Effects of malaria and anaemia during pregnancy on survival and morbidity in infants living in an area of low malaria transmission

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ABSTRACT

The effects of malaria and anaemia during pregnancy on infant survival and morbidity were investigated in an area of low malaria transmission on the western border of Thailand. A cohort of 1,495 mothers and their infants were followed weekly from admission of the mother to ante-natal clinics until one year of life of the infant.

37% (555/1495) of the women developed malaria during pregnancy and 77% (1134/1468) were anaemic (HCT<30%). Malaria during pregnancy was responsible for 23% (95% Confidence Interval:14-30) of Low Birth Weight (LBW), but did not reduce the duration of gestation. Infectious diseases in the week prior to birth were associated with premature birth (Population Attributable Fraction: 10%; 95%CI: 6-12).

Infant mortality rate was 78 (88/1127) per 1,000 live births. Risk factors for neonatal mortality were prematurity (Hazard Ratio: 9.8; 95% CI: 3.8-25.2), LBW (HR: 2.9; 95%CI: 1.2-7.3), and maternal infection in the week prior to delivery (HR: 4.3; 95%CI: 2.0-9.2). The latter remained associated with deaths occurring between one and three months of age (HR: 4.0; 95%CI: 1.2-13.7), whereas no risk factors could be identified for deaths occurring later in infancy. Malaria during pregnancy affected neonatal mortality solely by lowering birth weight, whereas fever in the week prior to birth had an additional independent effect as well as inducing premature birth.

Malaria incidence rate in infancy was low: 121 (148/1228) per 1,000 child-year (95%CI: 103-142). The stage of pregnancy at which malaria occurred influenced malaria in infants. Infants born to mothers who had falciparum malaria during the first trimester were 2.8 (1.5-5.1) times more likely to develop malaria in infancy, than infants born to mothers who had malaria later during pregnancy, or no malaria.

The long-term impact of malaria during pregnancy on the infant was mediated through LBW that was associated with higher morbidity, anaemia and impaired growth. However, as age increased, socio-economic factors became more important than LBW for the development of infant anaemia and for the risk of being underweight and/or stunted.

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ABBREVIATIONS

ANC	Ante-Natal Clinics
ARI	Acute Respiratory Infection
BBC	Burma Border Consortium
CFR	Case Fatality Rate
CI	Confidence intervals
ENT	Eyes Nose Throat
EPI	Expanded Programme of Immunization
Hb	Haemoglobin
HAZ	Height-for-Age Z score
HCT	Haematocrit
HR	Hazard Ratio
IR	Incidence Rate
IRR	Incidence Rate Ratio
IUGR	Intra-uterine growth retardation
IMR	Infant Mortality Rate
LBW	Low Birthweight
MSF	Médecins sans Frontières
MZO	Malaria Zone Office
NBW	"Normal" Birthweight
NCHS	National Center for Health Statistics
OR	Odds Ratio
PAF	Population Attributable Fraction
PCV	Packed Cell Volume
RR	Relative Risk
SD	Standard Deviation
SEM	Standard Error of the Mean
SES	Socio-Economic Status
SMRU	Shoklo Malaria Research Unit
UTI	Urinary Tract Infection
WAZ	Weight-for-Age Z score
WHO	World Health Organization
WHZ	Weight-for-Height Z score

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CHAPTER 1

INTRODUCTION

1.1. Statement of the problem

Malaria during pregnancy is harmful for both the mother and the foetus. Pregnant women living in malaria endemic areas have an increased risk of developing malaria when compared to their non-pregnant counterparts. The clinical features vary from asymptomatic infection to severe life-threatening malaria, depending on the level of transmission. The most common effect of malaria infection during pregnancy on the mother is anaemia. As a consequence, in malarious areas, malaria-attributable anaemia is the most important contributor to maternal anaemia. This is most marked in primigravidae who are more affected by malaria (Brabin, 1991). Nevertheless, maternal anaemia is usually multifactorial, and other factors such as iron deficiency, hookworms infestations, haemoglobinopathies, may all contribute (Fleming, 1989a). Their relative importance may depend on the number of pregnancies the mother has had.

Malaria and anaemia during pregnancy act together in reducing birthweight. Malaria has long been recognized as an important determinant of low birthweight (LBW) (<2500g) (McGregor *et al.*, 1983), as well as anaemia in both malarious and non-malarious areas. Until recently, the distinction between full-term and preterm (< 37 weeks) LBW was not clearly established. Accurate methods for the estimation of gestational age can now be employed. Since their introduction, it has been recognized that LBW due to malaria and anaemia are mostly due to intra-uterine growth retardation (IUGR) rather than preterm deliveries (Meuris *et al.*, 1993; Dolan *et al.*, 1993; Nosten *et al.*, 1994a; Steketee *et al.*, 1996b). However the incidence of premature births is also increased by severe maternal malaria.

The consequences of malaria and anaemia during pregnancy on maternal morbidity and mortality, and on the newborn are now well described (Brabin, 1991; Menendez, 1995). The long term effects on the infant are not so well established. As LBW is a major determinant of infant mortality (Ashworth & Waterlow, 1982), it has been assumed that malaria during pregnancy may indirectly increase infant mortality. In the neonatal period, maternal malaria has not been directly associated with perinatal or neonatal mortality, but both severe maternal anaemia and LBW have been shown to increase the risk of dying within the first month of life. Thus malaria-related neonatal mortality might be important. In the post-neonatal period, maternal anaemia has been

associated with infant mortality (Dolan *et al.*, 1993; Nosten *et al.*, 1994a; Verhoeff & Brabin, personal communication), but the effects of malaria *per se* have not been established.

The mechanisms through which maternal anaemia may increase the risk of infant death remain unclear. Are the children born to anaemic mothers more likely to develop anaemia during infancy than those born to non-anaemic mothers? Do they have impaired growth and/or an increased morbidity during infancy? What are the relative importance of factors such as the environment, and socio-economic status of the family?

The association between malaria during pregnancy and malaria in infancy is difficult to study. Infants have been shown to be susceptible to malaria infection (MacDonald, 1950) but protected against clinical malaria during the first months of life (Achidi *et al.*, 1996a; Snow *et al.*, 1998; McGuiness *et al.*, 1998). This protection is believed to be multifactorial, but mainly related to transplacentally acquired maternal antibodies. Nevertheless, the role of maternal antibodies in protecting the newborn baby remains controversial. Most investigators have used malaria-infection as their markers of protection, whereas the protective effect of maternal antibodies may only be on the development of symptoms. Recently, it has been shown that only some sub-groups of antibodies may be protective against clinical disease (Branch *et al.*, 1998). Materno-foetal transfer of antibodies and thus potential protection may also vary with the level of malaria transmission. There is also some evidence of prenatal immune priming of malaria. But, the complex materno-foetal relationships change during the course of pregnancy, and may differ between primi- and multigravidae. Hypothesis have been raised to suggest that the response to malaria during infancy may vary according to the age of first exposure to malaria *in utero* (Rasheed, 1994), and whether the infant is the first born child or from a multiparus woman. Longitudinal studies, in which pregnant women and their offspring are followed from early pregnancy, and throughout infancy are required in order to test these hypotheses. In the studies completed so far, women have only been followed from late pregnancy, and the results have often been conflicting.

In our study site on the Thai-Burmese border, malaria transmission is low and unstable. Malaria during pregnancy is a major public health problem. In 1984, it was recognized as an important cause of maternal morbidity and mortality. The Shoklo Malaria Research Unit (SMRU) implemented antenatal clinics (ANC) with weekly detection of malaria and treatment of all parasitaemic women. Since the clinics were initiated, maternal morbidity from malaria has been reduced and malaria-attributable mortality during pregnancy entirely suppressed (Nosten *et al.*, 1991). Nevertheless, the consequences on the infants have not disappeared. Both *P.falciparum* and *P.vivax* malaria during pregnancy are associated with reduced birthweight. In two randomised trials of malaria preventive measures during pregnancy, maternal anaemia at delivery and LBW have been shown to be major risk factors for infant mortality (Dolan *et al.*, 1993; Nosten *et al.*, 1994a). However, in these studies, morbidity during infancy, and socio-economic factors were not taken in account. Moreover, studied women were a selected group of all pregnant women and this may have introduced a bias. We therefore decided to follow closely all children born to women attending ANC. They represent 90% of pregnant women in the site and they often attend ANC early in pregnancy. Moreover the SMRU has a long experience of long term prospective studies. The longitudinal design would permit assessment of the long-term effects of malaria and anaemia during pregnancy, both on infant mortality and morbidity, and whether the stage at which malaria infection occurs during pregnancy influences malaria in infancy. The study was also designed to describe the main causes and risk factors of infant deaths, and when malaria was first acquired in infancy in an area of low malaria transmission. This thesis reports the results of these epidemiological evaluations.

1.2. Objectives

Using a cohort of liveborn babies, this work aims to assess the effects of malaria and anaemia during pregnancy on survival, morbidity and growth of the infants.

1.2.1. Main objectives:

1. To determine whether malaria and anaemia at various stages of pregnancy influence infant mortality in an area of low malaria transmission.
2. To determine whether malaria infections experienced during pregnancy influence malaria in children during the first year of life.
3. To determine whether malaria and anaemia during pregnancy have an impact on morbidity, haematological status, and growth of the offspring during the first year of life.

1.2.2. Specific objectives

1. To determine whether anaemia at delivery, irrespective of cause, is associated with infant deaths after other maternal morbid events during pregnancy and socio-economic factors are taken in account.
2. To determine the direct and indirect impact of malaria during pregnancy on survival of infants.
3. To describe the age distribution and causes of infant deaths in this population.
4. To describe the acquisition of malaria in infants living in an area of low and unstable transmission.
5. To determine whether malaria history at various stages of pregnancy influences malaria in infants.
6. To determine whether morbidity during pregnancy influences morbidity and growth during infancy

1.3. Organisation of the thesis

This thesis consists of 9 chapters and the results are divided into four main parts: (i) a description of morbidity during pregnancy and an analysis of the determinants of LBW and prematurity; (ii) a description of the level and causes of infant mortality and an assessment of the effects of malaria and anaemia during pregnancy on infant deaths; (iii) an analysis of the effects of morbidity during pregnancy on infant morbidity, with a special emphasis on the relationships between malaria during pregnancy and malaria during the first year of life; (iv) an analysis of the effects of morbidity during pregnancy and during infancy on infant growth.

