodies to both *Plasmodium* species in adults (Woodberry et al., 2008), we found that immunity had limited effect in preventing severe malaria from either species in early life.
Severe anaemia was the most frequent manifestation of severity for both *P. falciparum* and *P. vivax* infections and was more prevalent among infants with *P. vivax* infections than among those with pure *P. falciparum* (OR, 1.3). Although severe anaemia associated with *P. falciparum* in Africa generally manifests after 4 months of age (Snow et al., 1997), almost a third of young infants in Papua were severely anemic. This is likely to reflect a number of factors. Hookworm infection and malnutrition reduce mean hemoglobin concentrations; however, the rates of severe anaemia in infants admitted without malaria was only 3.9% and that of a parasitemic infants in a household survey was 0.6% (Karyana et al., 2008), suggesting that, in this age group, malaria was a major contributory factor. Both *P. vivax* and *P. falciparum* cause dyserythropoiesis and hemolysis, resulting in a combination of impaired red cell production and loss of parasitized and unparasitized erythrocytes (Looareesuwan et al., 1987, Collins et al., 2003, Anstey et al., 2009). Community studies conducted in parallel highlighted that only a third of infants with malaria infections were symptomatic (Karyana et al., 2008), making it likely that many infections remain undetected or undertreated before presentation with worsening anaemia. Persistent and recurrent infections are also important components arising from the emergence of multidrug resistant strains of both *P. falciparum* and *P. vivax* (Ratcliff et al., 2007b), associated with repeated bouts of malaria from vivax relapses as well as *P. falciparum* reinfections. Because severe anaemia has been shown to be an important determinant of infant mortality (Afolabi et al., 2001, Kitua et al., 1996), the prevalence and degree of anaemia in Papua are likely therefore to be a major factor in the high infant mortality rates reported locally (68 per 1000 live births).
Table 4-3. Details of young infants (< 3 months) admitted to the hospital with malaria who died

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Nutritional Status</th>
<th>Parasite species</th>
<th>Other Medical Condition</th>
<th>Malaria Treatment</th>
<th>Intravenous Antibiotic Treatment</th>
<th>Time before death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6 days</td>
<td>Female</td>
<td>Severely underweight</td>
<td><em>P. falciparum</em></td>
<td>Neonatal sepsis (clinical diagnosis)</td>
<td>Intravenous Quinine</td>
<td>Ampicillin, gentamicin</td>
<td>38 days</td>
</tr>
<tr>
<td>2</td>
<td>2 months</td>
<td>Male</td>
<td>Not recorded</td>
<td><em>P. falciparum</em></td>
<td>Bronchopneumonia</td>
<td>Intravenous Quinine</td>
<td>Ampicillin, gentamicin</td>
<td>4 days</td>
</tr>
<tr>
<td>3</td>
<td>2 months 17 days</td>
<td>Male</td>
<td>Not recorded</td>
<td><em>P. falciparum</em></td>
<td>Bacterial meningitis (CSF confirmed) Coma, severe anaemia, hypoglycemia, metabolic acidosis</td>
<td>Intravenous Quinine</td>
<td>Ampicillin, gentamicin</td>
<td>6 days</td>
</tr>
<tr>
<td>4</td>
<td>2 months 21 days</td>
<td>Female</td>
<td>Severely underweight</td>
<td><em>P. falciparum</em></td>
<td>Coma, severe anaemia, hypoglycemia, metabolic acidosis Severe anaemia with metabolic and respiratory acidosis</td>
<td>Intravenous Quinine</td>
<td>Ceftriaxone</td>
<td>Within 24 hr</td>
</tr>
<tr>
<td>5</td>
<td>3 months</td>
<td>Male</td>
<td>Normal</td>
<td><em>P. falciparum</em></td>
<td>No record of malaria treatment</td>
<td>Ceftriaxone and gentamicin</td>
<td>No record of malaria treatment</td>
<td>Within 24 hr</td>
</tr>
<tr>
<td>6</td>
<td>1 month 11 days</td>
<td>Male</td>
<td>Normal</td>
<td><em>P. vivax</em></td>
<td>Coma</td>
<td>Intravenous Artesunate</td>
<td>Ampicillin, gentamicin, ceftriaxone</td>
<td>Within 24 hr</td>
</tr>
<tr>
<td>7</td>
<td>2 month 16 days</td>
<td>Male</td>
<td>Underweight</td>
<td><em>P. vivax</em></td>
<td>Respiratory distress and severe anaemia</td>
<td>Intravenous Artesunate and then oral dihydroartemisin-piperaquine</td>
<td>Ampicillin</td>
<td>4 days</td>
</tr>
</tbody>
</table>
Another notable finding of our study was the high level of peripheral parasitaemia in the youngest infants with *P. vivax* infection. *P. vivax* is capable of inducing fever at parasite levels lower than those necessary to cause fever in *P. falciparum* infection. Indeed in associated community surveys and clinical studies in the same area the geometric mean *P. vivax* parasite counts in clinical infections were generally 1500-2000 per µL\(^{-1}\) (Hasugian et al., 2007, Ratcliff et al., 2007a), with the pyrogenic threshold estimated at ≈400-600 per µL\(^{-1}\) (Karyana et al., 2008, Poespoprodjo et al., 2008). In infants younger than 3 months the geometric mean parasite count was significantly higher (5814 per µL\(^{-1}\)) and similar to that observed with *P. falciparum*, suggesting an inherent susceptibility to infection in early infancy, which may reflect either a lack of immunity and/or a greater propensity for parasite multiplication.

In southern Papua, malnutrition generally manifests in infants 5-6 months old when mothers stop breast-feeding their children. In household surveys the overall prevalence of underweight infants is ≈8% in the first year of life (M. Karyana; unpublished data), but was significantly higher in young infants hospitalized with malaria in our study (28% with *P. falciparum* and 18% with *P. vivax*), a finding consistent with either *P. vivax* and *P. falciparum* malaria contributing to malnutrition in these young infants or with malnutrition increasing susceptibility to malaria. Although the relationship between malaria and low nutritional status is complex and likely bidirectional (Caulfield et al., 2004), our results in young infants concur with previous studies in children aged <2 years (Williams et al., 1997).

A limitation of the present study was the unavailability of related details on associated co-morbidities, particularly routine microbiology. A proportion of infants with malaria are likely to have been admitted with alternative diagnoses and incidental parasitaemia (Bejon et al., 2007). Approximately 40% of babies hospitalized with malaria had either leukocytosis or leukopenia, and this did not differ significantly among species of infection. Although sepsis itself may also have contributed to anaemia (Calis et al., 2008), malaria coinfection and its associated anaemia will have undoubtedly contributed to the underlying associated morbidity.

In conclusion, *P. vivax* is associated with major morbidity in early infancy. Malaria prevention strategies such as insecticide-treated nets and intermittent presumptive
treatment for infants, are mainly targeted at reducing the burden of *P. falciparum* infection (Greenwood, 2007). However the chronic and relapsing nature of *P. vivax* infection, and its very early acquisition are likely to undermine the effectiveness of these approaches. Additional studies are needed to confirm whether the degree of morbidity observed in southern Papua can be generalized to other vivax endemic settings. However in regions with mixed *P. vivax* and *P. falciparum* endemicity, improved strategies are needed urgently to reduce infection from all *Plasmodium* species, especially *P. vivax*. Our study suggests that these interventions should be initiated in the perinatal period.
Chapter 5

Severe Congenital Malaria
Acquired In Utero
Chapter 5 - Severe Congenital Malaria Acquired In Utero

Abstract
Vertical transmission of Plasmodium falciparum is under-recognized and usually associated with asymptomatic low-level parasitaemia at birth. We report symptomatic congenital malaria presenting as a neonatal sepsis syndrome. The presence at birth of a high asexual parasitaemia, gametocytemia and splenomegaly indicated in utero rather than intrapartum transmission. The neonate was successfully treated with intravenous artesunate followed by oral dihydroartemisinin-piperaquine, without apparent adverse effects.

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Introduction
Congenital malaria is increasingly recognized as a potentially serious, though usually delayed, complication of maternal malaria. Reported prevalence varies widely in malaria endemic areas from 0--33% (Menendez and Mayor, 2007, Larkin and Thuma, 1991, Lehner and Andrews, 1988). At birth, infections are usually asymptomatic with low parasitaemia and the diagnosis is often missed. Although described at birth (Larkin and Thuma, 1991, Lehner and Andrews, 1988, Vottier et al., 2008), symptoms usually do not appear until 10--30 days of age (Menendez and Mayor, 2007). Because of the very low parasitaemia usually found at birth it was previously hypothesized that infection occurs predominantly from trans-placental passage of parasites during disruption of the placental barrier at the time of delivery, with subsequent clinical illness in the infant attenuated by transfer of maternal antibodies (Menendez and Mayor, 2007). Recent evidence however suggests that antenatal transplacental transmission occurring prior to the onset of parturition is more frequent than previously realized (Malhotra et al., 2006), although the clinical consequences of in utero transmission are not well characterized and its management poorly defined.
In Papua, Indonesia, an area endemic for multidrug resistant *Plasmodium falciparum* and *P. vivax* (Ratcliff et al., 2007b), malaria is a major cause of morbidity in pregnancy (Poespoprodjo et al., 2008) and infants (Poespoprodjo et al., 2009). We report a case of a neonate with high-level *P. falciparum* parasitaemia and gametocytemia at birth demonstrating vertical transmission *in utero*, with severe disease requiring intravenous therapy. We describe the successful use of artemunate and dihydroartemisinin-piperaquine in this neonate.

**Case Report**

At birth, a female neonate weighing 2350 grams was pale, lethargic, unable to feed, hypothermic (36.3°C), and tachypneic (respiratory rate 96/minute), with chest indrawing and a normal heart rate (124/minute). An enlarged spleen was palpable at the umbilicus. Delivery was by uncomplicated vaginal delivery at 40 weeks gestational age estimated by Ballard score, with normal passage of meconium. A blood film performed on the day of delivery as part of routine hospital practice showed a *P. falciparum* peripheral parasitaemia of 7,575/µL. Her hemoglobin was 10.6 g/dL with a leukocyte count of 13,900 cells/µL.

The mother, a 35 year old grand-grand multiparous Papuan lowland woman (P11 A0), had not received any antenatal care but denied any history of fever or other complications during her pregnancy. Maternal peripheral blood examination was negative both on the day of delivery and 24 hours later, as was a *P. falciparum* histidine rich protein (HRP2) rapid antigen detection test (Paracheck™). The placenta was unavailable for analysis. At birth maternal hemoglobin was 8.5 g/dL with a leukocyte count of 9,600 cells/µL. Her ten other children were reportedly well.

The screening blood film from birth was reported at 36 hours, at which time the parasitaemia had risen to 26,700/µL. Because of the severity of illness, antimalarial therapy was commenced intravenously, using artemunate, standard treatment for severe malaria in older children at this hospital. Three doses (8 mg [3.4 mg/kg]) were administered at 24, 36 and 48 hours after birth. With clinical improvement, therapy was changed to oral dihydroartemisinin-piperaquine (DHP), 2 mg/kg
dihydroartemisinin and 16 mg/kg piperaquine crushed in a suspension of water, administered once daily for 3 days. Procaine penicillin was also given for 3 days.

The neonate had clinically improved within 24 hours and by 48 hours was aperasitemic; (Table 5-1). *P. falciparum* gametocytes were present on day 2 and days 4-8. In view of brown gastric aspirates, oral intake was restricted for the first 48 hours and intravenous ranitidine administered. On day 3 breast milk was initiated via an oro-gastric tube and the baby was breastfed from the following day. Because of progressive anaemia (Table 5-1), a transfusion of 25 ml of packed red cells was administered on day 4 and again on day 10.

At the time of discharge (day 11), the infant was afebrile, feeding well, with a hemoglobin of 14.4 g/dL. On follow up 8 days later, she was active, breastfeeding well, without any signs or symptoms. She was readmitted aged 9 months with acute diarrhoea and dehydration, with weight for age less than the 3rd percentile, but with no neurodevelopmental delay. She was anemic (Hb 9.4 g/dL) with a normal WCC (9,800 cells/µL) and no parasitaemia. She recovered from diarrhea and was discharged with nutritional education.
Table 5-1. Clinical course of congenital malaria during hospitalization

<table>
<thead>
<tr>
<th>Hospitalization day</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (day)</strong></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
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<tr>
<td><strong>Laboratory Results</strong></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asexual parasitaemia (µL⁻¹)</td>
<td>7,575</td>
<td>26,688</td>
<td>12,649</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Gametocytes (µL⁻¹)</td>
<td>Negative</td>
<td>556</td>
<td>Negative</td>
<td>278</td>
<td>Negative</td>
<td>208</td>
<td>Negative</td>
<td>208</td>
<td>139</td>
<td>Negative</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11.5</td>
<td>14.4</td>
</tr>
<tr>
<td>Leukocyte count (µL⁻¹)</td>
<td>13,900</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Clinical Data</strong></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (g)</td>
<td>2350</td>
<td>2200</td>
<td>2250</td>
<td>2250</td>
<td>2250</td>
<td>2400</td>
<td>2450</td>
<td>2500</td>
<td>2400</td>
<td>2500</td>
</tr>
<tr>
<td>Axillary temperature (ºC; range)</td>
<td>36.3-38.2</td>
<td>36-37</td>
<td>36-36.8</td>
<td>36-37</td>
<td>35.9-36.2</td>
<td>35.2-36.6</td>
<td>36.2-36.6</td>
<td>36.2-36.6</td>
<td>36.2-36.6</td>
<td>36.2-36.6</td>
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<tr>
<td>Respiratory rate/min (range)</td>
<td>42-96</td>
<td>40-48</td>
<td>42-56</td>
<td>48-60</td>
<td>52-80</td>
<td>40-68</td>
<td>38-44</td>
<td>32-44</td>
<td>40-52</td>
<td>40-40</td>
</tr>
<tr>
<td>Feeding</td>
<td>Unable to feed</td>
<td>Unable to feed</td>
<td>Breast milk</td>
<td>Breast milk</td>
<td>Breast milk</td>
<td>Breast milk</td>
<td>Breast milk</td>
<td>Breast milk</td>
<td>Breast milk</td>
<td>Breast milk</td>
</tr>
<tr>
<td>Urine output</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
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<tr>
<td><strong>Medications</strong></td>
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<tr>
<td>Artesunate IV</td>
<td>I</td>
<td>II-III</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Dihydroartemisin-piperaquine – Oral</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Procaine penicillin IM</td>
<td>I</td>
<td>II</td>
<td>III</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Ranitidine IV</td>
<td>√</td>
<td>√</td>
<td>√</td>
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<tr>
<td>Humidified oxygen</td>
<td>√</td>
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<tr>
<td>Packed red cell transfusion</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td>I</td>
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<td>II</td>
</tr>
</tbody>
</table>
Discussion
This report documents congenital malaria with severe manifestations at birth. The presence of relatively high parasitaemia within 24 hours of an uncomplicated delivery, gametocytemia (a marker of chronicity), and marked splenomegaly all indicate that vertical transmission occurred prior to delivery with parasite replication in utero. Although symptomatic malaria at birth has been reported (Larkin and Thuma, 1991, Lehner and Andrews, 1988, Vottier et al., 2008), the majority of infections are asymptomatic (Menendez and Mayor, 2007, Lehner and Andrews, 1988, Pengsaa, 2007) and severe manifestations, as in this case, are rare. In addition, the parasitaemia is usually low (Balaka et al., 2000, Akindele et al., 1993).

The lack of maternal parasitaemia and HRP2 antigenemia suggests that maternal infection was localized to the placenta and/or had cleared. Discordance between maternal peripheral blood microscopy/antigen testing and placental parasitization is well described (Rogerson et al., 2003a). Placental analysis was not routine and we could not determine whether there was associated placental infection. While the mother denied a history of febrile illness during the current pregnancy, in this area 66% of adults and 58% of pregnant women with P. falciparum infection are asymptomatic (Poespoprodjo et al., 2008). The incidence of malaria in Timika is estimated to be approximately 850 per 1000 person years (Poespoprodjo et al., 2008) making it highly likely that the 35 year old Papuan mother resident in the malaria-endemic lowlands had prior exposure to malaria infection.