Chapter 1 provides the introduction to the thesis and its objectives.

Chapter 2 reviews the relevant literature for this study and is organized in five parts. The first two sections describe the existing knowledge on the consequences of malaria and anaemia during pregnancy on the infant. A third part gives briefly available data on the risk factors and causes of infant mortality in developing countries. In the fourth section, published reports on morbidity and mortality from malaria in infancy are reviewed. The fifth section deals with current available data on the relationships between malaria during pregnancy and in infancy.

Chapter 3 describes the Karen Refugee camps where the study was conducted, the malaria situation and the main other health problems. The main objectives and the set-up of the SMRU are described. Methods employed in ANC, especially the detection and treatment of malaria and anaemia during pregnancy, are detailed.

Chapter 4 describes the methods of the study.

Chapter 5 provides a description of the mothers and their morbidity during pregnancy and explores the determinants of LBW and/or premature birth of the newborn.

Chapter 6 reports the results in term of infant mortality and describes the main causes of deaths in the cohort and the relationships with maternal characteristics.

Chapter 7 reports the results of morbidity during infancy, with a special emphasis on malaria and anaemia during the first year of life

Chapter 8 reports the results on growth of this cohort.

Chapter 9 discusses the main findings of the thesis and presents the conclusions.

CHAPTER 2

LITERATURE REVIEW

2.1. Malaria during pregnancy: consequences on the foetus or the infant

The increased susceptibility of pregnant women, especially primigravidae, to malaria has been recognized for more than a century and well described in the fifties (Brabin, 1991). In the last 20 years, the idea of malaria eradication was progressively abandoned and replaced by the objective of controlling the disease. Thus, the interest in the harmful effects of malaria during pregnancy was renewed, and research in this area intensified. The main effects of malaria on the pregnant woman and her offspring have been well described and regularly reviewed (Taufa, 1978; Brabin, 1983; McGregor, 1984; Brabin, 1991; Menendez, 1995; Steketee *et al.*, 1996a). The current review will focus on the consequences of malaria during pregnancy on the foetus or the infant.

2.1.1 Birthweight

Malaria during pregnancy reduces birthweight and this effect is most marked in primigravidae. Mean birthweight is lower in children born to infected mothers compared to newborn from non-infected mothers, and the proportion of babies with LBW is higher in the former group than in the latter (**Table 2.1**). The effects on the mother, in terms of morbidity and mortality, depend highly on the level of malaria endemicity, whereas the reduction of birthweight seems to be present at all level of transmission. Nevertheless, in areas of unstable and low endemicity, second and third gravidae are also affected.

In early studies (before 1980) samples sizes were relatively small and differences in mean birthweight were not often statistically significant. However, the trend toward a reduction of birthweight in children born to infected mothers was a constant theme. Moreover, the proportion of LBW was significantly higher in infected than non-infected mothers in most studies (Odds Ratio (OR) between 2 and 4). Nevertheless, the association between malaria during pregnancy and birthweight was weak and still questionable. All studies were hospital-based cross-sectional surveys that looked at the association between placental and/or peripheral maternal malaria at delivery and birthweight. There was therefore no information on malaria history earlier in pregnancy. This could lead to an underestimation of the problem.

McGregor *et al.* (1983) conducted the first large (N=6427) cross-sectional survey that confirmed a statistically significant reduction in birthweight of infected Gambian primigravidae. Babies born to multigravidae were not affected. Although further studies had shown similar results and a consensus was reached towards the effects of malaria in reducing birthweights, two studies had controversial results (Brabin *et al.*, 1990a; Desowitz *et al.*, 1992). They were conducted in a region of high transmission in Papua New Guinea. There were no differences in birthweights between children from infected and non-infected mothers, and this remained true after adjusting for parity. The authors concluded that maternal anaemia may have a more important effect on birthweight than malaria *per se*.

Information on the effects of malaria during pregnancy on birthweight in areas of low and unstable endemicity remained poor. All studies focused on *P.falciparum* malaria and the consequences of *P.vivax* malaria infection during pregnancy on birthweight were neglected. A prospective study was conducted on the Thai-Burmese border, an area of unstable endemicity where placental malaria was rare, despite a malaria incidence during pregnancy that reached 30%. Malaria infection, defined as peripheral parasitaemia at any time during pregnancy, was associated with a reduction in birthweight. Primigravidae were more susceptible to malaria than multigravidae, but second and third gravidae were also affected. As a consequence, reduction in birthweight was more marked in first born babies, but was also found in second and third pregnancies. There were not enough cases of vivax malaria to study the effects of this species on the foetus (Nosten *et al.*, 1991). In India, Nair & Nair (1993) reported a reduction in birthweight associated with vivax malaria, but did not mention the results of their statistical tests. After their first report, Nosten *et al.* (in press) continued to recruit pregnant women (N=11,004). They published the first evidence that *P.vivax* malaria also has a deleterious effect on birthweight, although smaller than that of *P.falciparum* infection. Primigravidae were more likely to be infected with *P.vivax* than multigravidae. But in contrast to falciparum malaria, the reduction in birthweight was greater in multigravidae.

Table 2.1: mean reduction in birthweight (g) and odds ratio of LBW newborn in relation to placental or peripheral malaria.

Study area	Study design	Gravidae	Mean reduction in birthweight (g)	OR (95%CI) LBW	Reference
South Nigeria	Cross-sectional at delivery	All gravidae	-145	2.1 (1.0-4.5)	Bruce-Chwatt, 1952
West Nigeria	Cross-sectional at delivery	All gravidae	-170	2.1 (1.1-3.9)	Archibald, 1956
North Nigeria	Cross-sectional at delivery	All gravidae	-298	3.0 (1.4-6.4)	Archibald, 1958
Nigeria	Cross-sectional at delivery	All gravidae	-310	4.2 (2.4-7.3)	Cannon, 1958
East Nigeria	Cross-sectional at delivery	All gravidae	-89	NA	Spitz, 1959
Tanzania	Cross-sectional at delivery	All gravidae	-55	2.1 (0.9-4.6)	MacLaren *et al.*, 1962
Uganda	Cross-sectional at delivery	All gravidae	-263	2.2 (1.2-4.1)	Jelliffe, 1968
Ghana	Cross-sectional at delivery	All gravidae	-178	NA	Jilly, 1969
Tanzania	Prospective	All gravidae	-75	1.6 (0.8-2.9)	Kortmann, 1972
Ivory Coast	Cross-sectional at delivery	All gravidae	-120	NA	Reinhard *et al.*, 1978b
Zimbabwe	Hospital-based; all admission for malaria	All gravidae	-333	NA	Herd & Jordan, 1981

The Gambia	Cross-sectional at delivery	All gravidae	-170	2.5 (2.1-2.9)	McGregor, 1983
Keneba, The Gambia	Prospective	Primigravidae	-570	NA	Watkinson & Rushton, 1983
Franceville, Gabon	Cross-sectional at delivery	All gravidae	-220	NA	Garin *et al.*, 1985
Lagos, Nigeria	Cross-sectional at delivery	All gravidae	-550	NA	Nnatu *et al.*, 1987
Papua New Guinea	Prospective	Primigravidae Multigravidae	-26 (a) -121 (b) [-12; + 244] (a) +1 (b)	NA	Brabin *et al.*, 1990a
North-West Thailand	Prospective	All gravidae	-123	NA	Nosten *et al*, 1991
Bedeci, Ivory Coast	Cross-sectional at delivery	All gravidae	-409	3.5 (0.3-25.3)	Bachschmid *et al.*, 1991
Benin	Cross-sectional at delivery	All gravidae	-270	3.6 (1.7-7.5)	Ibhanesebhor &Okolo, 1992
India	Cross-sectional at delivery	All gravidae	-520	13.7 (5.7-34.2) (c)	Kaushik *et al.*, 1992
Papua New Guinea	Cross-sectional at delivery	Primigravidae Multigravidae	+20 -33	NA	Desowitz *et al.*, 1992
Central Sudan	case-control	All gravidae	NA	1.6 (1.2-2.1) (d) 1.7 (1.3-2.3) (e)	Taha *et al.*, 1993a
Gujarat, India	prospective	All gravidae	-780 *P.falciparum* -390 *P.vivax*	2.0	Nair & Nair, 1993

Nord-Kivu, Zaire	Cross-sectional at delivery	All gravidae	-124 (a) -154 (b)	2.8 (1.5-5.4)	Meuris *et al.*, 1993
Maputo, Mozambique	Cross-sectional at delivery	All gravidae	-144	1.6 (0.6-4.2)	Bergström *et al.*, 1993
Sierra Leone	Cross-sectional at delivery	All gravidae	-265	2.7 (1.7-4.5)	Morgan, 1994
Burkina Faso	Cross-sectional at delivery	Primigravidae 2nd gravidae	-276 -45	NA	Gazin *et al.*, 1994
Zanzibar, Tanzania	Cross-sectional at delivery	All gravidae	-177	10.1 (2.9-35.4)	Matteelli *et al.*, 1996
Papua New Guinea	Cross-sectional at delivery	All gravidae	-130	NA	Allen *et al.*, 1998
North-West Thailand	Prospective	All gravidae	-108	1.7 (1.4-2.1)	Nosten *et al.*, in press

(a): placental malaria
(b): parasitaemia at delivery
(c): febrile women
(d): hospital-based
(e): community-based

In most studies designed to investigate the relationship between malaria during pregnancy and birthweight, potential confounding factors such as smoking, mother's nutrition, pre-eclampsia, socio-economic factors, were not taken in account. Recently, smoking was identified as an important determinant of LBW in malarious areas, with an effect similar to that described in developed countries (McGready *et al.*, 1998; Allen *et al.*, 1998). But these potential confounding factors would act on all pregnancies, whereas malaria selectively depresses birthweight to a greater extent in primigravidae, and this relates to a higher malaria prevalence in first pregnancies (Brabin, 1991). The plausibility of the causal role of malaria was also supported by an observational study in the British Solomon Islands where a successful malaria eradication campaign was followed by an increase in birthweight (McGregor & Avery, 1974). But this was a descriptive study with historical control.