Antenatal malaria transmission is associated with placental malaria particularly in primi- and secundi-gravidae (Malhotra et al., 2006). There are limited data on the influence of parity on congenital malaria (Larkin and Thuma, 1991, Menendez and Mayor, 2007). In the present case, vertical transmission occurred in an apparently well grand grand multiparous woman with longstanding malaria exposure. Relatively high in utero parasitaemia developed despite presumed maternal immunity and the reduced parasite growth rates associated with fetal haemoglobin (Menendez and Mayor, 2007). It was not possible to determine other factors associated with an increased the risk of vertical transmission, such as HIV infection (Villamor et al., 2005). Grand grand multiparous women are known to have smaller
and dysfunctional placentae, and this may have allowed a greater than normal maternofetal microtransfusion and a greater parasite inoculum than that hypothesized to occur in utero (Malhotra et al., 2006), resulting in a greater risk of symptomatic disease at birth.

The clinical manifestations in this case are similar to those seen with early neonatal sepsis, and without the policy of routine neonatal testing in this hospital, the diagnosis could easily have been missed (Ekanem et al., 2008). Although we cannot exclude concurrent bacterial sepsis, there were no risk factors for neonatal sepsis, the white cell count was normal, marked splenomegaly is very unusual in neonatal sepsis, and the level of parasitaemia made incidental parasitaemia unlikely. Over the four years of the routine neonatal malaria screening program at this hospital, median asexual parasitaemia among the other 29 neonates with detectable parasitaemia at birth was 75/uL (range 37-1,730/uL) (unpublished data), with this neonate having by far the highest parasitaemia. Although WHO criteria for severe malaria are not defined in neonates, the pallor, hypothermia, lethargy, inability to feed and respiratory distress justified a diagnosis of severe malaria and intravenous therapy. The clinical condition improved in parallel with a rapid clearance in parasitaemia with intravenous artesunate and oral DHP. Although safe and effective in reducing mortality from severe malaria compared to quinine in adults and children > 2 years (Dondorp et al., 2005), data on the safety and efficacy of intravenous artesunate in infants are limited (Poespoprodjo et al., 2009) with scant data on its use in neonates. Intravenous artesunate (three doses of 3.4 mg/kg over 24 hours) appeared safe, and rapidly cleared parasitaemia, in keeping with clinical experience in older age groups (Dondorp et al., 2005, Hasugian et al., 2007).

Due to the high prevalence of multidrug resistant P. falciparum and P. vivax in Papua, RSMM Hospital protocols for oral step-down therapy following intravenous artesunate therapy recommend DHP in children weighing more than 5 kg based on locally-derived safety and efficacy data with DHP in treating uncomplicated malaria in children in this weight range (Hasugian et al., 2007). However due to the limited effective antimalarial options in Timika (Ratcliff et al., 2007b), DHP was administered to this neonate by the treating pediatrician with close monitoring of potential adverse reactions. Although it appeared to be well tolerated, further studies
are required to evaluate the safety, efficacy and pharmacokinetics of DHP in very young infants.

Potential causes for the intrauterine growth retardation in this neonate include maternal anaemia (Poespoprodjo et al., 2008), congenital malaria infection per se (Menendez and Mayor, 2007) and the anaemia associated with congenital infection in this instance. In areas of high endemicity, fetal anaemia is associated with maternal hemoglobin concentrations below 8g/dl (Brabin et al., 2004a). Although *P. falciparum* placental parasitaemia and maternal peripheral parasitaemia increase the risk of fetal anaemia it is unclear in this case whether the maternal anaemia was associated with placental malaria or not (Brabin et al., 2004a). Low birth weight is also associated with higher susceptibility to infectious diseases and poor growth in later life as seen in this infant nine months later.

In summary, this case demonstrates vertical transmission *in utero* causing severe congenital malaria at birth, associated with neonatal anaemia and growth restriction. Symptomatic neonates in malaria endemic areas presenting with neonatal sepsis syndrome, should be screened for malaria. Although further data in neonates are required, intravenous artesunate followed by oral DHP treatment appeared safe and effective.
Chapter 6

Highly effective therapy for maternal malaria reduces the risk of vertical transmission
Chapter 6 - Highly effective therapy for maternal malaria reduces the risk of vertical transmission

Abstract

Background. The epidemiology, treatment and prevention of congenital malaria are poorly understood, particularly in malarious areas outside of Africa. We aimed to address this using data gathered from a hospital based malaria surveillance study in Papua, Indonesia.

Methods. From April 2005 to January 2010, 4878 women at delivery and their newborns were enrolled in the study and screened for malaria using microscopic examination of peripheral blood. A standardized form was used to document the clinical records of the mothers and newborns.

Findings. The prevalence of maternal malaria at delivery was 19% (929/4881) with *P. falciparum* present in 54% of infections and *P. vivax* in 34.5%. Congenital malaria occurred in 8 per 1000 (38/4884) of live births with *P. falciparum* accounting for 76.3% (29) of infections, *P. vivax* for 15.8% (6), *P. ovale* for 2.6% (1) and mixed infections present in 2 (5.3%) neonates. The geometric mean of parasitaemia in the newborns was 110 µl\(^{-1}\) (95%CI, 73-164 µl\(^{-1}\)) with no difference between *P. falciparum* and *P. vivax* infection (p=1.0). The majority (97%, 37/38) of these infections were asymptomatic. Maternal malaria at delivery (AOR=9.5; 95%CI, 4.2-21.5, p<0.001), mothers aged <16 years old (AOR=4; 95%CI, 1.4-12.1; p=0.011), and a prior history of malaria during pregnancy (AOR=2.2; 95%CI, 1.1-4.4, p=0.022) were independent risk factors for vertical transmission. Of 29 mothers and their babies with positive peripheral parasitaemias, 17% (5) had parasite species discordance suggesting possible antenatal malaria transmission. Newborns with malaria had 3.4 [95%CI 1.7-6.6] fold higher risk of having low birth weight than those without malaria, (p<0.001) and this remained after controlling for maternal malaria. Vertical malaria transmission was significantly lower in pregnant women with a history of dihydroartemisinin-piperaquine (DHP) treatment compared to those treated with chloroquine + sulfadoxine-pyrimethamine /quinine (OR= 0.04 [95%CI, 0.005-0.3]), p<0.001. Since the introduction of DHP for uncomplicated malaria in
the second and third trimesters of pregnancy, the incidence of congenital malaria has fallen from 3% to 0.2%.

**Conclusions.** Congenital malaria is an important cause of morbidity in early life in these region coendemic for *falciparum* and *vivax* malaria. The introduction of a highly effective artemisinin combination therapy has reduced significantly the risk of early congenital infection.
Results

Congenital malaria
Between April 2004 and January 2010, 4884 (85%) of 5728 babies born at RSMM were screened for malaria (Figure 6-1). In total 38 (0.8%) neonates were diagnosed with congenital infection within the first week of life. *P. falciparum* accounted for 76.3% (29) of infections, *P. vivax* for 15.8% (6), *P. ovale* for 2.6% (1) and mix for 5.3% (2) of the infections. There was one pair of twins with congenital malaria both of whom had *P. falciparum*. The geometric mean of parasitaemia of the newborns was 74 µl⁻¹ (range 37- 4023 µl⁻¹). All newborns were asymptomatic, with the exception of one baby with fever and hyperparasitaemia reported previously (Poespoprodjo et al., 2010). The geometric mean of parasitaemia was similar between infants infected with *P. falciparum* (121 µl⁻¹ [95%CI, 73 - 200 µl⁻¹]) and *P. vivax* (90 µl⁻¹ [95%CI, 40-200 µl⁻¹]), p=1.0.

Newborns with congenital malaria had a lower birth weight (mean=2764 g [95%CI, 2553-2975 g]), compared to newborns without malaria (mean=3031 g [95%CI, 2997-3028], p=0.02), the Odds Ratio (OR) for low birth weight being 3.4 [95%CI, 1.7-6.6]; p<0.001. The effect on low birth weight remained (AOR=2.8 [95%CI, 1.2-6.6], p=0.002) after controlling for other known risk factors in this population (maternal age, parity, maternal malaria and severe anaemia) (Poespoprodjo et al., 2008).

Antimalarials were given to all parasitaemic newborns, 32 receiving quinine (oral +/- intravenous), 2 intravenous artesunate followed with DHP and 4 oral DHP alone. All malaria smears were negative at time of discharge from hospital.
4878 pairs of mothers and newborns were screened for malaria
April 2005 – January 2010

2 mothers had missing newborn smear data

4839 mothers had malaria negative newborns
(4699 singletons and 140 sets of twins)

72 mothers had infants readmitted with malaria at ≤ 3 months old:
66 singletons:
13 *P. falciparum*
50 *P. vivax*
3 mix infection
4 half of twins: 4 *P. vivax*
2 sets of twins:
2 *P. falciparum*
2 *P. vivax*

37 mothers had malaria positive newborns:
36 singletons:
27 *P. falciparum*
6 *P. vivax*
1 *P. ovale*
2 mix infection
1 set of twin:
2 *P. falciparum*

Figure 6-1. Congenital Malaria Study profile
Maternal parasitaemia and vertical transmission

Data on the blood film examination of the mothers were available in 4878 women, the prevalence of maternal malaria being 19% (928); table 6-1. Of the 37 recorded cases of early vertical transmission 9 (24%) were born from aparasitaemic mothers. In the remaining 28 women with malaria at delivery, 5 had neonates with different parasite species, giving rise to discordance in 18% of cases (Table 6-1).

Maternal risk factors

Maternal risk factors for vertical transmission are shown in table 6-2. The risk of vertical transmission was 3% in women with peripheral parasitaemia at delivery compared to 0.2% in those aparasitaemic (OR=13.6 [95%CI, 6.4-28.9, p<0.001]) with the population attributable risk of congenital infection being 70%. The risk of transmission did not vary with degree of maternal peripheral parasitaemia even after controlling for the species of infection (table 6-3). Afebrile mothers with malaria had a risk of transmitting the parasites vertically (2.5%, 16/642) compared to symptomatic parasitaemic mothers (4.2%,12/284), p=0.21. Regardless of parasitaemia status at delivery, women with possible history of malaria infection during pregnancy had a 4.1 fold [95%CI, 2.1-7.9] greater risk of vertical transmission to the foetus compared to mothers without any history of malaria infections, p<0.001. In the multivariate models, maternal malaria at delivery, history of malaria during pregnancy and pregnant women aged ≤ 16 years old remained as independent risk factors for vertical transmission; table 6-2. Together these factors explained 89% of all congenital infections.

Malaria treatment in pregnancy

In total 905 pregnant women described a prior febrile episode during their pregnancy and were treated with antimalarial medication, with vertical transmission of infection was observed in 18 (2%) births. DHA-piperaquine has been the first line treatment of uncomplicated malaria in the 2nd and 3rd trimesters of pregnancy since March 2006. Babies born of mothers with a history of DHP treatment during pregnancy had significantly lower risk of malaria (0.2%, 1/471) compared to those with prior exposure to CQ/Quinine treatment (4.9%, 17/348), OR= 0.04 (95%CI, 0.005-0.3), p<0.001. The number of congenital malaria declined significantly following the
change in malaria treatment policy, from 3.2% (29/907) before policy change to 0.2% (9/3912) after, \( p<0.001 \) (OR=0.07 [95%CI, 0.03-0.15]). There has been no reported congenital malaria case since October 2008 (Figure 6-2). After controlling for other confounding factors, malaria treatment policy change remained as a protective factor for congenital malaria (AOR=0.18 [95%CI, 0.032-0.89]), \( p=0.036 \).

**Possible late manifestation of congenital infection**

Of 4878 hospital deliveries, 74 infants from 72 mothers (66 singletons, 4 half twins and two sets of twin) were readmitted to the hospital in the first 3 months of life with symptomatic malaria. All of these infants had been screened at birth with negative blood films. On readmission, *P. falciparum* accounted for 20% (15), *P. vivax* for 76% (56) and mix infections for 4% (3) of the infections. One baby boy (92 days old) with mix *P. falciparum* and *P. vivax* malaria had severe anaemia and died within 24 hours of his hospitalization. All of the other children were discharged from hospital after a median of 3 days (IQR 1-2) inpatient stay.

The major maternal factors associated with admission of the infant to hospital with malaria in the first three months of life were maternal malaria at delivery (OR=3.3 [95%CI, 2.1-5.3], \( p<0.001 \)) and a history of malaria during pregnancy (1.8 [95%CI, 1.1-3], \( p=0.024 \)). Low birth weight infants and twins had a 2.3 ([95%CI, 1.3-3.8], \( p=0.005 \)) and 3.5 ([95%CI, 1.6-7.9], \( p=0.01 \)) fold higher risk of malaria in early life compared to those with normal birth weight and singletons respectively.
Table 6-1. Vertical malaria transmission profile

<table>
<thead>
<tr>
<th>Maternal parasitaemia</th>
<th>Parasitaemia at birth*</th>
<th>Overall vertical transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td><em>P. falciparum</em></td>
</tr>
<tr>
<td></td>
<td>3939 (99.8)</td>
<td>8 (0.2)</td>
</tr>
<tr>
<td><em>P. falciparum</em> (n=501)</td>
<td>488 (97.2)</td>
<td>12 (2.4)</td>
</tr>
<tr>
<td><em>P. vivax</em> (n=321)</td>
<td>314 (97.8)</td>
<td>3 (0.9)</td>
</tr>
<tr>
<td><em>P. ovale</em> (n=3)</td>
<td>2 (66.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><em>P. malariae</em> (n=50)</td>
<td>49 (98)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Mix infections (n=53)</td>
<td>47 (88.7)</td>
<td>4 (7.5)/3</td>
</tr>
</tbody>
</table>

Note: All data are number of cases (%); * where deliveries included multiple births, vertical transmission was reported if at least one of the babies was found to be parasitaemic.
<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Prevalence in controls N/valid cases (%)</th>
<th>Prevalence of cases exposed to factor N/valid cases (%)</th>
<th>Univariate Analysis OR (95%CI) P value</th>
<th>PAR</th>
<th>Multivariate Analysis AOR (95% CI) P value</th>
<th>PAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal malaria</td>
<td>899/4838 (19)</td>
<td>28/37 (75)</td>
<td>13.6 (6.4-28.9) &lt;0.001</td>
<td>70%</td>
<td>9.5 (4.2-21.5) &lt;0.001</td>
<td>68%</td>
</tr>
<tr>
<td>• No</td>
<td>3939/4838 (81)</td>
<td>9/37 (24)</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>• <em>P. falciparum</em> malaria</td>
<td>487/4838 (10)</td>
<td>13/37 (35)</td>
<td>11.6 (4.9-27.4) &lt;0.001</td>
<td>32%</td>
<td>7.6 (2.9-19.4) &lt;0.001</td>
<td>30%</td>
</tr>
<tr>
<td>• <em>P. vivax</em> malaria</td>
<td>314/4838 (6.5)</td>
<td>7/37 (18.9)</td>
<td>9.7 (3.6-26.4) &lt;0.001</td>
<td>17%</td>
<td>6.9 (2.4-19.3) &lt;0.001</td>
<td>16%</td>
</tr>
<tr>
<td>Maternal Fever</td>
<td>352/4837 (7.3)</td>
<td>13/37 (35)</td>
<td>6.9 (3.5-13.7) &lt;0.001</td>
<td>30%</td>
<td>1.6 (0.8-3.4) 0.207</td>
<td>13%</td>
</tr>
<tr>
<td>Maternal History of febrile illness</td>
<td>906/4841 (19)</td>
<td>18/37 (48.6)</td>
<td>4 (2.1-7.9) &lt;0.001</td>
<td>36%</td>
<td>2.2 (1.1-4.4) 0.022</td>
<td>26%</td>
</tr>
<tr>
<td>Young mother (&lt; 16 yo)</td>
<td>114/4840 (2.4)</td>
<td>4/37 (10.8)</td>
<td>5 (1.7-14.4) 0.012</td>
<td>8.6%</td>
<td>4.1 (1.4-12.1) 0.011</td>
<td>8.2%</td>
</tr>
<tr>
<td>Primipara</td>
<td>1515/4838 (31)</td>
<td>12/37 (32.4)</td>
<td>1.05 (0.5-2.1) 0.860</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Severe anemia</td>
<td>329/4701 (7)</td>
<td>3/36 (8.3)</td>
<td>1.2 (0.4-3.9) 0.74</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>739/4825 (15.3)</td>
<td>8/37 (21.6)</td>
<td>1.5 (0.7-3.3) 0.260</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 6-3. Maternal parasitaemia at delivery and vertical malaria transmission

<table>
<thead>
<tr>
<th>Maternal Parasitaemia (Geometric mean/µL)</th>
<th>Vertical malaria transmission</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>• P. falciparum</td>
<td>403 (81-1998)</td>
<td>1480 (1211-1998)</td>
</tr>
<tr>
<td>• P. vivax</td>
<td>2186 *</td>
<td>665 (200-1998)</td>
</tr>
</tbody>
</table>

Note: * one case only

**Discussion**

This large surveillance study defines the epidemiology of congenital malaria and its outcome after treatment in Papua, Indonesia. Congenital malaria was observed in 0.8% of live births, the risk of vertical transmission rising almost 9 fold in women with peripheral parasitaemia at delivery. This incidence was significantly lower than that reported from areas of high malaria transmission in Africa, which generally range from 5 to 33% (Fischer, 1997, Falade et al., 2007, Okafor et al., 2006) and even higher in women with peripheral parasitaemia (19-50%) (Falade et al., 2007, Okafor et al., 2006, Larkin and Thuma, 1991). Contrary to previous hypotheses, these findings suggest that vertical transmission is likely to be determined by other factors than just the level of malaria exposure and associated maternal immunity (McGregor, 1984).