The causal role of malaria in reducing birthweight would be confirmed only by a reversible effect in randomised controlled trials of preventive antimalarial measures during pregnancy. Garner & Brabin (1994) reviewed the randomised controlled trials of antimalarial drug chemoprophylaxis during pregnancy, published between 1964 and 1994 (Morley *et al.*, 1964; Hamilton *et al.*, 1972; Martin & Nkwate, 1982; Nahlen *et al.*, 1982; Harrison *et al.*, 1984; Fleming *et al.*, 1986; Greenwood *et al.*, 1989; Mutabingwa *et al.*, 1991; Cot *et al.*, 1992; Nosten *et al.*, 1994a). There was an overall trend towards an increased birthweight in children born to mothers in the prophylaxis groups compared to the placebo (or receiving a less efficient drug) control groups. Nevertheless, only two trials reported a statistically significant difference when the outcome was birthweight in newborn from all parities (Morley *et al.*, 1964; Hamilton *et al.*, 1972) and only one (Greenwood *et al.*, 1989) when the authors restricted the analysis to birthweight in babies born to primigravidae. In this study, the difference reached statistical significance after stratification by season and age. Several hypotheses were raised to explain these poor results: insufficient sample sizes; prophylaxis started too late in pregnancy; *P.falciparum* resistant to the antimalarial chosen for prophylaxis. Results were more encouraging in studies that were published later. Gambian primigravidae who received pyrimethamine-dapsone prophylaxis delivered heavier newborn than those who received placebo (Menendez *et al.*, 1994a). When prophylaxis was given to Cameroonian primigravidae, the risk of having of LBW infant was reduced by 62% (95% Confidence Interval: 11-84) and the mean birthweight was significantly

higher than in the control group (Cot *et al.*, 1995). In Malawi, effective prophylaxis during pregnancy was also successful in reducing the proportion of LBW newborn (Steketee *et al.*, 1996b). In Mali, although the trial was not randomized, chloroquine prophylaxis increased mean birthweight and reduced the prevalence of LBW in para 0 and 1, but this effect could not be achieved with less than 15-20 weeks of prophylaxis (Bouvier *et al.*, 1997b).

LBW may be the result of IUGR, prematurity or both. A high risk of premature deliveries (27-60%) has been reported in epidemic situations (Wickramasuriya, 1937) and among women hospitalised with symptomatic and severe malaria (Le Van Hung, 1951; Herd & Jordan, 1981; Endeshaw, 1991; Nair & Nair, 1993). But in areas of stable transmission and in the absence of symptoms, malaria is thought to be associated with IUGR rather than prematurity. In early studies, it was difficult to estimate accurately the gestational age, and the definition of prematurity was that of LBW (<2500g), thus including full-term growth retarded infants. Since the introduction of the Dubowitz assessment of gestational maturity (Dubowitz & Dubowitz, 1977), a few studies of malaria during pregnancy were able to control for gestational age. In these studies, placental or peripheral malaria was not associated with preterm deliveries (Reinhardt *et al.*, 1978b; Watkinson & Rushton, 1983; Dolan *et al.*, 1993; Nosten *et al.*, 1994a; Steketee *et al.*, 1996b; Allen *et al.*, 1998). Similarly *P.vivax* infection during pregnancy did not reduce the duration of gestation (Nosten *et al.*, in press). Thus LBW due to malaria-infection appeared to be a consequence of IUGR rather than prematurity. Nevertheless, in the study conducted in Malawi, cord blood malaria infection was a risk factor for preterm LBW. The authors could not suggest any biological mechanism to explain their findings (Steketee *et al.*, 1996b). Another controversial result was obtained in Sudan. Taha *et al.* (1993a) showed an association between a maternal history of malaria and LBW. The study relied on the woman's recall of her malaria attacks during pregnancy at the time of delivery and had therefore possible misclassifications. Surprisingly, the malaria attributable risk of preterm-LBW was higher than that of full term LBW (35 and 11% respectively). A possible explanation might be that the method used to detect malaria (i.e. woman's own recall) only allowed to look at the association between symptomatic episodes and LBW. In the Gambia, the use of impregnated bed net by primigravidae was effective in reducing the proportion of babies classified as premature (D'Alessandro *et al.*, 1996). Again, this could be explained by the prevention

of symptomatic malaria occurring during the third trimester and leading to premature delivery. Another possible explanation is the misclassification of the newborn as gestational age was estimated by a simplified method of assessment, not validated in Africa. This effect was also marginal and seasonal.

Meuris *et al.* (1993) attempted to define which type of IUGR was associated with malaria by looking at the effects of the infection on different anthropometric measurements at birth. Circulating malaria parasites and placental infection were respectively associated with acute (i.e. effect on birthweight only) and subacute (effect on birthweight, head circumference and ponderal index) impairment of foetal growth. Gazin *et al.* (1994) reported an effect of placental malaria on birth height as well as birthweight, suggesting a prolonged effect.

2.1.2 Perinatal mortality

In areas of high malaria transmission peripheral or placental malaria was not associated with increased perinatal or early neonatal mortality (Bruce-Chwatt, 1952; Cannon, 1958; Spitz, 1959; Jilly, 1969). Numbers were small in these early studies and could not have reached significance. Moreover prematurity and periods of foetal and infant life were not well defined. Nevertheless the absence of association between maternal malaria and perinatal mortality was confirmed in larger recent studies (McGregor *et al.*, 1983; Taha & Gray, 1993b; McDermott *et al.*, 1996). In the Gambia, there were less stillbirths and neonatal deaths in children born to primigravidae who took antimalarial chemoprophylaxis than in those who took placebo, but this was not significant (Greenwood *et al.*, 1989). However, Nyirjesy *et al.* (1993) reported that maternal malaria at delivery and neonatal malaria were both associated with an increased risk of perinatal deaths and that the use of chloroquine prophylaxis significantly reduced this risk. Nevertheless, this was not a randomized study.

In areas of low or seasonal malaria transmission, severe malaria occurs during pregnancy and is associated with high foetal loss. Wickramasuriya (1937) described the disastrous effects of malaria during pregnancy during an epidemic in Ceylon. Malaria was associated with extremely high rates of abortion, foetal death and premature

delivery of infants. In Saigon, Le Van Hung (1951) recorded a foetal death rate of 14%. In Malaysia, Menon (1972) reported a foetal wastage in 35% (6/17; 5 stillbirths and one premature infant who died) of women hospitalised for symptomatic and sometimes severe malaria. In Zimbabwe, the foetal mortality rate reached 49% (24 abortions, 2 stillbirths and 4 LBW infants dying withing 1 hour) in 61 pregnant women admitted to hospital with malaria (Herd & Jordan, 1981). In Ethiopia 41% (15/37) pregnancies resulted in abortion (6), still birth (1) and preterm delivery with early neonatal deaths (8) (Endeshaw; 1991). In India, 4% of 104 women with symptomatic and sometimes severe malaria died, 60% delivered prematurely, 10% had an abortion and 6% a stillbirth. Stillbirths and maternal deaths were associated with *P.falciparum* only, whereas abortions and premature deliveries were common with both vivax and falciparum malaria (Nair & Nair, 1993). On the Thai-Cambodian border, the foetal loss in 193 pregnant women hospitalised with severe malaria was 66% (109 abortions; 10 stillbirths and 8 premature babies who died) (Meek, 1988). On the Thai-Burmese border, foetal deaths occurred in 27% (8/30) of severe malaria cases during pregnancy (3 abortions, 4 stillbirths and 1 premature delivery of twins who died within 48 hours) (Luxemburger *et al.*, 1997).

2.1.3. Infant mortality and morbidity (except malaria)

It is assumed that malaria during pregnancy may increase infant mortality, at least in first born infants, through its reduction in birthweight. The indirect effects of malaria during pregnancy on child's survival have been suggested in the report of a successful eradication of malaria in Guyana. During the campaign, neonatal mortality decreased from 49 to 12 per 1,000 live births. Neonatal deaths are in most cases related to maternal causes and this improvement was likely to be the indirect benefit of prevention of malaria during pregnancy. Moreover, in the post-neonatal period, the reduction of deaths was higher than expected if only the malaria-attributable deaths had been prevented (Giglioli, 1972). Two explanations for this are plausible. Prevention of malaria during pregnancy may have reduced the proportions of LBW infants who are more likely to die in infancy. Prevention of malaria during infancy may also have reduced the mortality indirectly associated with malaria, as did the use of impregnated bed nets some 20 years later in The Gambia (Alonso *et al.*, 1991). However, from recent

prospective studies, there is no evidence of a direct association between malaria during pregnancy and deaths in infancy, either in neonatal or post-neonatal periods (Dolan *et al.*, 1993; Nosten *et al.*, 1994a; Bloland *et al.*, 1995; Bloland *et al.*, 1996).

Placental malaria infection has been associated with anaemia in early infancy. In a prospective study of pregnant women and their children in rural Malawi, placental malaria infection was the strongest risk factor for an infant having anaemia (hct<25%) at 2 months of age (Redd *et al.*, 1994). This finding was confirmed by Cornet *et al.* (1998) who found that placental malaria was the strongest risk factor for anaemia in the six month-old children, independent of the infant's malaria history since birth (frequency of parasitaemia, parasitaemia at the time of haemoglobin measurement). At one year of age, parasitaemia at the time of haemoglobin measurement and microcytosis (reflecting iron deficiency) were significant risk factors for anaemia.