In almost 20% of cases the parasite species in the baby was discordant with the peripheral parasitaemia of the mother. Vertical transmission of malaria occurring antenatally is well described in both African and in South East Asian settings (Malhotra et al., 2006, Fischer, 1997, Falade et al., 2007, Tobian et al., 2000, Pengsaa, 2007, McGready et al., 2004) and this may explain some of the maternal and neonatal discordance in parasite species. In addition the vertical transmission of infection may reflect the species of placental infection, in which case the peripheral parasitaemia will be an incidental finding. However since we did not routinely examine placental tissue we were unable to investigate this further.

Most reports of congenital malaria originate from Subsaharan Africa where the majority of infections are due to *P. falciparum*. The epidemiology of congenital
infection in Asia and South America is less well described. In these regions, where *P. falciparum* and *P. vivax* are coendemic, vertical transmission can occur with either species (Pengsaa, 2007, McGready et al., 2004, Valecha et al., 2007, De Silva et al., 1982). In the present study we routinely screened all babies within 3 days of birth, and were thus able to reliably exclude infections acquired early in the postnatal period. *Plasmodium vivax* congenital infection (alone or mixed) occurred in 1.6 per 1,000 live births and accounted for 22% of all congenital infections. The median falciparum parasitaemia observed in newborns in Papua was higher (100 µl⁻¹, range 50-26,688 µl⁻¹) than that reported in Africa (48 µl⁻¹, range 8-200 µl⁻¹), although in contrast infections in Papua were mostly asymptomatic compared to a third of infections being symptomatic in Africa (Falade et al., 2007). These findings could not be explained by early detection since in both studies active screening was conducted at birth.

Recently foetal exposure to malarial parasites *in utero* have been shown to modify the immune response of the newborn, increasing their susceptibility to symptomatic malaria infections at birth and later in life (Broen et al., 2007, Dent et al., 2006, Malhotra et al., 2009, Mutabingwa et al., 2005). In Africa where peripheral parasitaemia and placental malaria are highly prevalent this increased susceptibility is associated with a greater risk of neonatal parasitaemia and clinical malaria (McGregor, 1984, Rogerson et al., 2003a, Mockenhaupt et al., 2002). In such settings neonatal screening is often reserved for symptomatic infants. However in lower transmission areas the consequence of leaving asymptomatic congenital malaria untreated may be significantly associated with greater malaria morbidity in early life (Poespoprodjo et al., 2009). As such early detection by active screening of all babies is warranted where resources permit.

African infants with congenital malaria are at greater risk of low birth weight than aaparasitemic infants (Larkin and Thuma, 1991, Okafor et al., 2006) and a similar observation was apparent in our Papuan study. Premature delivery associated with maternal malaria could not explain this increased risk suggesting that other factors such as placental infection and insufficiency may play a crucial role in reducing foetal growth (Adebami et al., 2007).
Our study highlights the importance ensuring the effective treatment of maternal malaria. The artemisinin combination therapy (dihydroartemisinin-piperaquine) has excellent efficacy against both multidrug resistant *P. falciparum* and *P. vivax*. In March 2006 local guidelines for the treatment of maternal malaria in the second and third trimesters of pregnancy were changed from chloroquine plus sulfadoxine-pyrimethamine or quinine to DHP. Over the ensuing 3 years this policy has been associated with a large reduction in the incidence of congenital malaria. Indeed we have not witnessed a case of congenital malaria since October 2008, despite screening almost 1416 babies for malaria. In most parts of Africa, chloroquine and sulfadoxine-pyrimethamine remain the main treatment options for maternal malaria. (Newman et al., 2003, Falade et al., 2007, Steketee et al., 1996b, Nosten et al., 2007) and yet this position is threatened by high rates of aminoquinoline and antifolate drug resistance. This may in part explain the high rates of reported congenital malaria found across the subcontinent (Larkin and Thuma, 1991, Okafor et al., 2006, Falade et al., 2007), and further emphasizes the need for exploring more effective and reliable treatment strategies in pregnancy.

There are a number of limitations to our study. We were reliant on microscopy of peripheral blood film for diagnosing malaria, and this is likely to have underestimated the real burden of congenital malaria by missing low parasitaemia infections and misdiagnosis of the species of infection (Mayxay et al., 2004). The use of PCR diagnostics in previous reports may have detected a higher proportion of infections (Adachi et al., 2000, Tobian et al., 2000, Wagner et al., 1998). In addition, the lack of cord blood parasitaemia and placental malaria data in our study will have also underestimated the true burden of congenital malaria. (Tobian et al., 2000, Falade et al., 2007). Interpretation and attribution of parasitaemia in early life is difficult, since parasitaemia of the mother results in increased risk of neonatal infection, both from vertical transmission and from environmental exposure. The risks to the baby documented in this paper are therefore conservative estimates.

Currently, there are no consensus international guidelines for the case management of congenital malaria. In Africa asymptomatic congenital malaria is often left untreated if the parasites are cleared within 3 days (Okafor et al., 2006). Chloroquine and sulfadoxine-pyrimethamine have been used previously for treating congenital
falciparum malaria (Larkin and Thuma, 1991, Hindi and Azimi, 1980), but with increasing antimalarial drug resistance these treatments can no longer be advocated (Ronn et al., 1996, Checchi et al., 2002, Wongsrichanalai et al., 2002, Ratcliff et al., 2007b).

At our study site all infants with positive blood films were treated with antimalarial drugs irrespective of symptomatology, however treatment options in this region are limited (Poespoprodjo et al., 2009). Due to the high prevalence of multidrug resistance in both *P. falciparum* and *P. vivax* (Ratcliff et al., 2007b), local guidelines recommend that infants less than 5 kg in weight with malaria should be treated with either a seven day course of oral quinine or, if unable to tolerate oral medication intravenous artesunate followed with DHP. In the current report all infants responded well to treatment although were not systematically following discharge from hospital. The reality is that unsupervised 7 day courses or oral quinine are rarely complied with and associated late recurrence rates are high (Ratcliff et al., 2007b). Current guidelines on the use of artemisinin combination therapy usually restrict its use to infants weighing more than 5kg, however evidence for the safety and efficacy of reliable short course treatment regimens is urgently required for malaria in early life.

In conclusion, our study provides insights into the epidemiology of congenital malaria in endemic areas outside Africa. The introduction of a highly effective artemisinin combination therapy for malaria treatment in the second and third trimesters of pregnancy was associated with a marked decrease in the risk of congenital malaria. In addition routine screening of newborns for asymptomatic parasitaemia followed by prompt, effective antimalarial treatment could reduce malaria burden in early life even further.
Chapter 7

Dihydroartemisinin-piperaquine use in pregnant women with malaria: a preliminary safety and toxicity report
Chapter 7 - Dihydroartemisinin-piperaquine use in pregnant women with malaria: a preliminary safety and toxicity report

Abstract

Background: Pregnant women and neonate are susceptible to adverse outcomes of malaria but treatment options are limited. Dihydroartemisinin-piperaquine (DHP) is known as safe and highly effective antimalarial in non-pregnant adults but limited information is available on its use in pregnancy. We report the safety and toxicity profile of antenatal DHP exposure and the impact on pregnancy outcomes following change in antimalarial treatment policy to artemisinin combination therapy.

Methods and Findings: From April 2004 to June 2009, 6519 pregnant women were enrolled in a hospital based malaria surveillance study and screened for malaria. Data for the safety analysis were available in 1217 pregnant women with acute antimalarials exposure on admission to hospital (765 exposed to DHP) and 847 women with a history of antimalarial exposure during the current pregnancy (395 with prior DHP exposures). Compared to women with prior exposure to quinine or chloroquine+- sulfadoxine-pyrimethamine, a history of DHP treatment during the current pregnancy reduced the risk of recurrent malaria at delivery (OR=0.37 (95%CI 0.27-0.52), p<0.001), congenital malaria (OR=0.06, [95%CI, 0-0.46], p=0.001) and perinatal death (AOR=0.32 [95%CI, 0.12-0.85]; p=0.03) when used in the second and third trimester of pregnancy. There was no increased risk of congenital malformations in pregnant women exposed to DHP. In women with a prior history of malaria treatment policy change to DHP in the second the third trimester of pregnancy was associated with a 3 folds reduction in women delivering with malaria and a 0.4 fold reduction in low birth weight babies.

Conclusions: DHP is safe and effective for malaria treatment in the second and third trimester of pregnancy. Further prospective studies are required to define DHP efficacy and safety for both malaria treatment and prevention in this high-risk group.
**Introduction**

As part of WHO recommended strategies,(WHO/AFRO, 2004) all pregnant women admitted to the maternity ward of RSMM have been screened for malaria since April 2004. All women are treated according to standard protocols, which until March 2006 were chloroquine or quinine. Following the declining efficacy of sulfadoxine-pyrimethamine (SP) and chloroquine (CQ) in curing malaria,(Adam et al., 2004, Collignon, 1991, Fryauff et al., 1997, Fryauff et al., 1998, Garavelli and Corti, 1992, Molta et al., 2003, Ratcliff et al., 2007b, Ronn et al., 1996, Checchi et al., 2002, Wongsrichanalai et al., 2002) WHO has recommended locally effective artemisinin combination therapy (ACT) as the first line treatment for *falciparum* malaria in the second and third trimester of pregnancy.(WHO, 2006a)

Clinical drug trials conducted in Timika in 2005 revealed the efficacy of standard regimens of chloroquine, sulfadoxine-pyrimethamine and unsupervised quinine were unacceptable with failure rates by day 28 exceeding 60% for both *P. falciparum* and *P. vivax.*(Ratcliff et al., 2007b) In view of these findings, ministry of health treatment guidelines were changed in the Mimika District in March 2006 to reflect rising antimalarial drug resistance to both *P. falciparum* and *P. vivax*. Dihydroartemisinin-piperaquine (DHP) became the recommended first line treatment for uncomplicated malaria from any species of infection.(Ronny et al., 2006) In view of the limited alternatives in pregnancy, DHP also became first line treatment of malaria in the second and third trimester of pregnancy.(Ronny et al., 2006) Intravenous artesunate is recommended for the initial treatment of pregnant women with severe malaria from any species of infection in the second and third trimester of pregnancy followed with DHP as soon as the patient can take oral drugs. The aim of this report is to document the exposure of pregnant women to DHP in pregnancy and assess its potential safety on both mother and baby and impact on malariometric parameters and pregnancy outcome.

**Methods**

The effect of antimalarials exposure during pregnancy in this study was evaluated in women with acute exposure (at the time of admission), as well as those with prior history, both individually and at a population level before and after policy change.
Definitions of the treatment group:

- Acute exposure of antimalarial drug is defined as malaria treatment received on hospital admission when the patient was enrolled.
- History of malaria treatment during pregnancy is defined as history of last malaria treatment during the current pregnancy obtained by a systematic interview method and when applicable, confirmed with hospital medical records.

The effect of malaria treatment to congenital malformation rate has only been analysed since May 2007. A trained research nurse performed a systematic evaluation on external congenital anomalies at birth and was closely supervised by a research paediatrician (Appendix 4) (Gomella et al., 1994).

**Results**

**Maternal malaria**

Between April 2004 and June 2009, 6519 women were admitted to the maternity ward and enrolled in the study. Of these women 5% (322) was admitted more than once (Figure 7-1). The reason for admission to the maternity ward was primarily for delivery (4963/6508, 76%) with the remaining women admitted for malaria (728, 11%), abortion (228, 3.5%), impending preterm delivery (PTD) (203, 3.1%), IUFD (110, 1.6%) and others (276, 4.2%). Over the period of five years, maternal and neonatal data were available in 79% (5292/6664) of all hospital deliveries (Figure 7-2). Of these women (5292), 5037 (95%) delivered on their first admission and 255 delivered on subsequent admission (Figure 7-1). Of the 639 women exposed and discharged with ongoing pregnancy 109 (17%) had documented pregnancy outcomes (Figure 7-1).

Of the 6475 (99%) pregnant women screened for malaria, 1050 (16.2%) were infected with *P. falciparum*, 456 (7%) with *P. vivax* and 113 (1.7%) with mixed infections. *P. malariae* parasitaemia was present in 62 cases and *P. ovale* in one. In total 893 (53%) women with malaria were symptomatic (fever or history of fever), with severe disease recorded in 106 (6.3%). Of those with severe disease, 73% (77) had *P. falciparum* infections and 9% (10) had *P. vivax*. The most common
manifestation of severity was severe anaemia (<5g/dl, present in 81 (76%)). Other presentations included coma in 5 women, jaundice in 11, acute renal failure in 3, hypoglycemia in 1, respiratory insufficiency in 3 and hyperparasitaemia in 15 women. Eight (7.5%) women had multiple markers of severity. The maternal mortality ratio in women without malaria was 18/10,000 live births (6/3398 live births) with the mortality ratio in women with malaria of 30/10,000 live births (5/1680); p=0.52.

The acute management of malaria varied over the duration of the study, as reflected by the drug exposure according to the reason for admission to hospital; see table 7-1. There were 1718 pregnant women treated for acute malaria during their admission to hospital (Figure 7-1). All antimalarials, including DHP, were well tolerated with no serious adverse reactions observed that required discontinuation of therapy. In total, 981 pregnant women were exposed to DHP treatment of whom 316 (32%) also received intravenous artesunate (Figure 7-3).
Figure 7-1. Overall Study Profile of Pregnant Women exposed with antimalarial drugs
Figure 7-2. Women at delivery and Neonates Enrolled in the Study
Figure 7-3. Malaria treatment in pregnant women admitted to the hospital (Arrow shows start time of treatment policy change)
<table>
<thead>
<tr>
<th>Reason for Admission</th>
<th>% with malaria</th>
<th>Oral route</th>
<th>Intravenous route</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CQ/Qu</td>
<td>DHP alone</td>
</tr>
<tr>
<td>Malaria</td>
<td>97% (708/728)</td>
<td>26% (90)</td>
<td>17.4% (116)</td>
</tr>
<tr>
<td>Delivery</td>
<td>16% (816/4963)</td>
<td>68% (237)</td>
<td>69% (456)</td>
</tr>
<tr>
<td>Abortion</td>
<td>23% (53/228)</td>
<td>3.4% (12)</td>
<td>2.8% (19)</td>
</tr>
<tr>
<td>Impending PTD</td>
<td>23% (47/203)</td>
<td>0.3% (1)</td>
<td>4.8% (32)</td>
</tr>
<tr>
<td>IUFD</td>
<td>23% (25/110)</td>
<td>0.9% (3)</td>
<td>2.7% (18)</td>
</tr>
<tr>
<td>UTI</td>
<td>0% (0/15)</td>
<td>0% (0)</td>
<td>0.2% (1)</td>
</tr>
<tr>
<td>Others</td>
<td>13% (33/261)</td>
<td>1.4% (5)</td>
<td>3.5% (23)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>26% (1682/6508*)</td>
<td>348</td>
<td>665</td>
</tr>
</tbody>
</table>

**Notes:** *No reason for admission was documented in 11 women; CQ= chloroquine; SP=sulphadoxine-pyrimethamine; DHP=dihydroartemisinin-piperaquine; Qu=quinine; Art=artesunate*
Acute exposures to antimalarial medication
Overall 1806 women were diagnosed with malaria with parasitological confirmation by microscopy in 93% (1682) of the cases. Of these women, 91% (1527) were treated in the second and third trimester of pregnancy and 9% (155) in the first trimester (<14 weeks of pregnancy gestation).