The long-term effects on morbidity from non-malarious causes in infants born to malaria-infected mothers has not been studied. Their growth has been described in only one study. In Nigeria, babies born to mothers who participated in a malaria prophylaxis study during pregnancy were prospectively followed from birth to 5 years of age. Although the protected mothers produced larger babies than did mothers who did not take prophylaxis, the weight differences disappeared within the first few months of life (Morley *et al.*, 1968).

2.2. Anaemia during pregnancy: consequences on the foetus or the infant

2.2.1. Anaemia during pregnancy in malaria endemic areas

Anaemia during pregnancy is a major health problem in developing countries and its causes are usually multifactorial. Nevertheless, in malarious areas, malaria is recognized as the leading cause of anaemia during pregnancy, mainly in primigravidae (Gilles *et al.*, 1969; Jilly, 1969; Harrison & Ibeziako, 1973; Rougemont *et al.*, 1977; Fleming *et al.*, 1986; Fleming, 1989b; Jackson *et al.*, 1991; Shulman *et al.*, 1996). Folate deficiency is often secondary to malarial haemolysis (Fleming, 1989a). Other factors, such as iron deficiency and hookworms, are relatively more important in

multigravidae (Isah *et al.*, 1985; Shulman *et al.*, 1996). The contribution of sickle-cell anaemia, thalassemia and Acquired Immune Deficiency Syndrome varies greatly between areas. The association between malaria and anaemia during pregnancy has been confirmed by several studies in which antimalarial prophylaxis or control measures had a beneficial effects on haematological markers (Gilles *et al.*, 1969; Kortmann, 1972; Fleming *et al.*, 1986; Spencer *et al.*, 1987; Greenwood *et al.*, 1989; Brabin *et al.*, 1990a; Dolan *et al.*, 1993; Mutabingwa *et al.*, 1993a; Nosten *et al.*, 1994a; Bouvier *et al.*, 1997a; Cot *et al.*, 1998). In the only detailed report of vivax malaria during pregnancy, anaemia was associated with infection with *P.vivax* (Nosten *et al.*, in press). In malarious areas, anaemia and malaria are therefore likely to act together on foetal growth.

2.2.2. Birthweight

Several studies conducted in malaria endemic areas have reported an association between maternal anaemia and LBW (primarily due to foetal growth retardation) (Macgregor, 1963; Ojo, 1965; Harrison and Ibeziako, 1973; Reinhardt, 1978a; Dawodu & Latidan, 1985; Oppenheimer *et al.*, 1986; Fleming, 1989b; Brabin *et al.*, 1990a; Dolan *et al.*, 1993; Nosten *et al.*, 1994a). Whatever the causes of anaemia, in both malarious and non-malaria developing countries, the risk of having LBW infants increase with increasing severity of maternal anaemia (Macgregor, 1963; Bhargava *et al.*, 1987; Fleming, 1989b; Mavalankar *et al.*, 1992). The duration of anaemia may be an important risk factor for delivering lighter babies. In Nigeria, Harrison & Ibeziako (1973) treated anaemic pregnant women at 20-28 weeks of pregnancy. At delivery women who were still anaemic (haematocrit (HCT) 26-30%) delivered lighter babies than those who responded to the treatment and were no longer anaemic (HCT>30%). Babies born to the latter group had similar mean birthweight than those born to non anaemic mothers.

In an attempt to estimate anaemia- and malaria-attributable LBW separately, Brabin & Piper (1997) compared two populations of Papua New Guinea. One was living in a non-malarious area and the other in a highly malaria endemic area. The prevalence of anaemia was much higher in the malaria endemic area than in the region

with no malaria. In both places, there was a trend towards increased risk of LBW with decreased haemoglobin level, but this was significant only in the malaria endemic area. In the latter study site, up to 40% of LBW was attributated to malaria whereas 10% were attributable to severe anaemia. The findings were similar in primi- and multigravidae. The authors concluded that in malaria endemic areas, malaria was a more important risk factor than anaemia for LBW.

As mentioned above, malaria-related anaemia during pregnancy has been corrected by using antimalarial prophylaxis, but whether this correction has an effect on birthweight is difficult to assess. In a randomized double-blind trial of antenatal oral antimalarial prophylaxis and haematinic supplements in primigravidae in Nigeria (Fleming, 1986), anaemia at 6 weeks after delivery was found in 61% of the women not receiving active treatment, in 39% of those protected against malaria but not receiving iron supplements and in only 18% of patients receiving both antimalarials and iron. The effect of antimalarial prophylaxis in reducing anaemia was higher than that of iron supplementation. Moreover, 9 out of 12 women who developed severe anaemia were in the group not receiving antimalarials (7) or not compliant to their prophylaxis (2). Folic acid had no significant effect on mean haemoglobin. The group receiving no active treatment delivered lighter babies and had a higher number of perinatal deaths than the women who took antimalarials alone or combined with haematinics but this was not significant. In Thailand, the use of impregnated bed nets was associated with a reduction in maternal anaemia, but had no effect on birthweight (Dolan *et al.*, 1993).

The effects of maternal iron deficiency on outcome of pregnancy are not well known in malarious areas but iron deficiency is associated with an increased risk of LBW in non-malarious areas (Singla *et al.*, 1997). In a randomized, double-blind, placebo-controlled community-based trial in the Gambia, iron supplementation given to multigravidae reduced the prevalence of anaemia (HCT<25%) and of iron deficiency at 36 weeks of gestation and one week after delivery. Mean birthweight was higher in the iron supplementation group than in the control group, but this was not significant. The proportion of children born with LBW did not change either. The absence of effects on birthweight could be related to the late attendance of women to ANC and thus of a short period of supplementation. When the analysis was restricted to women who had received at least 3 months of iron supplementation, birthweight was significantly higher

in treatment than in control group (Menendez *et al.*, 1994b). In areas endemic for hookworms, mebendazole therapy may also be required. In an area of high prevalence of hookworm infestation during pregnancy, the combination of mebendazole with iron and folate supplements was more efficient in improving haemoglobin concentration and iron-status than haematinics alone (Atukorala *et al.*, 1994). In a recent case-control study conducted in Sri Lanka, mebendazole therapy was associated with a 53% reduction in the incidence of LBW (de Silva *et al.*, 1999).

Maternal anaemia has been associated with IUGR rather than prematurity. In Papua New Guinea, severe anaemia was associated with LBW in primigravidae. The majority of these LBW babies were clinically growth retarded and not premature (Brabin *et al.*, 1990a). In the two studies in which the gestational age was assessed using the Dubowitz score, gestational age was not affected by maternal anaemia (Reinhardt, 1978a; Dolan *et al.*, 1993). In Zaire, Meuris et al (1993) reported that all anthropometric markers (birthweight, length and head circumference) of children born to anaemic mothers were reduced, but the ponderal index remained normal, indicating that there was a chronic impairment of foetal growth. More recently in Rwanda, maternal anaemia was an independent risk factor of having IUGR, but not of being born prematurely (Leroy *et al.*, 1998).

2.2.3. Infant mortality

There is compelling evidence that the risk of perinatal death increases in severely anaemic women. In malarious areas, first reports were done from large series of Malaysian pregnant women (Tasker, 1958; Llewellyn-Jones, 1965). Later, in a Kenyan hospital where malaria was low, severe anaemia (Haemoglobin (Hb) <7.4 g/dl) at delivery was associated with higher perinatal and neonatal mortality rates than moderate or absence or maternal anaemia (Hb>=8.9 g/dl) (Macgregor, 1963). Similarly, in Papua New Guinea, perinatal deaths were increased from 22 to 55 per 1,000 for women with and without severe anaemia (Hb < 8 g/dl) at delivery (Brabin *et al.*, 1990a).

Long-term survival of children born to anaemic mothers may also be impaired. In two consecutive prospective studies on the Thai-Burmese border, maternal anaemia at delivery was associated with a higher risk of infant deaths (Dolan *et al.*, 1993; Nosten *et al.*, 1994a). Although mortality rates for neonatal and post-neonatal periods were not specified, examination of the survival curves showed that the effect was more important in the post-neonatal period. These were the first published reports of an association between maternal anaemia and infant deaths in a malaria endemic area. But other potential confounding factors, such as morbidity during infancy and socio-economic environment were not taken in account. Nevertheless, a recent study in an area of much higher malaria transmission in Malawi, showed a similar association between maternal anaemia during pregnancy and post-neonatal mortality (Verhoeff & Brabin; personal communication).

2.2.4 Foetal and infant anaemia

Brabin (1992) reviewed published studies of cord Hb values in developing countries and compared findings for populations exposed with those not exposed to malaria. In non-malarious areas, maternal iron deficiency anaemia is associated with a significant degree of cord anaemia. In malarious areas, where women are likely to also have iron deficiency, the foetal anaemia appears to be higher than expected as a result of iron deficiency alone. Thus malaria, but also haemoglobinopathies, may contribute to foetal anaemia that itself is likely to be related to the risk of anaemia in infancy.

In developed countries, anaemia during pregnancy is a strong risk factor of developing iron deficiency in infancy (Colomer *et al.*, 1990). But in developing countries, the long term effects of anaemia during pregnancy on anaemia in infancy are unknown.

2.3. Infant mortality in developing countries

Infant and childhood mortality rates remain extremely high in developing countries. In a recent attempt to estimate the groups most at risk of dying worldwide and the main causes of deaths, Murray & Lopez (1997) demonstrated that a third of all deaths occurs in children under 5 years old from developing countries. The probability to die before ones fifth birthday is 22% in Sub-Saharan Africa whereas it is only 1% in developed countries. Communicable diseases remain a leading cause of deaths. In an earlier review, Ashworth & Waterlow (1982) had demonstrated that it is much more important to survey infant than childhood mortality, as the former is much higher than the latter. They reviewed 26,508 infant deaths from Latin America and Caribbean countries and calculated that 45% of them occurred during the neonatal period and 80% within 6 months. The leading cause of post-neonatal death was diarrhoea and the major risk factor was LBW (28% of deaths between 1 and 5 months). The situation was similar to that in 1920 in USA, when infant mortality rate (IMR) reached 117 per 1,000.