Second and Third Trimesters Exposure
The analysis of the acute exposure to antimalarial treatment in the second and third trimester was restricted to 1415 women with confirmed parasitaemia, excluding 112 women presenting with impending adverse outcomes (eg abortion, premature delivery, interuterine foetal death) (Figure 7-4). Of these women, the following analysis will focus on the 1217 women treated with either CQ+/−SP (n=97), oral/Iv quinine (n=271), DHP (n=486) alone or intravenous artesunate +/- DHP (n=363). In total 690 (56%) of these women were admitted for delivery, 525 (43%) for malaria and in two cases the reason for admission was not documented (Figure 7-4). The clinical characteristics of women treated with antimalarial drugs are presented in Table 7-2. Compared to women admitted for delivery and screened for malaria, women admitted primarily due to parasitaemia were more likely to be symptomatic (84.5%, 506/599 vs 29.5%, 241/816; OR=2.86 [95%CI, 2.56-3.19], p<0.001) and receive intravenous therapy (62.7%, 370/590 vs 11.8%, 88/747; OR=5.32 [95%CI, 4.33-6.54], p<0.001). Women treated with intravenous therapy, were at greater risk of adverse outcome (still birth and perinatal death) although this did not reached statistical significance (OR=1.78 [0.96-3.29], p=0.070) (Table 7-3).

The prevalence of early neonatal death was greater in women treated with oral quinine than in those receiving oral DHP alone; p=0.026, Table 7-3. Apart from this there were no significant differences in adverse outcomes between drug treatment for women admitted either for malaria or for delivery (Table 7-3). This remained in multivariate analysis after controlling for confounding factors listed in table 7-2.
First Trimester Exposure

In total 46% (152/332) of pregnant women hospitalized in the first trimester had malaria, of whom 38 were admitted with impending abortion. In the remaining 114 women, 53 (44%) were treated with oral antimalarials alone, 60 (53%) with intravenous antimalarials and one patient received antibiotics alone. Pregnant women in the first trimester treated with an artemisinin derivative were more likely to have severe malaria at presentation compared to those exposed to non artemisinin treatments (OR=4.7 [95%CI 1.3-17]; p=0.011); Table 7-4.

In total 69% (9/13) women receiving oral DHP or AAQ treatment aborted, compared to 2.5% (1/40) treated with quinine (p<0.0001). However none of the 10 women treated with intravenous artesunate with or without DHP aborted; Table 7-4.
Figure 7-4. Acute Malaria in the second and third trimesters of pregnancy
Table 7-2. Baseline Characteristics of pregnant women with acute malaria (2nd and 3rd trimesters)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Oral route</th>
<th>Intravenous route</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CQ+/-SP (n=97)</td>
<td>Qu (n=176)</td>
<td>DHP alone (n=486)</td>
</tr>
<tr>
<td>Age, mean year (95% CI of mean)</td>
<td>25.1 (23.8-26.3)</td>
<td>24.3 (23.6-25.1)</td>
<td>24.3 (23.8-24.8)</td>
</tr>
<tr>
<td>Papuan Ethnic groups</td>
<td>74.2% (72.97)</td>
<td>78.3% (137/175)</td>
<td>78.4% (381/486)</td>
</tr>
<tr>
<td>Primigravidae</td>
<td>39.2% (38/97)</td>
<td>35.8% (70/176)</td>
<td>35.6% (173/486)</td>
</tr>
<tr>
<td>Gestation of pregnancy, median week (range)</td>
<td>39 (17-41)</td>
<td>39 (13-41)</td>
<td>38 (13-42)</td>
</tr>
<tr>
<td>Haemoglobin concentration, g/dl (95% CI of mean)</td>
<td>9.1 (8.6-9.5)</td>
<td>8.5 (8.2-8.8)</td>
<td>9.4 (9.2-9.5)</td>
</tr>
<tr>
<td>Fever/History of fever</td>
<td>22.7% (22.97)</td>
<td>51.7% (91/176)</td>
<td>35.8% (174/486)</td>
</tr>
<tr>
<td>Severe Malaria</td>
<td>3.1% (3/97)</td>
<td>1.1% (2/176)</td>
<td>2.7% (13/486)</td>
</tr>
<tr>
<td>Duration of stay, median days (range)</td>
<td>3 (0-12)</td>
<td>2 (0-8)</td>
<td>2 (0-124)</td>
</tr>
</tbody>
</table>

Notes: CQ= chloroquine; SP=sulphadoxine-pyrimethamine; DHP=dihydroartemisinin-piperaquine; Qu=quinine; Art=artesunate
Table 7-3. Pregnancy outcomes in women with acute malaria (2nd and 3rd trimesters of pregnancy)

<table>
<thead>
<tr>
<th>Pregnancy Outcome</th>
<th>Oral route</th>
<th>Intravenous route</th>
<th>p value (oral vs iv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CQ+/-SP</td>
<td>Qu</td>
<td>DHP alone</td>
</tr>
<tr>
<td>Live births</td>
<td>99% (93/94)</td>
<td>96% (125/130)</td>
<td>97 (366/378)</td>
</tr>
<tr>
<td>Still births</td>
<td>0% (0/94)</td>
<td>0.8% (1/130)</td>
<td>1.3% (5/378)</td>
</tr>
<tr>
<td>Early Neonatal Deaths</td>
<td>1.1% (1/94)</td>
<td>3.1% (4/130)</td>
<td>1.6% (6/378)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Admitted for Treatment of Malaria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ongoing pregnancy</td>
<td>100% (3/3)</td>
<td>83% (38/46)</td>
<td>95% (102/107)</td>
</tr>
<tr>
<td>Still births</td>
<td>0% (0/5)</td>
<td>0% (0/46)</td>
<td>0% (0/107)</td>
</tr>
<tr>
<td>Early Neonatal Deaths</td>
<td>0% (0/3)</td>
<td>6.5% (3/46)</td>
<td>0% (0/107)</td>
</tr>
<tr>
<td>Preterm delivery *</td>
<td>0% (0/3)</td>
<td>2.9% (1/34)</td>
<td>1.2% (1/86)</td>
</tr>
</tbody>
</table>

Notes:
CQ= Chloroquine; SP= Sulphadoxine Pyrimethamine; DHP= Dihydroartemisinin piperazine; Qu= Quinine; Art= Artesunate
Compared with oral Quinine: * p<0.05
* In women presenting before 37 weeks gestation
Table 7-4. First Trimester Acute Exposures: Maternal characteristics and pregnancy outcome

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Oral route</th>
<th>Intravenous route</th>
<th>p value (oral vs iv)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Qu (n=40)</td>
<td>DHP alone (n=10)</td>
<td>AAQ (n=3)</td>
</tr>
<tr>
<td>Age, median year (range)</td>
<td>24.5 (17-37)</td>
<td>25.5 (18-38)</td>
<td>28 (23-32)</td>
</tr>
<tr>
<td>Papuan Ethnic groups</td>
<td>58% (23)</td>
<td>70% (7)</td>
<td>100% (3)</td>
</tr>
<tr>
<td>Primigravidae</td>
<td>38% (15)</td>
<td>40% (4)</td>
<td>33% (1)</td>
</tr>
<tr>
<td>Severe anaemia</td>
<td>12% (4/34)</td>
<td>25% (2/8)</td>
<td>33% (1/3)</td>
</tr>
<tr>
<td>Fever/History of fever</td>
<td>83% (33)</td>
<td>50% (5)</td>
<td>33% (1)</td>
</tr>
<tr>
<td>Severe Malaria</td>
<td>5% (2)</td>
<td>10% (1)</td>
<td>33% (1)</td>
</tr>
<tr>
<td>Abortion</td>
<td>2.5% (1)</td>
<td>70% (7)</td>
<td>67% (2)</td>
</tr>
</tbody>
</table>
History of febrile illness and antimalarial exposure

In total 5292 women were enrolled in the study with a known pregnancy outcome, of whom 884 (17%) reported a history of febrile illness during the pregnancy. Nearly all (847, 96%) of these women were able to state their prior antimalarial treatment, with diagnosis and treatment confirmed from hospital records in 404 (48%) of cases (Figure 7-5). Compared to women with no history of fever, those with prior febrile illness were more likely to be younger (mean age 25 years old [95%CI, 24.6-25.4] compared to 26 [95%CI, 25.8-26.1], p<0.001), Papuan (OR=1.4 [95%CI, 1.2-1.7], p<0.001) and primigravidae (OR=1.2 [95%CI, 1.1-1.4], p=0.006) Table 7-5.

---

**Figure 7-5.** Profile of Prior Febrile Illness in Pregnant Women Delivering

<table>
<thead>
<tr>
<th>5292</th>
<th>Women reaching the end of pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>4408 women</td>
<td>with no history of febrile illness</td>
</tr>
<tr>
<td>884 women</td>
<td>with history of febrile illness</td>
</tr>
<tr>
<td>847 (404 confirmed)</td>
<td></td>
</tr>
<tr>
<td>1 treatment episode</td>
<td>648</td>
</tr>
<tr>
<td>2 treatment episodes</td>
<td>151</td>
</tr>
<tr>
<td>&gt;=3 treatment episodes</td>
<td>48</td>
</tr>
<tr>
<td>37</td>
<td>unknown treatment history</td>
</tr>
</tbody>
</table>
Prior drug exposure during the same pregnancy ranged from 1 to 8 episodes, the last recorded exposure being a median of 10 weeks [Range: 1-39] prior to delivery. The majority of women had a single antimalarial exposure (77%, 648/847); figure 7-6.

![Figure 7-6. Prior antenatal exposure to antimalarial drugs](image)

Of the 199 women with multiple drug exposure, 61% (122) were exposed again to the same drug, whereas 77 received different drugs which was mostly quinine treatment followed by DHP (61%, 47/77). The potential effect of drug exposure on pregnancy outcome was therefore assessed in women according to last antimalarial exposure controlling for number of prior febrile episodes. The following analysis will focus on prior last treatment with CQ+/-SP, oral Quinine, DHP and Iv Artesunate +/- DHP.

The last treatment of malaria was documented in 784 women with 102 (13%) exposed in the first trimester, 238 (30%) in the second trimester, 441 (56%) in the third trimester of pregnancy. For three women the timing of exposure was unknown. Women with prior exposure to DHP were more likely to receive treatment closer to delivery and to be less symptomatic on admission compared to those treated with past CQ and oral Quinine (Table 7-5).
Risk of Malaria at delivery

Compared to women without prior febrile illness those with antimalarial exposure were at significantly greater risk of parasitaemia at delivery (OR=3.65 [95%CI, 3.07-4.34], p<0.001).

Of the pregnant women receiving oral DHP during the current pregnancy 26% (86/336) had malaria at delivery compared to 48% (178/370) of those with prior treatment with a non ACT regimen (oral quinine and CQ+/−SP); OR=0.37 (95%CI 0.27-0.52), p<0.001, Table 7-6 and Figure 7-7. The median time from last treatment to presentation with malaria was significantly longer (11 weeks, 1-27) following DHP treatment than following non ACT therapy (8 weeks, 1-37), p=0.05. The lower risk of parasitaemia at delivery was apparent for both *P. falciparum* (OR=0.34 [95%CI, 0.24-0.49], p<0.001) and *P. vivax* (OR=0.6 [95%CI, 0.37-0.96], p=0.034).

Figure 7-7. Risk of Maternal Malaria at Delivery according to prior antimalarial treatment
Maternal and Pregnancy Outcome

Prior antimalarial exposure was a significant risk factor for severe anaemia, low birth weight, preterm delivery and congenital malaria compared to those without any history of malaria during pregnancy (Table 7-6). The risk of maternal severe anaemia, low birth weight and preterm delivery were similar between treatment groups (table 7-6). However, the risk of congenital malaria was significantly greater in women with prior history of Quinine and CQ+/SP treatment (4.6%, 15/322) than in those exposed to DHP alone (0.3%, 1/326); OR=15.88 (95%CI, 2.19-324.32), p=0.001. After controlling for confounding factors, history of DHP treatment during pregnancy was not associated with any of the adverse outcomes (Table 7-7). On the other hand, women with a history of quinine exposure were at greater risk of perinatal deaths compared to those exposed to DHP (Table 7-7).

To address the potential for recall bias the comparative analysis was repeated for the 404 women in whom prior antimalarial treatment could be confirmed from hospital records (Table 7-8). After controlling for other risk factors, the only significant difference between treatment groups was the risk of perinatal deaths which was significantly greater in women with prior history of quinine (AOR=6.53 [95%CI, 1-42.81]; p=0.05).

Antimalarial exposure was also grouped according to whether any prior treatment of artemisinin derivatives had been prescribed (ie DHP, intravenous Artesunate and Artesunate-Amodiaquine) or a non-ACT regimen. Women receiving any ACT regimen were at lower risk of malaria at delivery (OR=0.43 [95%CI, 0.32-0.57], perinatal deaths (OR=0.45 [95%CI, 0.22-0.92]) and congenital malaria (OR=0.05 [95%CI, 0-0.36]) compared to those with exposure to a non-ACT regimens (Table 7-9). The lower risk of perinatal deaths following ACT regimens remained in the multivariable model.

Of the 16 pregnant women inadvertently exposed to DHP in the first trimester of pregnancy 12 had documented pregnancy outcomes: 7 (58.3%) had live births, 4 (33.3%) aborted, 1 (8.3%) had a stillbirth. In comparison 97 women with history of quinine treatment in the first trimester had known pregnancy outcomes: 78 (80.4%)
live births, 11 (11.3%) abortion, 5 (5.2%) stillbirths and 3 (3.1%) neonatal deaths. There were no significance difference in any of the adverse pregnancy outcomes; $p>0.05$. 
Table 7-5. Baseline characteristic of pregnant women delivering with history of last antimalarial exposure

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No history of malaria (n=4408)</th>
<th>Last previous malaria treatment</th>
<th>CQ±SP (n=24)</th>
<th>Oral Qu (n=347)</th>
<th>DHP alone (n=336)</th>
<th>Iv Art +/- DHP (n=77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean year (95%CI of mean)</td>
<td>25.9 (25.8-26.1)</td>
<td>23.7 (21.3-26.2)</td>
<td>25.4 (24.8-26.0)</td>
<td>24.8 (24.1-25.4)</td>
<td>24.6 (23.3-25.9)</td>
<td></td>
</tr>
<tr>
<td>Papuan Ethnic groups</td>
<td>64.7% (2846/4399)</td>
<td>75% (18/24)</td>
<td>61.6% (213/346)</td>
<td>83% (279/336)</td>
<td>71.4% (55/77)</td>
<td></td>
</tr>
<tr>
<td>Primigravidae</td>
<td>31% (1365/4404)</td>
<td>45.8% (11/24)</td>
<td>37% (129/347)</td>
<td>32.4% (109/336)</td>
<td>39% (30/77)</td>
<td></td>
</tr>
<tr>
<td>Gestation of pregnancy, median week (range)</td>
<td>39 (19-44)</td>
<td>39 (34-40)</td>
<td>39 (20-43)</td>
<td>38 (24-44)</td>
<td>38 (21-41)</td>
<td></td>
</tr>
<tr>
<td>Febrile at delivery</td>
<td>5.2% (231/4406)</td>
<td>33.3% (8/24)</td>
<td>28% (97/347)</td>
<td>10.7% (36/336)</td>
<td>9% (7/77)</td>
<td></td>
</tr>
<tr>
<td>Number of febrile episodes during pregnancy, median times (range)</td>
<td>-</td>
<td>1 (1-2)</td>
<td>1 (1-8)</td>
<td>1 (1-7)</td>
<td>1 (1-4)</td>
<td></td>
</tr>
<tr>
<td>Time from last exposure to delivery, median weeks (range)</td>
<td>-</td>
<td>16 (1-35)</td>
<td>12 (1-39)</td>
<td>10 (1-29)</td>
<td>10 (1-23)</td>
<td></td>
</tr>
</tbody>
</table>