2.3.1. Infant mortality rates

In most developing countries reliable statistics are lacking and estimations of IMR are questionable (Lumbiganon *et al.*, 1990; Amin *et al.*, 1992). Community-based surveys give more realistic information and allow comparisons within areas. **Table 2.2** summarised IMR obtained from such studies. Overall, IMR tend to be higher in Africa than in other parts of the world and neonatal mortality represents nearly half of infant deaths.

2.3.2 Main causes of infant deaths

In most studies, diagnosis facilities are lacking and infants die at home. Moreover, some causes may overlap (respiratory infections and malaria) or induced different final episode (deaths from measles may be due to respiratory infections or malnutrition). General and imprecise classifications are therefore usually used and the diagnosis of the cause of death remains unknown for a large proportion of them. This is more problematic in the neonatal period, the cause of death often being reported as "LBW" or "premature".

Nevertheless, there are some similarities within areas (**table 2.2**). A large proportion of deaths occurs in the neonatal period. Infectious diseases are the most common causes of infant or post-neonatal deaths, with two leading causes: acute respiratory infections (ARI) and diarrhoea. The importance of tetanus varies largely, probably reflecting the immunization coverage in the area. In malaria endemic areas, malaria-attributable deaths vary from 0 to 35%.

Table 2.2.: Infant Mortality Rates and main causes of infant or early childhood deaths in developing countries.

Year of study	Country	Method	Infant and neonatal mortality rates (/1,000)	Main infectious causes (% of total deaths)	Reference
1935-1950	Nigeria	Vital statistics Autopsies	Infant: 140 to 86	ARI: 41% Malaria: 9% Gastro-Intestinal: 5%	Bruce-Chwatt, 1952
1969-1979	Sierra Leone	Vital statistics Survey	NA	Tetanus: 17% ARI: 15% Diarrhoea: 10% Malaria: 5%	Kandesh, 1986.
1971-1975	Nigeria	Vital statistics	Infant: 88	NA	Ayeni & Oduntan, 1978
1975-1978	Kenya	Longitudinal	Infant: 50	ARI: 23% Diarrhoea: 23% Mealses: 7% Malaria 2%	Omondi-Odhiambo *et al.*, 1990.
1976	Congo	Retrospective	Infant: 71 Neonatal: 47	0-5y: Measles: 41% Diarrhoea: 11% ARI: 3% Malaria: none	Carme *et al.*, 1984
1978	Cameroon	longitudinal	Infant: 53	Malaria: 9%	Kuate Defo, 1995

1980-1984	Nigeria	cohort	Infant: 42 Neonatal: 5	0-5 y: Measles 35% Malaria: 15% Diarrhoea: 15%	Adedoyin & Watts, 1989
1982-1983	The Gambia, Farafenni	Surveillance system Verbal autopsies	Infant: 142 Neonatal: 66	1-23 m: ARI 23% Diarrhoea 14% Malaria 13%	Greenwood *et al.*, 1987b
1984	Sudan	Cohort	Infant: 118	Diarhoea: 53% ARI: 7% Malaria: 7%	Woodruff *et al.*, 1984
1987-1989	Somalia	Surveillance system	Infant: 142 Neonatal: 48	0-1m: Tetanus: 80% 1-11m: ARI 28% Diarrhoea: 28% "Fever": 20%	Ibrahim *et al.*, 1996
1987-1990	Malawi	Cohort	Infant: 157 Neonatal: 47	Diarrhoea: 40% "Fever": 18% ARI: 15%	Bloland *et al.*, 1996 Slutsker *et al.*, 1996b
1988-1989	The Gambia, North	Surveillance system Verbal autopsies	Infant: 97 Neonatal: 41	ARI 29% Diarrhoea: 9% Malaria: 8%	De Francisco *et al.*, 1993

1988-1989	The Gambia, South	Surveillance system Verbal autopsies	Infant: 120	1-5 m: ARI 22% Diarrhoea: 11% Malaria: 5% 6-11m: Malaria 26% Diarrhoea 26% ARI 18%	Alonso *et al.*, 1993
1989- 1993	The Gambia, East	Surveillance system Verbal autopsies	Infant: 80 Neonatal : 38	ARI 21% Malaria 12% Diarrhoea 7%	Jaffar *et al.*, 1997
1990-1991	Burundi	Longitudinal	Infant: 108	Malaria: 35% ARI: 16% Diarrhoea: 15%	Delacollette & Barutwanayo, 1993
1990	Sierra Leone	Survey+ cohort	Infant: 154	NA	Amin *et al.*, 1992
1991	Nigeria	Retrospective	NA	0-1 m: Sepsis 38% Tetanus 21% 1-12 m: Malaria 47% ARI 11% Diarrhoea 11%	Ekanem *et al.*, 1994
1994	Tanzania	Surveillance system Verbal autopsies	Infant: 133	0-5y: Malaria 36%	Salum *et al.*, 1994
1982	Brazil	Cohort	Infant: 39 Neonatal: 21	ARI: 12% Diarrhoea: 12%	Barros *et al.*, 1987

1988-1989	Guatemala	Cohort	0-3 months: 30 neonatal: 12	0-3m: ARI (50%) Sepsis (10%)	Bartlett *et al.*, 1991
1975-1977	Bangladesh	Surveillance system	Infant: 143	Tetanus: 26% Diarrhoea: 14% ARI: 7%	Chen *et al.*, 1980
1976-1978	Indonesia, Java	Cohort	Infant: 78 Neonatal: 30	ARI 25% Tetanus 10% Diarrhoea 8%	Handayani *et al.*, 1983
1976-1977	Bangladesh	Cohort	Infant: 160 Neonatal: 71	0-1m: Tetanus: 31% 1-11m: ARI 33% Diarrhoea: 10%	Islam *et al.*, 1982
1977-1984	Bangladesh	Vital statistics Verbal autopsies	Infant: 90-130 Neonatal: 57-67	ARI: 20-22% Diarrhoea: 13% Tetanus: 7%	Bhatia, 1989
1979	India (urban)	Cohort	Infant: 47 Neonatal: 21	NA	Ghosh *et al.*, 1979
1982-1984	Indonesia, Madura	Cohort	Infant: 121 Neonatal: 52	NA	Kusin *et al.*, 1989
1982-1985	Papua New Guinea	Surveillance system Verbal autopsies	Infant: 46 Neonatal: 22	1-11m: ARI 31% Measles 13% Malaria: 2%	Moir *et al.*, 1989

1986-1987	Thailand	Cohort	Infant: 23	NA	Lumbiganon *et al.*, 1990
1988-1990	Vietnam	Demographic and Health Survey	Infant: 33-36 Neonatal: 18-24	NA	Swenson *et al.*, 1995

2.3.2. Relationship between LBW and infant mortality and morbidity.

LBW is the main risk factor for infant deaths. In developed countries, McCormick (1985) showed that LBW was the most important predictor of neonatal and post-neonatal mortality. Children born in the United States with birthweights < 2500g had a 40-fold and a five-fold increased risk to die during the neonatal and post-neonatal period than infants with birthweights of 3001-3500g. In developing countries, the association between LBW and infant deaths was also extensively reported (Ayeni & Oduntan, 1978; Rao & Inbaraj, 1978; Ghosh *et al.*, 1979; Barros *et al.*, 1987; Datta *et al.*, 1987; Victora *et al.*, 1988; Kusin *et al.*, 1989; Bartlett *et al.*, 1991; Downes *et al.*, 1991; Greenwood *et al.*, 1992; Dolan *et al.*, 1993; Lira *et al.*, 1996).

It is generally assumed that LBW in developed countries are mainly due to preterm births, whereas most LBW in developing countries are due to IUGR (de Onis *et al.*, 1998). In the absence of gestational age, populations have been classified by their prevalence of LBW: <7.5% is assumed to reflect preterm births and >15% is considered as a proxy for IUGR prevalence (World Health Organization (WHO), 1995). Premature infants are assumed to be more likely to die than term infants of similar birthweight. If this is the case, the risk of infant deaths attributable to LBW in developing countries may have been overestimated, as in these countries most LBW are growth retarded infants. In a large cohort conducted in Brazil, mortality rates were much higher for pre-term than for small-for-gestional age infants (Victora *et al.*, 1987). In Indonesia, infants weighing less than 2000g at birth remained at higher risk of death during the whole infancy, whereas those between 2000 and 2500 had a higher mortality only during the neonatal period (Kusin *et al.*, 1989). Similar results were reported in the Gambia. Children weighing less than 2000g were at increased risk of both neonatal and infant mortality when compared to babies weighing more than 2500g. But newborn weighing between 2000 and 2500g had higher but not significant neonatal and infant mortality rates than babies weighing more than 2500g (Greenwood *et al.*, 1992). This might reflect the different risks of dying of premature and full-term LBW babies as it is likely that the group of children weighing less 2000g comprises many premature infants whereas most newborn weighing between 2000 and 2500g were probably growth-retarded. But this might also be only the consequence of lower birthweight. On the Thai-Burmese border, premature birth, assessed by using the Dubowitz score, was the

strongest risk factor for infant deaths, independent of birthweight but the authors did not compared mortality of full-term and preterm infants of similar weight (Dolan *et al.*, 1993). In Malawi, prematurity and LBW were both identified as risk factors for neonatal deaths, but only birthweight remained in the multivariate analysis (Bloland *et al.*, 1996). In a recent review, Ashworth (1998) demonstrated that term and preterm LBW infants of the same birthweight had risks of neonatal and post-neonatal death of similar magnitude. Infants weighing 2000-2499g had a 4-fold increased risk of neonatal deaths and a 2-fold greater risk of post-neonatal death than infants 2500-2999g. But most pre-term infants were from developed countries, whereas most IUGR infants were from developing countries and thus a direct comparison was difficult. Moreover, the type of growth-retardation might differ greatly between developed and developing countries. In conclusion, LBW is the main risk factor for infant deaths, but it is not clearly established whether the excess of IUGR in developing countries distorts the estimations of risk calculated from developed countries.

LBW infants have been shown to be at higher risk of death from infectious diseases than children with appropriate birthweight. A cohort of 50,000 US births was followed from 7 days to 7 years of age. Moderate LBW (1,500 to 2,499g) was associated with an increased risk of post-perinatal infectious disease mortality and this relation was attributable to preterm birth rather than to IUGR (Read *et al.*, 1994). In developing countries, LBW is an important determinant of mortality (Victora *et al.*, 1988) and morbidity from diarrhoeal diseases (Victora *et al.*, 1990; Bukenya *et al.*, 1991; Ittiravivongs *et al.*, 1991; Barros *et al.*, 1992; Lira *et al.*, 1996). LBW has also been associated with mortality (Barros *et al.*, 1987; Victora *et al.*, 1988) and morbidity from respiratory infections (Victora et al., 1990; Barros *et al.*, 1992). The increased risk of LBW infants of suffering from infectious diseases has been related to the findings that they have a severely compromised immune function (Chandra, 1981).

2.4. Morbidity and mortality from malaria during infancy

The acquisition of malaria infection in infancy has been recognized for long time as a marker of the level of malaria transmission in an area. Longitudinal studies using infant parasite rates have therefore been established in the 1950's and 1960's in order to evaluate the effects of the malaria eradication campaigns. But few of them reported on clinical malaria. More recently, the attention has focused on morbidity and mortality from malaria rather than malaria-infection. But this required longitudinal studies that are logistically difficult. Another method for estimating the burden of malaria disease is through hospital-based studies, but it comprises an important potential bias: in some areas, many children with severe and fatal malaria do not reach a hospital (Greenwood *et al.*, 1987a).

Numerous studies have confirmed early anecdotal reports that infants are protected against clinical malaria during the first months of life. In a small longitudinal study in Ghana, clinical malaria was absent before 4 months of age (Akanmori *et al.*, 1989). In Benin, Velema *et al.* (1991) showed that fever associated with parasitaemia was less common in babies under 6 months of age than in older children. In Liberia, parasite rates and densities were low during the first 3 months of life, and then maximum from 6 months to 2 years (Petersen *et al.*, 1994). In Ghana, both malaria infection (measured by using parasite rates) and malaria illness (measured by parasite densities and association with fever) were higher in infants 6-11 months than those under 5 months. (Binka *et al.*, 1994). In a small cohort of Nigerian infants, clinical malaria did not appear before 2 months of age and was rare until 3 months (Achidi *et al.*, 1996a). In a recent study conducted in Ghana, McGuinness *et al.* (1998) confirmed that clinical malaria was truly uncommon in the first 5 months of life, even after a lower parasite density threshold for the definition of clinical malaria in infants was defined and applied. Lastly, Snow *et al.* (1998) compared incidence of malaria hospital admissions in four areas of Africa with marked differences in the intensity of transmission. In all four areas, children were protected against severe disease in the first two months of life. From 6 months onwards, morbidity and mortality from malaria varied with the level of transmission (Snow *et al.*, 1997a; Snow *et al.*, 1998). The risk of severe disease increased little with age in low to moderate areas whereas reduction of risk already appeared after 6 months where the malaria transmission was intense.

Severe malaria is rare in infancy, although when it does occur, the mortality is high. Duren (1951) reviewed hospital-based reports from Congo Belge and found a case fatality rate (CFR) of 30%. This situation was unchanged fifty years later when Greenberg *et al.* (1989), in Zaire, reported the highest CFR (32%) in infants followed by a regular decrease with age. In an area of very low malaria transmission on the Thai-Burmese border, where severe malaria occur at all ages, CFR for infants was higher than that in the children aged 1-4 years (18.2 and 4.4 per 1,000 respectively) (Luxemburger *et al.*, 1997).

In areas where *P.vivax* co-exists with *P.falciparum*, the former seems predominant in infancy. In Pakistan, infants were first infected with *P.vivax* (Khan & Talibi, 1972). In Papua New Guinea, 75% of malaria cases (defined as fever and parasitaemia) in infants were due to *P.vivax*, whereas *P.falciparum* predominated in older age groups (Genton *et al.*, 1995). In infants from Vanuatu, morbidity from vivax malaria was higher than that from *P.falciparum* (0.9 and 0.5 episode per child-year respectively) (Maitland *et al.*, 1996).

Malaria is an important factor in the development of infant anaemia. It was the major cause of anaemia in infants from Tchad, although iron deficiency was also common (Renaudin & Lombart, 1994). In Ghanian infants, Hb levels fell sharply from the first to the second half of infancy and this was thought to be mainly caused by the increase in malaria, but there was only a weak correlation between parasite density and Hb level (Binka *et al.*, 1994). Malaria parasitaemia lowered Packed Cell Volume (PCV) levels in infants 4-10 months old living in an area of high transmission in Nigeria (Achidi *et al.*, 1996b). In Tanzania, an area of intense and perennial malaria transmission, PCV levels were related to parasite densities. The infants with chronic low parasite densities recovered after 7 months and had similar PCV levels to children without detectable parasitaemia, whereas children with high parasite densities had constantly lower PCV levels than infants with low parasite densities (Kitua *et al.*, 1997). Malaria-related anaemia did not develop in Nigerian children protected against malaria by chemoprophylaxis, who had higher Hb and HCT levels than those who were not protected (Bradley-Moore *et al.*, 1985b).

Malaria attacks temporarily stop infant growth (Garnham, 1949; Bruce-Chwatt, 1952; Williams *et al.*, 1997). The long-term effects of malaria on growth are more difficult to assess as, in malaria endemic areas, it is not possible to find a control group. In most intervention studies, growth was similar in the group of children protected against malaria by the intervention and in the control group. Gambian children successfully protected against malaria during the first three years of life had an overall growth similar to that of their non-protected counterparts, although weight gain of the latter showed seasonal variations that could be related to clinical malaria attacks (McGregor *et al.*, 1956). Similarly, children receiving pyrimethamin during the first five years of life and controls who did not take prophylaxis reached the same weights at 2 and 4 years of age (Morley *et al.*, 1968). In another study, the protected group was taller and had a larger arm circumference than the non-protected one at 1 year of age but weight for age and weight for height were similar in both groups (Bradley-Moore *et al.*, 1985a). Draper & Draper (1960) reported that a successful malaria control in an hyperendemic area in East Africa was not associated with any changes in rates of growth. But more recently, the use of impregnated bed nets was associated with a more rapid growth in the 5 months post-intervention (Shiff *et al.*, 1996) and a reduction in the proportion of stunted children (Snow *et al.*, 1997b).

2.5. Relationship between malaria during pregnancy and malaria in infancy.

2.5.1. Materno-foetal immunological relationship

Various non-immunological factors have been proposed to explain the apparent protection of young infants against malaria: the milk diet deficient in *p*-amino-benzoic acid (Hawking, 1965), selective biting by mosquitoes of different age groups (Muirhead-Thompson, 1951) and haematological factors such as an ageing red cell population and the presence of foetal haemoglobin (Pasvol *et al.*, 1976; Wilson *et al.*, 1977). The first two hypothesis did not appear as important factors in the intervention study conducted by Colbourne (1956) who found that infants supplemented with *p*-amino-benzoic acid acquired malaria as rapidly as those not supplemented, and that all infants were bitten by mosquitoes. It is generally assumed that, although protection may be multifactorial, *in-utero* immunological mechanisms are the more important factors.

Macdonald (1950) and Brabin (1990b) have reviewed the studies of malaria parasite rates in infants. Despite differences due to the level of malaria transmission, they showed that the increase of parasite rates is regular during the first year of life and similar to that expected from a model which does not take into account passively acquired immunity. They concluded that infants are susceptible to malaria infection. Nevertheless, as pointed out by Greenwood (1991) and confirmed by the recent studies already mentioned, protection against clinical attacks of malaria, which is probably separated from protection against the infection, does exist in the first months of life (Akanmori *et al.*, 1989; Velema *et al.*, 1991; Petersen *et al.*, 1994; Binka *et al.*, 1994; Kuate Defo, 1995; Achidi *et al.*, 1996b; McGuiness *et al.*, 1998; Snow *et al.*, 1998; Wagner *et al.*, 1998). Moreover, congenital malaria is relatively frequent in malaria endemic areas. The prevalence of cord blood infections varies from 0 to 21% and parasites are present in 0-29% of infant's peripheral blood at birth, but newborn rarely develop symptomatic and/or severe malaria (Bruce-Chwatt, 1952; Kortman, 1972; Reinhard *et al.*, 1978b; Marshall, 1982; Larkin & Thuma, 1991; Nyirjesy *et al.*, 1993; Egwunvenga *et al.*, 1995).