Notes: Compared with prior history of last antimalarial exposure: a p<0.001, b p<0.05; Compared with Oral Quinine: c p<0.001, d p<0.05; Compared with CQ+/−SP: e p<0.05
<table>
<thead>
<tr>
<th>Adverse Outcomes</th>
<th>No exposure and history of malaria illness (n=4408)</th>
<th>CQ+/- SP (n=24)</th>
<th>Oral Qu (n=347)</th>
<th>Iv Art +/- DHP (n=77)</th>
<th>DHP alone (n=336)</th>
<th>P value a vs b</th>
<th>P value within b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Malaria at delivery</td>
<td>13.6% (596/4378)</td>
<td>56.5% (13/23)</td>
<td>47.6% (165/347)</td>
<td>28.6% (22/77)</td>
<td>25.6% (86/336)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Maternal severe anaemia</td>
<td>8% (344/4291)</td>
<td>17.4% (4/23)</td>
<td>12% (41/341)</td>
<td>11% (8/72)</td>
<td>12.2% (39/319)</td>
<td>&lt;0.001</td>
<td>0.880</td>
</tr>
<tr>
<td>Low Birth weight</td>
<td>14.7% (642/4382)</td>
<td>25% (6/24)</td>
<td>20.4% (70/343)</td>
<td>14.3% (11/77)</td>
<td>18% (60/333)</td>
<td>&lt;0.001</td>
<td>0.507</td>
</tr>
<tr>
<td>Mean Birth Weight, 95%CI of mean</td>
<td>3028 (3011-3045)</td>
<td>2726 (2478-2974)</td>
<td>2884 (2819-2950)</td>
<td>2859 (2759-2958)</td>
<td>2905 (2844-2967)</td>
<td>&lt;0.001</td>
<td>0.698</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>10.8% (450/4158)</td>
<td>21.7% (5/23)</td>
<td>12.9% (41/318)</td>
<td>18.7% (14/75)</td>
<td>16.9% (55/326)</td>
<td>&lt;0.001</td>
<td>0.337</td>
</tr>
<tr>
<td>Median Gestational Age, range</td>
<td>39 (26-43)</td>
<td>39 (32-40)</td>
<td>38 (28-43)</td>
<td>38 (32-41)</td>
<td>38 (28-43)</td>
<td>&lt;0.001</td>
<td>0.077</td>
</tr>
<tr>
<td>Perinatal death</td>
<td>4% (177/4408)</td>
<td>0% (0/24)</td>
<td>6.6% (23/347)</td>
<td>2.6% (2/77)</td>
<td>3.3% (11/336)</td>
<td>0.510</td>
<td>0.091</td>
</tr>
<tr>
<td>Still Births</td>
<td>3% (134/4408)</td>
<td>0% (0/24)</td>
<td>4.6% (16/347)</td>
<td>2.6% (2/77)</td>
<td>2.7% (9/336)</td>
<td>0.625</td>
<td>0.386</td>
</tr>
<tr>
<td>Early Neonatal Deaths</td>
<td>1% (43/4408)</td>
<td>0% (0/24)</td>
<td>2% (7/347)</td>
<td>0% (0/77)</td>
<td>0.6% (2/336)</td>
<td>0.800</td>
<td>0.222</td>
</tr>
<tr>
<td>Congenital malaria</td>
<td>0.6% (20/3409)</td>
<td>5% (1/20)</td>
<td>4.6% (14/302)</td>
<td>0% (0/75)</td>
<td>0.3% (1/326)</td>
<td>&lt;0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Congenital Malformation</td>
<td>0.6% (11/1794)</td>
<td>0% (0/1)</td>
<td>1.4% (1/73)</td>
<td>0% (0/10)</td>
<td>0% (0/209)</td>
<td>1.000</td>
<td>0.388</td>
</tr>
<tr>
<td>Predictors</td>
<td>Maternal Malaria at delivery</td>
<td>Maternal severe anaemia</td>
<td>Low Birth weight</td>
<td>Preterm delivery</td>
<td>Perinatal death</td>
<td>Congenital malaria</td>
<td></td>
</tr>
<tr>
<td>----------------------------</td>
<td>------------------------------</td>
<td>-------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td>----------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>Maternal Malaria at delivery</td>
<td>-</td>
<td>3.03 (1.83-5.01), p&lt;0.001</td>
<td>2.08 (1.34-3.22), p=0.001</td>
<td>1.74 (1.06-2.87), p=0.029</td>
<td>2.43 (1.10-5.39), p=0.029</td>
<td>3.25 (0.56-19.06), p=0.191</td>
<td></td>
</tr>
<tr>
<td>Maternal severe anaemia</td>
<td>3.05 (1.83-5.07), p&lt;0.001</td>
<td>-</td>
<td>2.61 (1.57-4.33), p&lt;0.001</td>
<td>2.01 (1.13-3.55), p=0.018</td>
<td>1.22 (0.47-3.15), p=0.684</td>
<td>1.29 (0.32-5.16), p=0.721</td>
<td></td>
</tr>
<tr>
<td>Fever/History of fever</td>
<td>9.04 (5.62-14.53), p&lt;0.001</td>
<td>0.78 (0.44-1.39), p=0.404</td>
<td>1.38 (0.83-2.29), p=0.208</td>
<td>2.45 (1.39-4.29), p=0.002</td>
<td>0.93 (0.38-2.28), p=0.881</td>
<td>1.48 (0.47-4.68), p=0.508</td>
<td></td>
</tr>
<tr>
<td>Primigravidae</td>
<td>1.17 (0.78-1.76), p=0.448</td>
<td>1.05 (0.62-1.78), p=0.868</td>
<td>2.56 (1.63-4.02), p=0.001</td>
<td>2.16 (1.31-3.54), p=0.002</td>
<td>1.87 (0.83-4.23), p=0.132</td>
<td>0.52 (0.14-1.97), p=0.336</td>
<td></td>
</tr>
<tr>
<td>Papuan Ethnicity</td>
<td>1.23 (0.82-1.85), p=0.322</td>
<td>2.42 (1.25-4.68), p=0.009</td>
<td>0.99 (0.62-1.59), p=0.975</td>
<td>0.92 (0.55-1.56), p=0.757</td>
<td>2.66 (0.99-7.12), p=0.051</td>
<td>0.68 (0.19-2.48), p=0.560</td>
<td></td>
</tr>
<tr>
<td>Maternal age ≤ 18 y.o</td>
<td>0.97 (0.93-1.00), p=0.065</td>
<td>0.97 (0.92-1.01), p=0.152</td>
<td>1.0 (0.96-1.04), p=0.910</td>
<td>0.99 (0.95-1.04), p=0.856</td>
<td>1.02 (0.95-1.09), p=0.567</td>
<td>1.04 (0.95-1.14), p=0.421</td>
<td></td>
</tr>
<tr>
<td>Year of observation</td>
<td>0.79 (0.68-0.94), p=0.005</td>
<td>0.89 (0.72-1.11), p=0.309</td>
<td>1.02 (0.85-1.22), p=0.826</td>
<td>1.17 (0.96-1.43), p=0.128</td>
<td>1.26 (0.92-1.73), p=0.143</td>
<td>0.35 (0.16-0.75), p=0.007</td>
<td></td>
</tr>
</tbody>
</table>

**Prior Last Treatment Exposures**

<table>
<thead>
<tr>
<th></th>
<th>CQ+/− SP</th>
<th>Qu+/−Iv Qu</th>
<th>Iv Art+/−DHP</th>
<th>DHP alone</th>
</tr>
</thead>
<tbody>
<tr>
<td>CQ+/− SP</td>
<td>1.52 (0.51-4.49), p=0.450</td>
<td>0.87 (0.24-3.18), p=0.831</td>
<td>1.03 (0.33-3.16), p=0.966</td>
<td>1.10 (0.33-3.71), p=0.874</td>
</tr>
<tr>
<td>Qu+/−Iv Qu</td>
<td>1.56 (0.97-2.50), p=0.008</td>
<td>0.75 (0.39-1.48), p=0.411</td>
<td>0.90 (0.52-1.55), p=0.707</td>
<td>0.59 (0.32-1.1), p=0.097</td>
</tr>
<tr>
<td>Iv Art+/−DHP</td>
<td>1.13 (0.61-2.12), p=0.690</td>
<td>0.89 (0.39-2.07), p=0.798</td>
<td>0.69 (0.32-1.45), p=0.325</td>
<td>1.02 (0.49-2.10), p=0.967</td>
</tr>
<tr>
<td>DHP alone</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 7-8. Previous malaria treatment and adverse pregnancy outcomes in women with confirmed history of malaria

<table>
<thead>
<tr>
<th>Exposure History</th>
<th>Maternal malaria at delivery</th>
<th>Maternal severe anaemia</th>
<th>Low Birth weight</th>
<th>Preterm delivery</th>
<th>Perinatal death</th>
<th>Congenital malaria</th>
<th>Congenital malformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qu+/IV Qu (n=47)</td>
<td>27.7% (13/47)</td>
<td>8.7% (4/46)</td>
<td>14.9% (7/47)</td>
<td>15.6% (7/45)</td>
<td>4.3% (2/47)</td>
<td>0% (0/44)</td>
<td>0% (0/38)</td>
</tr>
<tr>
<td>IV Art+/DHP (n=58)</td>
<td>24.1% (14/58)</td>
<td>9.4% (5/53)</td>
<td>13.8% (8/58)</td>
<td>19.6% (11/56)</td>
<td>3.4% (2/58)</td>
<td>0% (0/56)</td>
<td>0% (0/6)</td>
</tr>
<tr>
<td>DHP Alone (n=245)</td>
<td>23.3% (57/245)</td>
<td>11.7% (27/230)</td>
<td>14.8% (36/244)</td>
<td>14.2% (34/240)</td>
<td>2% (5/245)</td>
<td>0.4% (1/240)</td>
<td>0% (0/198)</td>
</tr>
<tr>
<td>No exposure and history of malaria illness</td>
<td>13.6% (596/4378)</td>
<td>8% (344/4291)</td>
<td>14.7% (642/4382)</td>
<td>10.8% (450/4158)</td>
<td>4% (177/4408)</td>
<td>0.6% (20/3409)</td>
<td>0.5% (7/1378)</td>
</tr>
</tbody>
</table>

Univariate Analysis

<table>
<thead>
<tr>
<th>P value for no exposures vs history of antimalarial exposure</th>
<th>&lt;0.001</th>
<th>0.08</th>
<th>0.95</th>
<th>0.016</th>
<th>0.23</th>
<th>1.00</th>
<th>0.603</th>
</tr>
</thead>
<tbody>
<tr>
<td>P value within treatment</td>
<td>0.811</td>
<td>0.774</td>
<td>0.981</td>
<td>0.589</td>
<td>0.611</td>
<td>0.811</td>
<td>0.744</td>
</tr>
</tbody>
</table>

Multivariate Analysis (AOR, 95%CI)

<table>
<thead>
<tr>
<th>Qu+/IV Qu</th>
<th>1.09 (0.41-2.89), p=0.867</th>
<th>2.02 (0.52-7.80), p=0.309</th>
<th>1.40 (0.42-4.72), p=0.587</th>
<th>2.81 (0.86-9.14), p=0.087</th>
<th>6.53 (1-42.81), p=0.05</th>
<th>-</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV Art+/DHP</td>
<td>0.98 (0.46-2.09), p=0.966</td>
<td>0.67 (0.22-2.07), p=0.490</td>
<td>0.89 (0.34-2.37), p=0.823</td>
<td>1.47 (0.60-3.62), p=0.397</td>
<td>2.41 (0.40-14.38), p=0.336</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DHP alone</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Multivariate analysis controlled for the following confounding factors: Maternal malaria, maternal severe anaemia, fever/history of fever, primigravidae, Papuan ethnicity, age of the mothers and year of observation.
Table 7-9. Previous malaria treatment and adverse pregnancy outcomes in women with any artemisinin exposures

<table>
<thead>
<tr>
<th>Exposure History</th>
<th>Maternal malaria at delivery</th>
<th>Maternal severe anaemia</th>
<th>Low Birth weight</th>
<th>Preterm delivery</th>
<th>Perinatal death</th>
<th>Congenital malaria</th>
<th>Congenital malformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any antimalarial, but not artemisinin (n=412)</td>
<td>46.8% (192/410)</td>
<td>12.9% (52/403)</td>
<td>20% (81/406)</td>
<td>13.3% (50/377)</td>
<td>6.3% (26/412)</td>
<td>4.2% (15/359)</td>
<td>3.1% (2/65)</td>
</tr>
<tr>
<td>Any artemisinin (n=471)</td>
<td>27.4% (129/471)</td>
<td>11.2% (50/445)</td>
<td>16.2% (76/468)</td>
<td>17% (78/458)</td>
<td>3% (14/471)</td>
<td>0.2% (1/458)</td>
<td>0.4% (1/241)</td>
</tr>
<tr>
<td>No malaria at delivery and history of malaria illness (n=3695)</td>
<td>0% (0/3695)</td>
<td>7.2% (260/3614)</td>
<td>13.6% (501/3678)</td>
<td>10.1% (353/3500)</td>
<td>3.5% (129/3695)</td>
<td>0.3% (7/2789)</td>
<td>0.9% (12/1381)</td>
</tr>
<tr>
<td>P value for no malaria vs history of antimalarial exposure</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.001</td>
<td>&lt;0.001</td>
<td>0.170</td>
<td>&lt;0.001</td>
<td>0.743</td>
</tr>
<tr>
<td>P value within treatment</td>
<td>&lt;0.001</td>
<td>0.522</td>
<td>0.181</td>
<td>0.159</td>
<td>0.026</td>
<td>&lt;0.001</td>
<td>0.115</td>
</tr>
</tbody>
</table>

**Univariate Analysis**

**Multivariate Analysis**

Any antimalarial, but not artemisinin, AOR (95%CI) | 1.33 (0.86-2.05), p=0.199 | 0.79 (0.42-1.49), p=0.794 | 0.97 (0.58-1.62), p=0.895 | 0.59 (3.40-1.06), p=0.076 | 2.78 (1.09-7.04), p=0.031 | 1.61 (0.13-19.89), p=0.709 |

Any artemisinin | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
Birth defects/Congenital Malformation

From May 2007 systematic physical examinations were performed on all neonates delivered at RSMM by a trained research nurse and supervised closely by a paediatrician. This screening for congenital malformations included external examination (skin and body parts morphology and palpation and auscultation of the internal organs) and thus predominantly focused on external malformations, such as craniofacial anomalies or anal atresia. Congenital heart disease or genitourinary tract anomalies may manifest later in life and, unless symptoms and signs were apparent at birth, would have been missed by this examination.

Table 7-10. Congenital Anomalies (International Classification of Disease 10)

<table>
<thead>
<tr>
<th>Type of defect/s (ICD 10)</th>
<th>Number of cases</th>
<th>Outcome on discharged</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral talipes equinovarus (Q66.0)</td>
<td>3</td>
<td>Survived</td>
</tr>
<tr>
<td>Cleft lip and palate (Q37)</td>
<td>3(^a)</td>
<td>Survived</td>
</tr>
<tr>
<td>Anal atresia (Q42.2)</td>
<td>2</td>
<td>Survived</td>
</tr>
<tr>
<td>Bilateral talipes equinovarus (Q66.0) and bilateral lobster claw hand (Q71.6)</td>
<td>1(^b)</td>
<td>Survived</td>
</tr>
<tr>
<td>Bilateral longitudinal reduction defect of femur (Q72.4), tibia (Q72.5) and fibula (Q72.6) with fused right toes (Q70.2)</td>
<td>1</td>
<td>Survived</td>
</tr>
<tr>
<td>Anencephaly (Q00.0)</td>
<td>1(^c)</td>
<td>Died</td>
</tr>
<tr>
<td>Micrognathia (K07.0) and anteversion of femoral neck (Q65.8)</td>
<td>1</td>
<td>Died</td>
</tr>
<tr>
<td>Triorchidism (Q55.2)</td>
<td>1</td>
<td>Survived</td>
</tr>
<tr>
<td>Syndactili in both hands (Q70)</td>
<td>1</td>
<td>Survived</td>
</tr>
<tr>
<td>Congenital hypotonia (P94.2)</td>
<td>1</td>
<td>Died</td>
</tr>
</tbody>
</table>

Note: \(^a\)One mother was exposed to quinine in the first trimester of pregnancy; \(^b\)Mother was exposed to artesunate-amodiaquine in the second trimester of pregnancy; \(^c\)Mother was exposed to unknown herbal medicine in the first trimester of pregnancy

Of the 1945 live births, 15 congenital malformations were reported with an overall incidence rate (IR) of 8 [95%CI: 5 to 12] per 1000 live births. The risk of congenital
defect was evaluated in pregnant women with prior antimalarial exposures at any
time during the current pregnancy. The risk of malformation was 0.9% (12/1381) in
babies without known in utero exposure to any antimalarial drugs (IR 9 [95%CI: 5-
15] per 1000 live births) , 0% (0/207) in babies in utero exposure to DHP (IR 0
[95%CI: 0-20] per 1000 live births, 1% (1/92) for amodiaquine-artesunate (IR 10
[95%CI: 0.3-60] per 1000 live births) and 1.5% (1/65) for non-ACT antimalarial
exposure (IR 15 [95%CI, 0.4-80] per 1000 live births; p=0.515. The 15 cases of
malformation are listed in table 7-10.