The transplacental transfer of *P.falciparum*-specific antibodies directed against sporozoites (Nardin *et al.*, 1981) or blood stages (Richard-Lenoble *et al.*, 1988; Chizzolini *et al.*, 1991; Achidi *et al.*, 1995; Rasheed *et al.*, 1995) has been confirmed. Collins *et al.* (1977) and Campbell *et al.* (1980) have also reported that newborn of El Salvador had transplacentally derived *P.vivax* antibodies and Biggar *et al.* (1980) detected maternal antibodies against *P.malariae* and *P.ovale* in newborn from Papua New Guinea. The efficacy of transplacentally acquired maternal antibodies in protecting against malaria in infancy is still controversial. In some longitudinal studies, the age at first malaria infection in infancy was not associated with the level of cord antibody titres at birth (Campbell *et al.*. 1980; Mutabingwa *et al.*, 1993b; Achidi *et al.*, 1996a; Wagner *et al.*, 1998). Biggar et al. (1980) demonstrated that the first malaria infection in 31 Ghanian infants appeared when maternal antibodies had already vanished. Yet symptoms were absent or mild. They concluded that the moderation of symptoms of malaria in infants was due to factors other than maternal transmitted antibodies. On the contrary, in Papua New Guinea, most infections (67%) in infants that were detected in the presence of maternal antibodies were asymptomatic with scanty parasitaemias, whereas 73% of the heavy *P.falciparum* infections developed when maternal antibodies

had vanished. The authors concluded that transplacentally transferred antibodies might be important for the protection against clinical malaria (Seghal *et al.*, 1989). In other studies, the acquisition of infection and clinical malaria clearly followed the disappearance of passively acquired IgG antibodies against *P.falciparum* blood stages (Akanmori *et al.*, 1995; Kitua *et al.*, 1996).

Various hypotheses were proposed to explain these controversial results. Among the hypothesis that may explain the limited protective effect of maternally derived antibodies against infant's malaria, is the short half-life of these antibodies. Most authors have estimated it to be less than 2 months (Campbell *et al.*, 1980; Biggar *et al.*, 1980; Omanga *et al.*, 1982; Collins *et al.*, 1987; Høgh *et al.*, 1995) and no longer detected by 6 months of age (Nardin *et al.*, 1981). The levels of antibodies in infants may vary widely in areas of low malaria endemicity (Collins *et al.*, 1987). Prolonged antimalarial chemoprophylaxis may affect the level of transplacentally acquired maternal antibodies in the newborn (Ibeziako & Williams, 1980; Collins *et al.*, 1987). Moreover, the transfer through *Plasmodium*-infected placentas is reduced (Bray & Anderson, 1979; Brair *et al.*, 1994; de Moraes-Pinto *et al.*, 1998) and selective to some immunoglobulin isotypes (Chizzolini *et al.*, 1991; Deloron *et al.*, 1997) which seem to have little importance in the control of the infection. On the contrary, Kitua *et al.* (1996) proposed that maternally derived antibodies act by controlling the asexual stage parasites. They showed that the attack rate did not change with age but that the duration of infection was shorter in neonates 0-2 months old than in older infants. Thus parasite and fever-parasite prevalence, parasite densities increased with age as passively transferred immunity vanished and recovery became slower.

More recently, some authors have showed that protection from transplacentally derived antibodies against clinical malaria in infancy existed but was associated with specific sub-classes of antibodies. In a prospective study conducted in Cameroon, *P.falciparum*-specific IgG1 and IgG3 were transferred to the offspring more often than IgG2 and IgG4, but only the IgG2 subclass was associated with a reduced risk of acquiring falciparum malaria infection during the first 6 months of life (Deloron *et al.*, 1997). This was a surprising result, as in adults only anti-malarial IgG3 and/or IgG1 had been associated with some degree of protection (Aribot *et al.*, 1996; Taylor *et al.*, 1995). Høgh *et al.* (1995) reported a lower risk of developing an episode of clinical malaria

during the first year of life in the infants with high levels of anti-$MSP1_{19}$ antibodies at birth. The study was conducted in an area of high malaria transmission where 96% of the mothers and 80% of their infants had anti-$MSP1_{19}$ antibodies at delivery. The proportion of children with antibodies declined rapidly until 3 months of age (66%) and increased again to be higher than at birth (85-93%) from 6 months onwards. Infants who had a positive anti-$MSP1_{19}$ antibody response at birth were less likely to develop clinical malaria during infancy than those who had a negative response, after adjustement for age and season. The protection was longer than that expected to be achieved by transplacentally aquired antibodies but the authors discussed the possibility that these antibodies did not interfere with the natural boosting and protected the infant until the acquisition of natural immunity. Other maternally derived antibodies (anti-schizont antigen antibodies) did not seem to play a role in the risk of acquiring malaria infection or disease during infancy. More recently, in a carefully designed prospective study of mothers and infants, the presence of anti-$MSP1_{19kD}$ IgG antibodies one month prior and at the time of the first infection in life was associated with a lower parasite density, a reduced risk to develop febrile disease, a higher proportion of parasite clearance without treatment and a lower infection-related loss of haemoglobin. Thus the presence of anti-$MSP1_{19kD}$ IgG antibodies in infants clearly reduced morbidity from malaria. As the study was longitudinal, the authors were able to demonstrate that the first IgG and IgM peaks coincided with the first malaria infection in infancy in 92% of the infants. Therefore IgG present one month prior the first malaria infection were likely to be transplacentally derived maternal antibodies. Moreover, mother's and cord's IgG levels were very similar and the presence of maternal IgG at the time of delivery was associated with a lower incidence and parasite density of placental malaria and a delayed time to first infection in infants (Branch *et al.*, 1998).

Soluble *P.falciparum* antigens are transferred to the foetus (Druilhe *et al.*, 1976; Jakobsen *et al.*, 1998) and induce the production of parasite-specific IgM (Chizzolini *et al.*, 1991; Achidi *et al.*, 1995; Deloron *et al.*, 1997; King *et al.*, 1998). They also induce cell-mediated immunity, as shown by the response of cord blood cells to *P.falciparum* antigens extracts (Desowitz *et al.*, 1988; Fievet *et al*, 1996). The blastogenic responses of cord blood lymphocytes from newborn of Papua New Guinea showed wide variations that could be related to the sequence of infection during pregnancy (Desowitz *et al.*, 1988). In one study (Rasheed *et al.*, 1995) mean lymphoproliferation and the proportion

of responders to *P.falciparum* antigens were even higher in neonates than in mothers. But the neonatal response is believed to be biaised towards Th2-cell (Carlier &Truyens, 1995) and to produce interleukine 4 (Il-4), a cytokine required for the synthesis of IgE and IgG4. Th2 cells act antagonistically on the Th1-cell subset involved in the activation of macrophages essential in the control of infection. There is also a negative regulation in the gene expression of interferon gamma (IFN-γ). Overall, this neonatal's biaised towards Th2-cell response may lead to altered anti-parasite immunity. Indeed, lymphocytes from babies born to *P.falciparum*-infected mothers have been shown to synthesize more IgE antibodies in their response to *P.falciparum* antigens (Desowitz *et al.*, 1993) than did the maternal cells. In Cameroon, the percentage of responders and the mean level of positive responses after stimulation of cord blood lymphocytes by malaria antigens were of the same order as those observed in adults from the same area for IL-2 production, while proliferation and IL-4 responses were moderately reduced and IFN-γ highly reduced. Surprisingly these findings were similar in primi and and multigravidae and whether mothers presented with a *P.falciparum* infection at delivery or not (Fievet *et al.*, 1996). On the contrary, in another study, the neonatal TH2-biased response did not exist. Cytokine responses to blood stage malaria antigen $MSP1_{19}$ (IFN-γ secreting lymphocytes and IL-4) by cord blood lymphocytes of 92 Kenyan neonates were present in 23 and 8% of the samples and mirrored that observed in paired maternal blood. There were no $MSP1_{19}$-specific IgE antibodies in the cord blood but significant levels of $MSP1_{19}$-specific IgM (compared to American cord plasma) in 4.5% of the samples. The authors concluded that "prenatal exposure to malaria may not lead to tolerance or altered foetal immunity and could stimulate partial protection to malaria in infancy" (King *et al.*, 1998).

The vertical transmission of parasite antigens can induce T-cell tolerance and this has been clearly shown in filariasis. As a result, children born to infected mothers were two or three times more susceptible to filarial infection than those born to uninfected mothers (Lammie *et al.*, 1991). In the case of filariasis, microfilaraemic patients with T-cell hyporesponsiveness are usually asymptomatic. Therefore, maternally-induced T-cell tolerance probably conferred a beneficial effect to the offspring, by increasing the risk of infection but not that of severe clinical manifestations. There are no study supporting this hypothesis in malaria.

The relationships between malaria during pregnancy and malaria in infants may depend on the timing of malaria infection during pregnancy. Rasheed (1994) reviewed the knowledge on the influence of maternal infections on infection susceptibility in childhood, from published studies in malaria and other infections during pregnancy. The time at which *in utero* exposure occurs will depend on the likelihood of maternal infection (greater during the second trimester for malaria), on the flow of products from mother to foetus, which is not constant throughout pregnancy (e.g. increased IgG transfer close to term), and on the maternal immune status, which changes with time (tolerance in the first part of pregnancy) and gravidity (immunosuppression and different hormonal environment in primi- compared to multigravidae). The foetal response will depend on the maturity of its immune system at the time of exposure. Thus a complex materno-foetal immunoregulation will occur that may lead to either tolerance or protection against further infection in infancy.

2.5.2 Malaria during pregnancy and malaria in infancy

A few longitudinal studies following pregnant women and their offspring were conducted in order to assess whether malaria during pregnancy was associated with higher or lower risk to develop malaria in infancy. In Malawi, Redd *et al.* (1994) found that children born to mother with placental malaria were more likely to have *P.falciparum* infection at 2 months of age than those born to mothers without placental infection. Le Hesran *et al.* (1997) also found that children born to a mother with placental malaria were more likely to have *P.falciparum* infection than those born to mothers without placental infection, but this difference occurred later in life, from 4 to 6 months. From 6 to 9 months, the difference was reduced and from 9 months onwards, the children born to mothers with or without placental malaria had the same risk to develop parasitaemia. Throughout infancy, the risk of having clinical malaria was similar in both groups. Slutsker *et al.* (1996a) did not find an association between malaria during pregnancy and in infants during the first three months of life. Some authors compared malaria in infancy in children born to mothers who received efficient antimalarial prophylaxis and in those whom mothers had not received prophylaxis or a less efficient drug. Malaria during infancy was not influenced by chemoprophylaxis during pregnancy (Spencer *et al*, 1987; Mutabingwa *et al.*, 1994; Achidi *et al.*, 1996a)

nor by the number of malaria attacks during pregnancy (Mutabingwa *et al.*, 1994). None of these studies could recruit pregnant women in early pregnancy. Therefore they could not determine whether malaria at various stages of pregnancy influences malaria in infancy.

CHAPTER 3

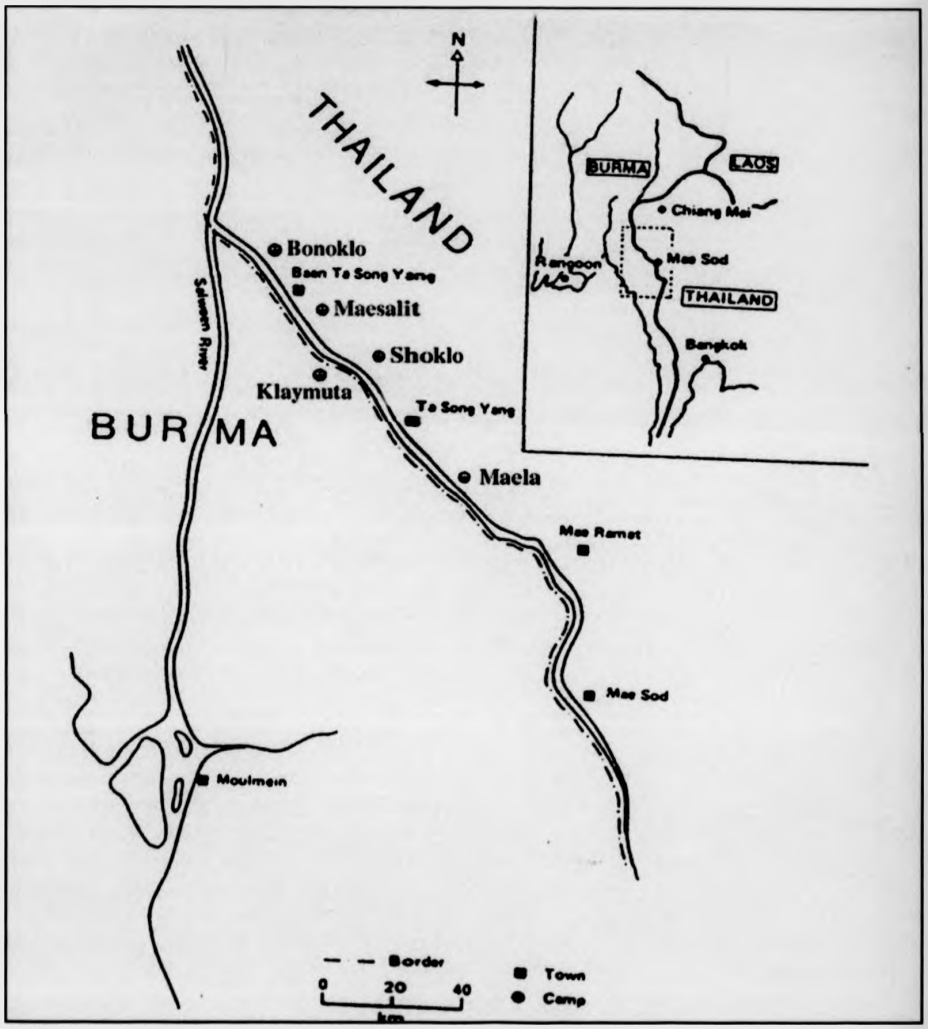
BACKGROUND

3.1. The Karen refugee camps

3.1.1 Situation and organisation

In 1984, around 18,000 Karen in conflict with the Burmese junta took refuge in Thailand. On their arrival, they settled in a few opened camps situated in forested areas and accessible through out the year by four-wheel drive vehicles, near the Thai town of Mae Sod (**fig 3.1**). Since then, the situation in Burma has deteriorated and Karen, as well as refugees from other ethnic groups, have continuously arrived in Thailand. New camps have been created, located all along the border and the number of refugees from Burma is estimated to have reached 100, 000. The Karen were never given the status of refugees, and are officially considered as illegal migrants by the Thai authorities. These had both negative and positives consequences. The United Nations High Commission for Refugees (UNHCR) was not allowed to be in-charge of the refugees. The camps were therefore set-up closer to the border than stated by the UN security norms. The refugees were not allowed to apply for asylum in a third country. The distribution of food rations and the implementation of a health care system relied only on the action of a few Non Governmental Organizations (NGO) that received permission from the Thai authorities. On the other hand, the Karen camps were created and administered by the Karen. They therefore built houses, schools and health structures very similar to that in the Karen state. As the camps were opened and close to the border, they maintained their commercial system. Farmers could go back to their own farms in Burma or work in Thai farms. Merchants could renew their stock in Burma and sell it in the camps. Families regularly moved back to their villages and men to the army. At the end of 1994, the Karen split in two groups and during the following dry season (February to April 1995), the camps were attacked and sometimes burnt by the dissident fraction. The Thai army intervened and, in order to protect the camps, restricted population movements, and established a curfew. Some camps were closed and their inhabitants relocated to one of the other sites, Maela, which now comprises around 25,000 refugees.

Fig 3.1. Map of the area



3.1.2. Population

The refugees are mainly women (54%) and children: 45% of the population is under 15 years old. About 90% of the refugees are Karen, most of whom (80%) are Buddhists or animists and the others are Christians. The remaining 10% of the refugees compose a distinct group, who may have originated in Bangladesh but have been living with the Karen for generations. They are Muslims and call themselves the "Black Karen". Whilst most of the Karen were farmers prior to fleeing to Thailand, the Muslims were usually merchants and run the small markets established in the camps. Both groups are well educated, with only 30% illiterate and access to education is equal between men and women. As a consequence, all camp administrators, teachers and health workers arise from within the community.

A wide range of background socio-economic status are found. If the differences diminish once families arrive in Thailand, there are still obvious degrees of income in the camps. Food, school furniture, blankets and mosquito nets are given by a consortium of charities based in Bangkok (Burma Border Consortium; BBC). The food ration comprises rice, fermented fish paste and salt. Most of the refugees grow vegetables and fruit and some own poultry, pigs and goats. They find roots and leaves in the surrounding forest. The families usually send at least one of their members to work outside the camps, which gives them small cash income and leads to a local economy in the camps.

3.2. Health facilities

3.2.1 General organisation

Health care is provided by the French medical organization Médecins Sans Frontières (MSF) and in two camps, Shoklo and Maela, the SMRU has opened health structures in order to treat patients enrolled in the trials. In each camp, all houses are at walking distance from a dispensary. Most camps also have an in-patient department where patients can stay overnight. More severe cases are transferred to Shoklo or Maela hospitals, where most common medical problems can be treated. Patients who need

surgery or pregnant women with obstetrical complications are referred to the Thai hospital of Mae Sod. Expatriate physicians were on call around the clock in the main camps until 1995. Since 1996, they had to go back to Mae Sod every evening for safety reasons. Preventive programmes have been implemented by MSF: Expanded Programme of Immunization (EPI) and sanitation. The SMRU has been running ANC since 1986. All MSF and SMRU health facilities are run by Karen staff: medical assistants, nurses, laboratory technicians and logistic officers. All services are free of charge for the refugees and for Thai-Karen from the neighbouring villages. A few health workers have private practices in the camps, and medicines are available from the shops. Nevertheless, most of the refugees cannot afford them.

3.2.2. The Shoklo Malaria Research Unit

The work described here is part of a large community-based malaria research project. The SMRU was set-up in 1986 and is part of the Oxford-Mahidol Tropical Medicine Research Programme, directed by Pr. Nicholas J. White. The SMRU is directed by Dr. François Nosten. The objectives of the Unit are:

- to determine the optimum antimalarial treatment in an area of multi-drug resistant *P.falciparum*.
- to describe the epidemiology of malaria in an area of low and unstable endemicity where *P.vivax* and *P.falciparum* co-exist.
- To evaluate preventive antimalarial measures such as impregnated mosquito-nets or vaccine candidates (SPf66).
- to describe malaria during pregnancy and its consequences on mother and foetus, in this context of low endemicity, co-existence of *Plasmodium* species and multi-drug resistance.
- to assess the efficacy of antimalarial treatments and preventive measures in pregnant women with emphasis on potential toxicity on the foetus or infant.

Equipment, communications and staffing are common for all the trials conducted in the Unit. The main base was located in Shoklo camp and partly moved to Maela in June 1995. In both places, the Unit comprises a laboratory, a hospital, a delivery room, a dispensary, clinics ascribed to specific trials (Antenatal Clinic, Infant Clinic, Drug Trials Clinic...), an office with computing facilities and staff houses. Electricity is provided by a generator, running water is available in all buildings. Each camp has a radio liaison with the office in Mae Sod. Another laboratory is also located in Mae Sod. The Karen personal (200 persons) comprises medical assistants, midwives, nurses, laboratory technicians, computer data entry clerks and home-visitors. There are 5 expatriate scientists (4 physicians and 1 technician). Further logistic and administrative support is provided at the Mahidol University in Bangkok and at the University of Oxford.

3.3. Malaria situation

3.3.1. Overview

In 1984, malaria was immediately identified as the main health problem in this community. The camps are located in forested areas, often surrounded by rice fields cultivated by the neighbouring Thai-Karen villagers. The average minimum and maximum annual temperatures are 21 and 29^{0}C and the relative humidity varies from 65 to 87%. The monsoon begins in May and lasts until October. Average annual rainfall (1987-92) is 1900 mm. Conditions are therefore optimum for anopheline vector breeding sites in the stagnant pools along the rivers and in rice fields. The vectors are predominantly *Anopheles minimus* (44%) and *An.maculatus* (43%) with relatively few *An.dirus* (2%). Most feed outdoors in the evening (6-10 pm) and in the early morning (4 am) (McGready; personal communication).