Discussion
Malaria in pregnancy is associated with adverse outcomes for both mother and
baby.(Desai et al., 2007) Early detection and prompt treatment with effective
antimalarial reduces the risk of poor outcomes.(Nosten et al., 2007) In Papua
Indonesia we have reported that both P. falciparum and P. vivax infections occur in
up to 17% of pregnant women and even when associated with asymptomatic
parasitaemia, increase the risk of maternal anaemia, still birth, prematurity, low birth
weight and perinatal death.(Poespoprodjo et al., 2008) However, the proportion of
malaria in this study was only taken at delivery, thus the cumulative proportion of
malaria throughout pregnancy would be higher.

Treatment options are limited since high grade multidrug resistance has emerged in
both P. falciparum and P. vivax to chloroquine, amodiaquine, and sulfadoxine-
pyrimethamine.(Ratcliff et al., 2007b, Hasugian et al., 2007)  The use of quinine in
pregnancy is well document and although not known to cause adverse fetal
outcomes, adequate cure is dependent upon giving it 3 times a day for 7 days.  Such
treatment regimens coupled with reliable side effects such as nausea and chinconism,
result in poor adherence. When unsupervised the efficacy of a quinine falls to less
than 45%.(Ratcliff et al., 2007b) The necessary revision of antimalarial policy in
March 2006, saw the introduction of dihydroartemisinin-piperaquine (DHP) for
the treatment of uncomplicated malaria due to any species, including the second and
third trimesters. Although the data on the efficacy and safety of DHP in pregnant
women is limited, preliminary studies in Thailand (Rijken et al., 2008) and
reproductive toxicity studies in animals are reassuring.(Clark et al., 2004, Clark et
In this report we document our experience of in utero exposure to DHP in more than 1000 pregnant women.

Our observations show that compared to standard antimalarial treatment such as chloroquine or quinine, DHP is a well tolerated, efficacious and safe in the second and third trimesters of pregnancy. In women with documented acute exposure, the use of DHP reduced risk of early neonatal death (OR=0 [95%CI, 0-0.94], p=0.025), although other outcomes in the acute admission differed little between groups. The main difference between treatment regimens was observed in women with history of prior antimalarial exposure during pregnancy, representing to hospital either with recurrence and/or to deliver. Compared to women with prior exposure to chloroquine or quinine, women treated with DHP had a lower risk of malaria at delivery (OR=0.37 (95%CI 0.27-0.52), p<0.001), an effect that was observed from treatment received up to 9 weeks prior to delivery. This benefit is likely to rely on the superior efficacy of DHP against drug resistant isolates of both *P. falciparum* and *P. vivax* and the prolonged post treatment prophylaxis associated with piperaquine’s long terminal elimination half life ~28 days.(Hung et al., 2004, Davis et al., 2005, Price and Douglas, 2009) These data highlight DHPs potential for novel intermittent presumptive therapies.(White, 2005) The reduced risk of recurrent malaria was associated with a significantly lower risk of the newborn babies having congenital malaria (OR=0.06, [95%CI, 0-0.46], p=0.001) and perinatal death (AOR=0.32 [95%CI, 0.12-0.85]; p=0.03).

In utero exposure to artemisinin derivative have raised concerns over teratogenesis particularly in the 4th to 10th week of pregnancy.(White and Clark, 2008, Clark et al., 2008, Clark, 2009) Less is known regarding the use of piperaquine in early pregnancy. Our study shows that the risk of congenital defects in the neonate were similar irrespective of with and without exposure. One of the 258 babies born of women with documented ACT exposure had congenital malformations, the incidence being 0.4% [95%CI: 0.01%-3%]), similar to that observed following chloroquine or quinine exposure in this study (1.5% [95%CI, 0.04%-8%]) and those of others.(McGready et al., 2002, McGready et al., 2005, McGready et al., 2001) There were no birth defects reported following in utero exposure to DHP (Incidence 0% [95%CI, 0%-2%]). These data are reassuring, although our screening method was
limited to an examination at birth and will not have identified late congenital defect manifestations such as cardiac malformations. Additional larger, longitudinal studies are needed to address this.

Toxicity in rats, rabbits and monkeys suggest that artemisinin results in more foetal deaths than congenital malformations, although the former can be a consequence of severe malformation. (Clark et al., 2004, Clark et al., 2008) In contrast piperaquine exposure in pregnant mice was not associated with any severe congenital malformation, abortion or stillbirths. (Batty et al., 2009) Information on the presence of congenital malformation in stillbirths from this study was not available.

The risk of abortion following first trimester exposure was similar between women receiving any artemisinin derivative and those presenting without malaria (~40-47%), but was markedly higher than that in women treated with quinine (1%). Interestingly the risk of abortion was greatest in the women receiving DHP alone (70%) with none of those receiving iv artesunate, either with and without DHP, aborting (although numbers were small). The abortion risk of women treated with quinine in our study was significantly lower than that described in women on the Thai-Burmese border (25%), (McGready et al., 2002) suggesting a possible systematic bias in prescribing alternative medication in this risk group, for instance prescription of oral DHP alone reserved for those women who were more unwell, even though this was not recommended in local protocols. The markedly higher rates of abortion in women with first trimester exposure to DHP is of concern, and although this may signify fetal toxicity, it may also reflect intrinsic biases within our non-comparative observational study.

There are a number of limitations to this study. Information from prior antimalarial exposure was derived from a history from the mother and thus open to a potential recall bias. We used a systematic interview method to obtain information on the timing of exposures (approximate pregnancy gestational age in months) and treatment received (shape and colour of the tablets). Our experiences show that in this region women are familiar with the various antimalarial drugs available in the area. Furthermore when we restricted the data to the 50% of women in whom
documentation could be confirmed from hospital records, the observations of adverse outcomes were similar (Table 7-7).

Another limitation of our study is that pregnant women were not followed up prospectively following exposure. This is notoriously difficult to achieve and our study like many others, only manage to document birth outcome in 17% of pregnant women exposed to antimalarials and discharged with ongoing pregnancy. We can not discount the possibility of women exposed to DHP having adverse outcomes away from hospital, but we anticipate this to be similar for DHP as for chloroquine and quinine and hence the comparison between treatments provides a useful indication of relative adverse outcomes. Lack of prospective follow up also limited our ability to determine the efficacy of treatment regimens. Despite excellent cure rates in local clinical trials these excluded pregnant women, in whom pharmacokinetic drug profiles can vary considerably.(Nosten et al., 2006, Tarning et al., 2009, McGready et al., 2006b, McGready et al., 2006a) Reassuringly a study from Thailand found 92% cure rate at day 63, in a cohort of 47 pregnant women.(Rijken et al., 2008)

Conclusions
In conclusion, our results show an extremely favourable outcome when DHP is used in the second and third trimesters, reducing recurrent malaria and thereby perinatal death and congenital malaria. Further prospective studies defining the clinical and pharmacokinetic studies of DHP in pregnant women will help ensure optimal efficacy of this antimalarial in this important at risk group. The increased rates of abortion in women with exposure in the first trimester is of concern, and although may reflect the non comparative nature of our observational study, the use of DHP in the first trimester should continue to be avoided until further safety information is available.
Chapter 8

Temporal Trends of the Adverse Pregnancy Outcomes
Chapter 8 - Temporal Trends of the Adverse Pregnancy Outcomes

Abstract
Background. Pregnant women with malaria are at higher risk of having adverse pregnancy outcomes. A primary focus of current malaria control programs in pregnancy in Timika, Papua (Indonesia) is the early diagnosis and prompt treatment of malaria. There has been an active screening policy in the maternity ward at the local hospital since April 2004. In March 2006 the treatment of maternal malaria in the second and third trimesters of pregnancy was changed to dihydroartemisinin-piperaquine (DHP). In this chapter I report the impact of this change in treatment policy on associated adverse outcomes in pregnancy and early life.

Methods. From April 2004 to June 2009, a standardized questionnaire was used to collect information on pregnancy outcomes, clinical and laboratory data. A thorough physical examination was performed to all hospital live birth newborns. Pregnancy outcomes were documented in 847 women with a history of malaria treatment. The relative risks (RR) and the associated population attributable risks (PAR) of adverse outcomes in women with a history of malaria treatment to the risk in those without a history of malaria during the current pregnancy were examined to evaluate the temporal trends before and after DHP deployment.

Results. Over the 63 months period of the study 5292 women were enrolled in the hospital based surveillance program with known pregnancy outcome. Of these 847 (16%) reported prior antimalarial treatment during their pregnancy. By 2009 the proportion of women with malaria reporting treatment with DHP rose to 66% (154/234). The increasing use of DHP was associated with a 53% fall in the overall proportion of maternal malaria at delivery, the associated population attributable risk (PAR) of malaria treatment during pregnancy falling from 34% (95%CI, 29%-40%) before policy change to 16% (95%CI, 12%-19%) after. There was a modest reduction in the risk of low birth weight (6.5% [95%CI, 3%-10%] to 2.5% [95%CI, 0.1%-5%]) and severe anaemia (9% [95%CI, 4%-13%] to 7% [95%CI, 3%-11%]) attributed to history of malaria treatment during pregnancy before and after policy
change. In addition the risk of congenital malaria fell by 94%, from 3% prior to 0.2% after policy change (p<0.001).

**Conclusions.** We observed marked improvements in maternal health and pregnancy outcomes following change in treatment policy to DHP. Ensuring universal access to ACT in pregnancy through novel treatment and prevention strategies is likely to impact significantly on maternal child health.
Methods

An important finding in chapter 7 is the demonstration that pregnant women with history of malaria are at significantly greater risk of maternal malaria at delivery and other adverse pregnancy outcomes compared with women without any history of malaria (see table 6 in chapter 7). The impact of antimalarial policy change will be most apparent in women treated for malaria during pregnancy, the magnitude of this impact dependent upon the proportion of women with malaria receiving the new treatment regimen. Therefore overall temporal trends of the adverse outcomes were presented for the whole population of women delivering at the hospital and also after stratifying by those with prior malaria illness. Each year the proportion of women receiving each type of treatments was presented graphically in a pie chart.

To control for background fluctuations in the risk of adverse outcomes, the relative risk (RR) of adverse pregnancy outcomes were derived for women with history of malaria treatment compared to those without prior malaria. The proportion of women with malaria during pregnancy and its associated RR, were used to calculate the population attributable risk (PAR) of prior malaria during pregnancy on each adverse outcome before and after policy change. The significance of differences in relative risks was calculated according to the method of Altman and Bland (Altman and Bland, 2003). The 95% confidence interval for PAR was calculated according to the delta method (Hildebrandt et al., 2006).

A multiple logistic regression model was used to examine the relative contribution of the change in treatment policy on the occurrence of adverse pregnancy outcomes. All potential confounding factors, including a dichomotous variable denoting pre and post DHP introduction, were entered into the analysis.

Results

In total, 6519 pregnant women were enrolled in the hospital based malaria surveillance study. Of the 5292 (81%) women with known pregnancy outcomes, 847 (16%) reported a history of malaria treatment during the current pregnancy.
Figure 8-1. Prior antimalarial treatment during pregnancy in women delivering

Numbers above columns represent the proportion of known treatments within each year attributed to each drug regimen

Over the duration of the study, the number of women delivering with a history of malaria and malaria treatment during pregnancy increased significantly from 94 (of 931 women, 10%) in the first 12 months of observation to 218 (of 1057 women, 21%) in year 4 (April 2007 to March 2008), p<0.001. See figure 8-1. In those women with a history of malaria treatment the proportion receiving DHP rose from zero prior to policy change to 66% at the end of the study. See Figure 8-1.

**Temporal Trends in Confounding Factors**

The proportion of young mothers (age ≤ 16 years old) was similar between women with a history of malaria treatment (3.4%, 27/783) and those without prior exposures to antimalarial during pregnancy (2.3%, 102/4405), p=0.08. First time mothers were more frequently found in women with history of malaria treatment (36%, 279/784) than in those without history of antenatal malaria treatment (31%, 1365/4404), p=0.01. Overall, there was no difference in the annual proportion of pregnant women either aged ≤ 16 years old or primigravidae over the study period (Figure 8-2).
Figure 8-2. The yearly proportion of primigravidae (solid line) and pregnant women age ≤16 years old (dashed line)

The pie chart above the figures denote the proportions of women with prior antimalarial exposure: Quinine: dark grey, DHP: black, Cq+/-SP: light grey, AAQ: white. Numbers above the lines are RR [95%CI]. Black arrow marks the start of policy change. Black arrow marks the start of policy change.

The temporal trends of adverse outcomes in women with prior antimalarial drug exposure (solid line) and in those without history of malaria during pregnancy (dashed line) are presented in figures 8-3 and 8-4.

Lastly, the climate in the lowland Mimika district varies little throughout the year. Rainfall in the lowlands is approximately 5,000mm/year and tends to occur in the afternoons and evenings. Mean lowland temperatures are between 22°C and 32°C.

**Maternal Malaria**

The introduction of DHP for malaria in pregnant women was associated with a reduction in the prevalence of malaria at delivery. In women with a history of prior malaria treatment, the proportion with malaria at delivery declined from 60% (137/227) before DHP deployment to 28% (180/633) after policy change, p<0.001 (Figure 8-3). The relative risk of malaria at delivery associated with treatment was 4.9 [95%CI: 4.2-5.9] before policy change to 1.9 [95%CI: 1.7-2.3] after DHP deployment, p<0.001. Although the trend in decreasing RR began to emerge in year 2, before policy change was implemented. Overall the corresponding Population Attributable Risks (PAR) were 34% (95%CI, 29%-40%) before March 2006 and
16% (95%CI, 12%-19%) thereafter; in other words 18% of malaria at delivery was prevented by changing policy to ACT.

**Severe Maternal Anaemia**

A similar pattern was noted in the prevalence of severe maternal anaemia (Figure 8-3), the risk of severe anaemia declining steadily over the 5 years of the study in both women with and without a history of malaria treatment. However the relative risks associated with prior malaria treatment fell from 1.7 [95%CI, 1.3-2.4] prior to ACT to 1.4 [95%CI, 1.1-1.8] after DHP deployment, p=0.17. The corresponding Population Attributable Risks (PAR) were 9% (95%CI, 4%-13%) before DHP and 7% (95%CI, 3%-11%) after DHP; in other words 2% of severe anaemia was prevented by improving treatment.

**Figure 8-3. Maternal Malaria at Delivery and Severe Anaemia**

The pie chart above the figures denote the proportions of women with prior antimalarial exposure: Quinine: dark grey, DHP: black, Cq+/−SP: light grey, AAQ: white. Numbers above the lines are RR [95%CI]. Black arrow marks the start of policy change.
Adverse Outcomes of the Newborn

The proportion of low birth weight babies born of mothers without a history of malaria remained static over the study period (14.7%, 642/4382). In mothers with prior malaria treatment the prevalence of low birth weight fell from 20% (50/244) in the first two years of the study, to 11% (27/233) in year 5, \( p=0.017 \) (Figure 8-4). Prior to DHP deployment, the risk associated with a history of malaria treatment was 1.5 [95%CI, 1.2-2.0] compared to 1.1 [95%CI, 1-1.4] after policy change, \( p=0.024 \). The population attributable risks of low birth weight associated with prior malaria treatment decreasing from 6.5% (95%CI, 3%-10%) to 2.5% (95%CI, 0.1%-5%) following treatment policy change.

The prevalence of preterm delivery rose significantly prior to DHP deployment, but was followed by a decline after year 3 (Figure 8-4). These patterns were apparent in both women with and without a history of malaria. The relative risk associated with prior history of malaria fell from 1.6 [95%CI, 1.1-2.4] to 1.2 [95%CI, 1.1-1.6] after policy change, \( p=0.017 \), the population attributable risk decreasing from 7.8% (95%CI, 3%-13%) to 3.8% (95%CI, 2%-6%).

Over the study period perinatal death was recorded in 4.1% (217/5295) women delivering. There was no apparent increase in the risk of perinatal death associated with prior antimalarial treatment: 4.6% (36/784) in women with history of malaria treatment versus 4% (177/4408) in those women without prior antimalarial exposure, \( p=0.43 \). The low number of event leads to a wide confidence intervals in estimates of prevalence and no discernable temporal trend identifiable with change in policy.

The results of the temporal trends in congenital malaria have been presented in Chapter 6. The overall incidence was 3.1% (28/901) before DHP deployment, and fell to 0.2% (7/3322) after DHP was implemented; \( p<0.001 \). There have been no congenital cases reported since October 2008 (Figure 8-5).
**Figure 8-4. Adverse Pregnancy Outcomes**

The pie chart above the figures denote the proportions of women with prior antimalarial exposure: Quinine: dark grey, DHP: black, Cq+/SP: light grey, AAQ: white. Numbers above the lines are RR [95%CI]. Black arrow marks the start of policy change.
Figure 8-5. Congenital Malaria Cases
Discussion
Since the local Ministry of Health revised the antimalarial policy in Timika in March 2006, the public health care sector has knowingly treated more than 2900 pregnant women with DHA-piperaquine. At our sentinel site at the RSMM the clinical research team have observed administration of DHP to 765 women, and recorded deliveries in 847 women known to have been exposed to DHP during pregnancy. We have used this surveillance network to evaluate the impact of the policy change on adverse pregnancy outcomes at the hospital maternity ward.

The interpretation of temporal trends in malariometric burden is complex and dependent both on the management and health seeking behaviour of women presenting to the antenatal clinics as well as other risk factors. Our study showed that the number of women delivering with history of malaria and malaria treatment during pregnancy increased significantly from 94 in the first 12 months of observation to 218 in year 4 (April 2007 to March 2008). Community studies conducted in parallel also demonstrated a paradoxical increase in the number of malaria cases in the general population during the same period of time (May 2007 to November 2007) (unpublished data). A shift in health seeking behaviour following treatment policy change and an increase in vector numbers by 77% is likely to have contributed to this rise in malaria. People in this region are more likely to visit public health care for a good and free malaria treatment provided by the government. Despite the increased number of women presenting with malaria after introduction of DHP, we did not observe any corresponding increase risks in the adverse outcomes associated with malaria infection during pregnancy. In addition, our analyses of the temporal trend data were based on simple before-and–after comparisons and did not take into account the trajectory of trends nor the likely autocorrelation of the data. For these reasons, interpretation of our findings requires caution.

In women with antenatal malaria, the introduction of DHP correlated with a drop in the proportion of women with peripheral parasitaemia at delivery from 60% to 28%. Prior to policy change a history of antental malaria infection accounted for almost a third of cases of malaria at delivery but this fell to 16% following the introduction of
a more efficacious treatment regimen. The fall in the proportion of women with peripheral parasitaemia at delivery was associated with a dramatic reduction in the risk of congenital malaria from 3% to 0.2%. Indeed there have been no more congenital cases of malaria since October 2008.

We also demonstrated a modest reduction in the relative risk of low birth weight and a non significant fall in severe anaemia associated with antenatal malaria. The corresponding population attributable risk (PAR) falling from 6.5% to 2.5% and 9% to 7% respectively. Low birth weight and severe anaemia in pregnant women with *falciparum* malaria are associated with frequent malaria episodes and the timing of infections (Kalilani et al., 2010), hence the modest effect found in this study could be attributed to the limited access to early treatment. Inadequate quality of antenatal care would also compromise the pregnancy and health outcomes of pregnant women with relapsing *P. vivax* infections, which accounts for a third of maternal malaria in this area (Poespoprodjo et al., 2008). The introduction of DHP was also associated with a slight reduction in the risk of preterm delivery associated with prior malaria treatment during pregnancy. However an unexplained increased in the proportion of this adverse outcome before policy change confounds the interpretation of this observation.

Our study demonstrates an encouraging effect of improving maternal and pregnancy outcomes in women with the introduction of more efficacious antimalarial treatment regimens. However in order to have a significant impact on health targets, both case management and prevention program of malaria in pregnancy must be optimized to deliver universal coverage (WHO/AFRO, 2004, Brabin et al., 2008). In Papua, even though malaria control programs in pregnancy focus on providing early diagnosis and prompt treatment, in reality this capacity is available in less than half of the local health facilities (Karyana et al., 2008). There is very limited activity on the prevention of malaria in pregnancy. At the end of 2007 long lasting Insecticide Treated Nets (ITN) were distributed to 52% pregnant women in the district, but data on ITN use and its efficacy in pregnant women is still lacking. Currently, there is still no policy for introducing an Intermittent Preventive Treatment (IPT) program.
In view of the burden of disease and high infant and maternal mortality, optimizing malaria control programs remains a high priority for local healthcare providers. Our previous study (chapter 7) highlights that early detection and prompt treatment with an effective drug during pregnancy reduces the risk of malaria at delivery, perinatal deaths and congenital malaria. This suggests that intermittent screening and treatment would be a suitable option for malaria control programs, particularly in areas where a high proportion of maternal malaria is asymptomatic. On the Thai-Burmese border, intensive weekly screening and treatment during pregnancy reduced the local malaria burden in pregnancy (Nosten et al., 1991). However, the optimal timing and frequency still needs to be defined in areas with limited capacity and accessibility to health care such as is found in Papua.

Our study also highlights the excellent post treatment prophylaxis effect of DHP in pregnancy, the delay in recurrent malaria being observed for up to 9 weeks (Chapter 7). These findings demonstrate the potential for using effective long acting ACTs for IPT (see also Chapter 7) (White, 2005), both in pregnancy and school children (Cisse et al., 2009).

Our study has several limitations. Firstly, we used hospital-based surveillance and were unable to evaluate the overall program impact in the community. Although 76% of women came to the hospital for delivery, selection bias and treatment seeking behaviour remain potential confounders of the analysis. However we attempted to control for this by comparing the relative risks of adverse outcomes in women with and without a history of malaria treatment and using multivariate analysis to control for confounding risk factors.

Secondly, a history of symptomatic malaria and antimalarial treatment during pregnancy was obtained by interview, a method that is subjected to recall bias. However pregnant women in this region are very familiar with malaria symptoms and alternative antimalarial drugs. In addition we used a systematic interview method and review of the clinical notes. The history of malaria treatment could be confirmed in almost 50% of women with excellent concordance between reported drug history and that documented from the records (see chapter 7). Unfortunately, the interview method did not gather information on possible asymptomatic and untreated malaria
in pregnant women that may have underestimated the effectiveness of treatment policy change.

In conclusion, our findings highlight the declining trend of adverse maternal outcomes associated with deployment of DHP for malaria in the second and third trimesters of pregnancy. This study paves the way for scaling up both treatment and prevention programs using ACT. The latter will require identifying the most effective method of service delivery and translating research into practice.
Chapter 9

Summary and Recommendations
Chapter 9 - Summary and Recommendations

Summary
The work from this thesis has resulted in three major contributions to the knowledge of maternal and infant malaria. First, we have quantified for the first time the burden of malaria in pregnant women and infants in Papua (Indonesia) and highlighted the associated severe adverse outcomes in these vulnerable populations. Secondly, our studies highlight that in a coendemic area with emerging drug resistance, *Plasmodium vivax* accounts for a significant burden of disease. Thirdly, this region is the first in the world to recommend dihydroartemisinin piperaquine (DHP) as first line treatment for malaria in the second and third trimester of pregnancy for uncomplicated malaria from any species, providing unique opportunity to document important preliminary data on the relative safety and toxicity profile of this new antimalarial drug. We have shown that wide scale deployment of DHP has been associated with a reduction in key adverse outcomes in both mother and baby.

Maternal malaria at delivery is highly prevalent (17%) in this region with *P. vivax* accounting for almost a third of malaria infections. Maternal severe anaemia was a key determinant of adverse infant outcomes and was two folds more prevalent in women with malaria than in those without malaria. Peripheral *Plasmodium* parasitaemia at delivery together with severe anaemia was associated with low birth weight (OR=1.9), preterm delivery (OR=1.5) and stillbirths (OR=2.3). More than two thirds of the women with malaria were asymptomatic, highlighting the need for routine active surveillance to detect and treat infections as soon as possible. When symptomatic, malaria increased the risk of having preterm delivery and stillbirths by two folds. Women infected with *P. vivax* were at higher risk of having low birth weight babies (OR=1.9) and moderate anaemia (OR=1.8) compared to women without malaria. All parity groups are similarly affected.

In Papua the risk of malaria starts in the neonatal period with mother to child malaria transmission rate of 3%. Of the 38 newborns with congenital malaria, only one had symptom. Symptomatic malaria was commonly found in the first 3 months of life.
with severe anaemia as the major marker of severity present in 30% of infants needing admission to hospital. *P. vivax* is a predominant cause of morbidity in early infancy accounting for 56% of all plasmodium infections and associated with an increased risk of severe anaemia (OR=2.4) and severe thrombocytopenia (OR=3.3) compared with those infected with *P. falciparum*. Malaria is associated with undernutrition in this age group, which was present in 28% of infants with *P. falciparum* and 18% with *P. vivax* compared to ≈8% in the community. Case fatality rate was similar between *P. vivax* and *P. falciparum* infections (1-2%).

In Timika high levels of multidrug resistance exists to both *P. falciparum* and *P. vivax*. Malaria treatment policy was changed from sulfadoxine-pyrimethamine and chloroquine to highly effective dihydroartemisinin-piperaquine (DHP) in March 2006. Our surveillance system has helped demonstrate that the introduction of ACT was associated with favourable impact to maternal and child health outcomes reducing both morbidity and mortality. After the introduction of DHP, the relative risk of recurrent malaria at delivery and the prevalence of congenital malaria are markedly reduced by two and 15 folds respectively.

Despite concerns regarding the use of dihydroartemisinin-piperaquine in pregnancy, the regimen appears relatively safe for malaria treatment in the second and third trimester of pregnancy and preliminary evidence suggests that it was not associated with an increased risk of congenital malformation.

**Recommendations**

Although the introduction of ACT has had notable benefits on community health, the impact on maternal and infant outcomes has been variable. It is likely that this reflects a lack of access of women to early and effective treatment. In Papua where the quality of antenatal care can be severely compromised, greater efforts are needed to provide better access, early diagnosis and prompt treatment. These factors are crucial if universal impact is to be achieved in the general health of pregnant women and infants.
The studies of our results are very reassuring, but additional studies are needed to quantify the pharmacokinetics of piperaquine in the second and third trimester of pregnancy to ensure optimal dosing strategies. Tolerability of DHP in both mother and foetus needs to be monitored particularly the outcomes of inadvertent exposure to dihydroartemisinin-piperaquine in the first trimester.

In view of the high prevalence of asymptomatic parasitaemia in pregnant women, methods in delivering timely antimalarial therapy as treatment or prevention should be identified. The long term role of the post treatment prophylactic benefits of DHP in preventing reinfection and *P. vivax* relapses needs to be explored in pregnant women and infants. Studies of the effectiveness of intermittent malaria screening and treatment (IST) are warranted and, if successful, whether extrapolating the use of DHP to intermittent preventive treatment in pregnancy and infants (IPTp and IPTi) is appropriate. In addition to the optimization of drug therapy and delivery strategies, attention need to be placed on the role of ITNs, particularly with regard to controlling *P. vivax* malaria in pregnant women and infants.

In conclusion, this thesis addresses global health priorities in improving maternal and child survival that is stated in the Millennium Development Goals number 4 and 5. Our findings have paved the way to identifying the most effective intervention strategy and service delivery that would reduce malaria associated morbidity and mortality in both mothers and infants.
References
References


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USMAN. 2009. **RE: Malaria Officer at Mimika District Health Authority - Personal Communication.**


Appendices
Appendix 1.1  
HOSPITAL SURVEILLANCE: Information Sheets & Consent Forms

These forms are for all hospital patients, who are pregnant or less than 3 months of age. Malaria is a common infection in Indonesia and may cause serious illness. This form is for you to keep and tells you about planned research projects investigating which patients (particularly pregnant women and babies) are at highest risk of developing malaria.

“Which pregnant women and babies are more likely to get malaria?”

Malaria can be a serious disease if it is not treated quickly and effectively. Pregnant women and babies are particularly vulnerable to malaria infection. We are conducting studies in this Hospital to investigate which pregnant women and babies are more likely to get malaria and how sick they become. If you have malaria you/your child will receive current hospital standard therapy; this will depend upon how sick you / your child is and what type of malaria you are infected with.

If you agree to be in the study the following procedures will happen:

1. You will be asked a questionnaire about your pregnancy / illness and you will be examined.

2. If you agree to give us a drop of blood from a finger prick, it will be used to confirm that you have malaria, test the parasite type and if your baby gets malaria to see whether you both have the same parasite. From the sample blood sample we will check to see whether you are anaemic.

3. If you agree to your baby having a heel prick blood sample it would be used to see if you and your baby have the same parasite.

Benefits of these Studies: Being part of this study may help us to detect as early as possible if you / your child have malaria. If we identify that you or your baby are at high risk of malaria then we will be able to monitor you more closely so that treatment can be started as soon as possible.

If you do not wish to participate in these studies it will NOT affect your right to receive standard health care administered at this clinic.

Any time you can withdraw you/your child from the study and still receive the treatment as you would normally.

If you have any questions about this study, you may contact Dr Rini on 901-301-827 or the study doctors in this clinic.

In case of an emergency you should return to this clinic or if it is after hours present yourself to the Rumah Sakit Mitra Masyarakat Hospital Emergency Room and inform the doctor that you have been a participant in this study.

If you have a complaint about the study then these should be addressed to Dr Liliana Kurniawan, the Chairperson of the Ministry of Health Ethics Committee, Jakarta. Telephone Number: 021 – 425 9860

If you wish to know what this study showed: - you can write to the Medical Director of RSMM. There will be no name or other patient identification in any study report which will be published later on.
Appendix 1.1
HOSPITAL SURVEILLANCE: Information Sheets & Consent Forms

“Which pregnant women and babies are more likely to get malaria?”

Consent Form for ADULTS to participate in malaria research studies:

This form means I can say “No”
I have read and understood the information sheets attached, and have been given the opportunity to discuss it and ask questions. I agree that a sample of my blood can be used in the research outlined on the information sheet. I understand that I do not have to participate in this study.
If I do not participate in the study I will still receive the standard health care administered at this clinic.

I ……………………………………………….agree to participate in the above study.

I DO / DO NOT * agree to having a finger prick sample taken.
Signature of Patient: ..........................................................
Date: ..........................................................................
Witness Name: ..........................................................
Signature: .................................................................

Consent Form for BABIES to participate in community research studies:

This form means I can say “No”
I have read and understood the information sheets attached, and have been given the opportunity to discuss it and ask questions. I agree that my babies blood can be used in the research outlined on the information sheet. I understand that we do not have to participate in this study.
If I do not participate in the study I will still receive the standard health care administered at this clinic.

I agree as the PARENT / LEGAL GUARDIAN of………………………………………………..that he / she can be enrolled in the above studies.

I DO / DO NOT * agree to my baby having a heel prick sample taken.
Signature of Parent/Guardian: ..........................................................
Name of Parent/Legal Guardian: ..........................................................
Date: ..........................................................................
Witness Name: ..........................................................
Signature: .................................................................
FORM INI UNTUK SEMUA PASIEN DI RUMAH SAKIT/KLINIK YANG SEDANG HAMIL ATAU BERUSIA KURANG DARI 3 BULAN.

Malaria adalah penyakit yang sering terjadi di Indonesia dan dapat menyebabkan sakit berat. Form ini dapat anda simpan dan isinya akan memberikan informasi tentang penelitian yang bertujuan untuk mengetahui pasien mana (terutama ibu hamil dan bayi) yang mempunyai risiko tinggi terkena malaria.

“IBU HAMIL DAN BAYI MANA YANG RENTAN TERHADAP MALARIA?”

Malaria berpotensi untuk menjadi penyakit berat jika tidak diobati dengan cepat dan efektif. Ibu hamil dan bayi sangat rentan terkena malaria. Kami melakukan penelitian di RS/Klinik ini untuk mengetahui ibu hamil dan bayi mana yang rentan terhadap malaria dan seberapa jauh mereka menjadi sakit.

Jika anda mengalami malaria, anda/anak anda akan mendapatkan pengobatan sesuai standard pengobatan RS/Klinik; ini akan tergantung seberapa berat sakit anda/anak anda dan jenis malaria apa yang menginfeksi anda/anak anda.

Jika anda setuju untuk ikut serta dalam penelitian ini maka kami akan melakukan beberapa prosedur penelitian dibawah ini:

1. Anda akan ditanyakan mengenai kondisi kehamilan dan kesehatan serta akan dilakukan pemeriksaan fisik.
2. Jika anda setuju untuk diambil darah dari ujung jari, darah tersebut akan digunakan untuk pemeriksaan malaria, jenis parasit dan jika bayi anda juga terkena malaria, sampel darah itu juga akan digunakan untuk mengetahui apakah jenis parasit nya sama. Dari sampel darah kami juga akan memeriksa apakah anda mengalami kurang darah.

KEUNTUNGAN PENELITIAN INI: Keikut sertaan pada penelitian ini dapat membantu anda/anak anda untuk mendeteksi malaria secara dini. Jika kami mendapatkan bahwa bayi anda mempunyai risiko tinggi untuk terkena malaria, maka kami dapat memonitor secara ketat dan memulai pengobatan sesegera mungkin.

JIKA ANDA TIDAK INGIN BERPARTISIPASI DALAM PENELITIAN INI, HAL INI TIDAK AKAN MEMPENGARUHI HAK ANDA DAN BAYI ANDA UNTUK MENDAPATKAN PELAYANAN Kesehatan Standard di RS/Klinik Ini.

Anda dapat setiap saat mengundurkan diri dari penelitian ini dan tetap mendapatkan pengobatan seperti biasa.
Jika anda mempunyai pertanyaan mengenai penelitian ini, anda dapat menghubungi Dr. Rini (0811491699) atau peneliti lain di klinik ini.

Dalam keadaan emergensi anda harus kembali ke RSMM dan memberi tahu dokter bahwa anda merupakan partisipan penelitian ini.

Jika anda mempunyai keluhan terhadap penelitian ini, maka harus dilaporkan kepada tim Etik Departemen Kesehatan: Dr. Liliana Kurniawan, Ketua Tim Etik di 021- 425 9860

Jika anda ingin mengetahui hasil penelitian ini: anda dapat menyurat ke Direktur RSMM. Nama anda tidak akan disebutkan dalam setiap laporan/publikasi penelitian ini.
“Ibu hamil dan bayi mana yang rentan terhadap malaria?”

Lembar persetujuan pasien dewasa untuk ikut berpartisipasi dalam penelitian:

Lembar ini berarti anda dapat berkata “Tidak”

Saya telah membaca dan mengerti informasi yang tercantum pada lembar informasi dan telah diberi kesempatan untuk mendiskusikan dan menanyakan hal tersebut. Saya setuju jika sample saya dapat digunakan untuk penelitian yang tercantum dalam lembar informasi. Saya mengerti bahwa saya dapat menolak untuk ikut dalam penelitian. Saya mengerti bahwa sample saya tidak akan digunakan untuk kepentingan lain. Saya sadar bahwa saya dapat mengundurkan diri dari penelitian ini kapan saja saya mau.

Jika saya tidak mengikuti penelitian ini saya akan tetap menerima pelayanan kesehatan standard di RS ini

Saya ……………………………………………. setuju untuk berpartisipasi dalam penelitian ini.

Saya SETUJU / TIDAK untuk diambil darah dari ujung jari

Tanda tangan pasien : .................................
Nama : .................................
Tanggal : .................................
Nama Saksi : .................................
Tanda tangan : .................................
Lembar persetujuan untuk bayi yang ikut dalam penelitian malaria:

Lembar ini berarti anda dapat berkata “Tidak”

Saya telah membaca dan mengerti informasi yang tercantum pada lembar informasi dan telah diberi kesempatan untuk mendiskusikan dan menanyakan hal tersebut. Saya setuju jika sample saya dapat digunakan untuk penelitian yang tercantum dalam lembar informasi. Saya mengerti bahwa saya dapat menolak untuk ikut dalam penelitian. Saya mengerti bahwa sample saya tidak akan digunakan untuk kepentingan lain. Saya sadar bahwa saya dapat mengundurkan diri dari penelitian ini kapan saja saya mau.

Jika saya tidak mengikuti penelitian ini saya akan tetap menerima pelayanan kesehatan standard di RS ini

Saya setuju sebagai **ORANG TUA/WALI** dari …………………… untuk berpartisipasi dalam penelitian ini

Saya **SETUJU / TIDAK** bayi saya untuk diambil darah dari ujung jari

**Tanda tangan OT/Wali:** ………………………………………

**Nama OT/Wali** : …………………………………………

**Tanggal** : ………………………………………

**Nama Saksi** : ………………………………………

**Tanda tangan** : ………………………………………
CLINICAL DETAILS

STUDY/CODE: MP________

Date of admission: 

HRN: ________

Name:______________________________

Address:______________________________

Age:______ Suku:________

Place of Birth:  Highland Lowland NonPapuan

How long have you been in Timika?______________years

Reason for Admission:  Malaria Delivery Other:______________________________

Parity:______ Week of Pregnancy (approx):_________

Clinical Features

History of Fever (48Hrs): Y / N

Febrile Illness During this Pregnancy Y / N How Many?________

If Yes What Month?________ What treatment?____ Where? ________

ANC: None RSMM Private doctors Midwives Other:

HIV Status: + -- UK Smoker Y / N How Many?_______

Other Comorbidities:_________________ PPH : Y / N

Markers of Severity (see RSMM List):________

Temperature on Admission: ________ BP : __________ mmHg

Treatment: 1. Quinine 2. Chloroquine 3. SP 4. Other_______

Outcome: Mother: Alive Died

Pregnancy: Delivery Miscarriage Stillbirth Neonatal Death Ongoing Pregnancy

IF Delivery...

Date of Delivery:__________ Placental Weight:_______ g

Birth weight1:______________ Birth weight2:_________

Name:______________________________

Dubowits score :__________ Gestational age:________

Age When Examined (Hours): Sex1: M / F

Baby Symptoms: Sex2: M / F

Fever Restless Unable To Suck Other:______________________________

Baby Code MB
APPENDIX 2

PREGNANT WOMEN FORM

LABORATORY DETAILS

THE MOTHER

<table>
<thead>
<tr>
<th>Date</th>
<th>Blood Film</th>
<th>FBC</th>
<th>WCC</th>
<th>ParaCheck</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg Pf Pm Po P200:P1000:G200:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

THE BABY

<table>
<thead>
<tr>
<th>Date</th>
<th>Blood Film</th>
<th>FBC</th>
<th>Others:</th>
<th>Slides Saved:</th>
<th>WB Saved :</th>
<th>PCR Spot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neg Pf Pm Po P200:P1000:G200:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

0. Not severe, 1. GCS < 11/15 Blantyre<2 / 5, 2. Hb<5g/dl/Hct<15% , 3. Visible Jaundice and >100,000 parasites /ul, or Cr>1.5 4. Cr>3mg/dl +/- Urine Output <400ml day-1 5. Plasma Glucose < 40mg/dl 6. Asexual Parasitaemia > 10%. 7. BP<80mmHg and cool peripheries 8. Blackwater fever 9. Acidosis HCO3<15 10. Resp Insufficiency (central cyanosis or RR>32min or deep breathing in children) 11. Prostration
### Ballard Score

**MATURATIONAL ASSESSMENT OF GESTATIONAL AGE** (New Ballard Score)

<table>
<thead>
<tr>
<th></th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>Date/Time of birth</td>
</tr>
<tr>
<td>Hospital No</td>
<td>Date/Time of exam</td>
</tr>
<tr>
<td>Race</td>
<td>Age when examined</td>
</tr>
<tr>
<td>Apgar score: 1 minute</td>
<td>5 minutes</td>
</tr>
<tr>
<td></td>
<td>10 minutes</td>
</tr>
<tr>
<td>Sex</td>
<td>Birth weight</td>
</tr>
<tr>
<td></td>
<td>Length</td>
</tr>
<tr>
<td></td>
<td>Head circ.</td>
</tr>
<tr>
<td></td>
<td>Examiner</td>
</tr>
</tbody>
</table>

#### Neuromuscular maturity

<table>
<thead>
<tr>
<th>Neuromuscular maturity sign</th>
<th>Score</th>
<th>Record score here</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picture</td>
<td>-1</td>
<td></td>
</tr>
<tr>
<td>Square window (wrist)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Arm recoll</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Popliteal angle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scarl sign</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heel to ear</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Maturity rating**

<table>
<thead>
<tr>
<th>Score</th>
<th>Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td>20</td>
</tr>
<tr>
<td>-5</td>
<td>22</td>
</tr>
<tr>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>26</td>
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<td>50</td>
<td>44</td>
</tr>
</tbody>
</table>

#### Physical maturity

<table>
<thead>
<tr>
<th>Physical maturity sign</th>
<th>Score</th>
<th>Record score here</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>-1</td>
<td></td>
</tr>
<tr>
<td>Lanugo</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plantar surface</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye/ear</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genitals (male)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Genitals (female)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**FIGURE 3-1: Maturation assessment of gestational age (New Ballard Score). (Reproduced, with permission, from Ballard JL et al. New Ballard Score, expanded to include extremely premature infants. J Pediatr 1991;119:417.)**
Appendix 4.1

Newborn Check

Mothers Name and ANC code

Date and Time of Birth (dd/mm/year/hh/min)

Date and time of Examination (dd/mm/year/hh/min)

Sex

Weight (kg)  HC (cm)  Length (cm)

Home delivery  YES/NO

History of Labour and Delivery

1. Gestation at birth  _______ Weeks

2. Has mother had any previous early neonatal deaths? YES/NO

3. Length of time membranes ruptured  _______ Hours

4. Any evidence of maternal sepsis? YES/NO

5. Any antibiotics or antimalarials during labour? YES/NO

6. Any foetal distress? YES/NO

7. Type of delivery?

- Normal
- Forceps
- Vacuum
- Breech
- C-section

Resuscitation of baby

8. Apgar Score  1 minute  _______  5 minutes  _______

9. Any resuscitation needed? YES/NO

Details

Newborn Examination

Observations

1. What is the colour of the infant? (circle all that apply)

- Pale
- Cyanosed
- Jaundiced
- Normal

2. Is there a rash present? YES/NO

Describe ___________________________

3. Are there any birth marks present? YES/NO

Describe ___________________________

4. Describe the anterior fontanelle

- Raised
- Normal
- Depressed

5. Is there any bruising to the head or face? YES/NO

Describe ___________________________

6. Is the baby’s face including palate and mouth normal? YES/NO

Describe ___________________________

7. Are the limbs, fingers and toes normal? YES/NO

Describe ___________________________
Cardiovascular Examination

8. Heart rate (per minute) ______ If >160 or < 100 call the medic or doctor

9. Describe the heart sounds
   Normal    Murmur, describe ________________

10. Are the femoral pulses present? YES/NO

Respiratory Examination

11. Respiratory rate (per minute) ______ If >60 call the medic or doctor

12. Listen to the chest is it clear? YES/NO
   If no describe the noise and location on the diagram

13. Are any of the following present? (circle all that apply)
    Chest indrawing    Nasal flaring    Head bobbing    None

Abdominal Examination

14. On palpation of the abdomen is there a mass / liver/ spleen palpable? YES/NO
    If yes please draw on the diagram

Neurological examination

15. Does the infant have normal muscle tone? YES/NO
    If no describe __________________________________________

16. Is the infant moving all limbs normally? YES/NO
    If no describe __________________________________________

Others

17. Is the genitalia normal (both testes descended in a boy)? YES/NO
    Describe ____________________________________________

18. Does the infant have a normal anus? YES/NO
    Describe ____________________________________________

19. Is there any other abnormalities found? YES/NO
    If yes, describe ____________________________________________

Is this a normal infant? YES/NO
If NO refer to a doctor or medic

Outcome? (please circle)
   Admitted IPD    Observation 24hours    Home

Form completed by:
Date
Appendix 4.2

Pemeriksaan Neonatus

<table>
<thead>
<tr>
<th>MP Code</th>
<th>__________</th>
</tr>
</thead>
</table>

Tanggal dan jam Lahir:  
Tanggal dan Jam Pemeriksaan:  
Jenis Kelamin  Berat Badan (kg)  Lingkar Kepala (cm)  
Panjang (cm)  
Lahir di rumah  Ya/Tidak

Riwayat Persalinan

1. Masa gestasi saat lahir  _________ Minggu  
2. Apakah ibu mempunyai riwayat kematian neonatus awal (umur 0-7 hari)?  
   Ya/Tidak  
   Jika Ya, beritahu perawat atau dokter
3. Lama pecah ketuban  _________ Jam  
   Jika lebih dari 18 jam, beritahu perawat atau dokter
4. Ada bukti sepsis pada ibu?  
   Ya/Tidak  
   Keterangan  _________
5. Apakah ibu diberi antibiotik atau antimalaria selama persalinan?  
   Ya/Tidak  
   Keterangan  _________
6. Apakah ada gawat janin?  
   Ya/Tidak
7. Jenis Persalinan?  
   Normal  Forceps  Vacuum  Sungkang  Operasi Caesar

Resusitasi pada bayi

8. Skor Apgar  
   1 menit _________  5 menit _________
9. Apakah dilakukan tindakan resusitasi?  
   Ya/Tidak  
   Keterangan  _________

Pemeriksaan bayi

Observasi keadaan bayi

1. Warna kulit bayi (lingkari):  
   Pucat  Mottled  Sianosis  Normal  Ikterik
2. Apakah ada rash (lingkari)?  Ya/Tidak  
   Jika ada, jelaskan  _________
3. Apakah ada tanda lahir (lingkari)?  Ya/Tidak  
   Jelaskan  _________
4. Jelaskan fontanel (ubun2) anterior (lingkari) :  
   Normal  Cembung  Cekung
5. Apakah terdapat lebam di daerah kepala dan muka (lingkari)?  Ya/Tidak  
   Jika Ya, Jelaskan  _________
6. Apakah muka bayi terlihat normal (lingkari)?  Ya/Tidak  
   Jika tidak, Jelaskan  _________
7. Apakah ke empat anggota badan dan jari tangan-kaki terlihat normal (lingkari)?  
   Ya/Tidak  
   Jika Tidak, Jelaskan  _________
Appendix 4.2

Pemeriksaan Neonatus

**Pemeriksaan Kardiovaskuler**
8. Heart rate (per menit) __________ (jika >160x/menit atau <100x/menit, lapor)
9. Bunyi Jantung (lingkari)
   a. Normal  b. Murmur, Jelaskan ________________
10. Apakah denyut nadi femoral teraba (lingkari)? **YA/TIDAK**

**Pemeriksaan Sistem Pernapasan**
11. Respiratory rate (per menit) __________ (jika >60x/menit atau <30x/menit, lapor)
12. Auskultasi dada:
   a. Normal  b. Suara tambahan
   Jelaskan, kualitas dan lokasi pada diagram
13. Apakah terdapat satu hal dibawah ini (Lingkari)? Retraksi intercostal Retraksi subcostal Tracheal tug Pernapasan Cuping Hidung Head bobbing

**Pemeriksaan Abdomen**
14. Palpasi Abdomen
   - Teraba massa (lingkari)? **YA/TIDAK**
   - Teraba hepar (lingkari)? **YA/TIDAK**
   - Teraba limpa (lingkari)? **YA/TIDAK**
   Jika ada, gambarkan dalam diagram

**Pemeriksaan Neurologis**
15. Apakah tonus otot bayi normal (lingkari)? **YA/TIDAK**
   Jika tidak normal, Jelaskan ________________
16. Apakah pergerakan extremitas bayi normal (lingkari)? **YA/TIDAK**
   Jika tidak normal, Jelaskan ________________

**Lain-lain**
17. Apakah terdapat kelainan pada genitalia? **YA/TIDAK**
   Jika ya, Jelaskan ________________
18. Apakah terdapat anus? **YA/TIDAK**
19. Apakah terdapat kelainan lain yang belum disebutkan diatas?
   Jelaskan ________________

**Apakah secara keseluruhan bayi ini tampak normal?** **YA/TIDAK**

**Jika Tidak, lapor ke dokter**

**Outcome? (lingkari)**
*Dirawat*  *Observasi 24 jam*  *Pulang*

**Formulir diisi oleh:**
**Tanggal :**
### Active Malaria Surveillance Data Collection Form

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Appendix 6

MALARIA IN BABIES (0-3m)

CLINICAL DETAILS

Date of admission:_________________ HRN: _________
Name:__________________________________
Address:________________________________
Date of Birth:_______ Sex:_______ Age:_______
Suku:_____________

Clinical Features

History of Fever : Y / N
Duration of Illness:_______
Temperature on Admission:_______ Weight:____
Sign:    Fever     Splenomeg     Hepatomeg    Restless    Unable to Suck    Cough
        RR_______ Other:_________

Markers of Severity (see RSMM List):___________

Treatment:

1. Quinine   2. Chloroquine   3. SP   4. Other____
Outcome:    Survived    Died
Date of Discharge / Death:______________

Maternal Details

Name:__________________________________ HRN:___________
Age:______ Suku:____________
Parity:____________

Do you spend more time in Highland or Lowland?    Highland   Lowland
Febrile Illness During Pregnancy:  Y / N
Known Malaria during Pregnancy:Y / N
    If Yes When?__________
    If Yes Where?__________

Delivered at RSMM?    Y / N
Pregnancy Code   MP
### LAB DETAILS (BABY)

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**Others:**

- Slides Saved: Y / N
- Clinical WB Saved (if available): Y / N
- PCR Spot: Y / N

### LAB DETAILS (Mother)

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**Others:**

- Slides Saved: Y / N
- Clinical WB Saved (if available): Y / N
- PCR Spot: Y / N

### COMMENTS: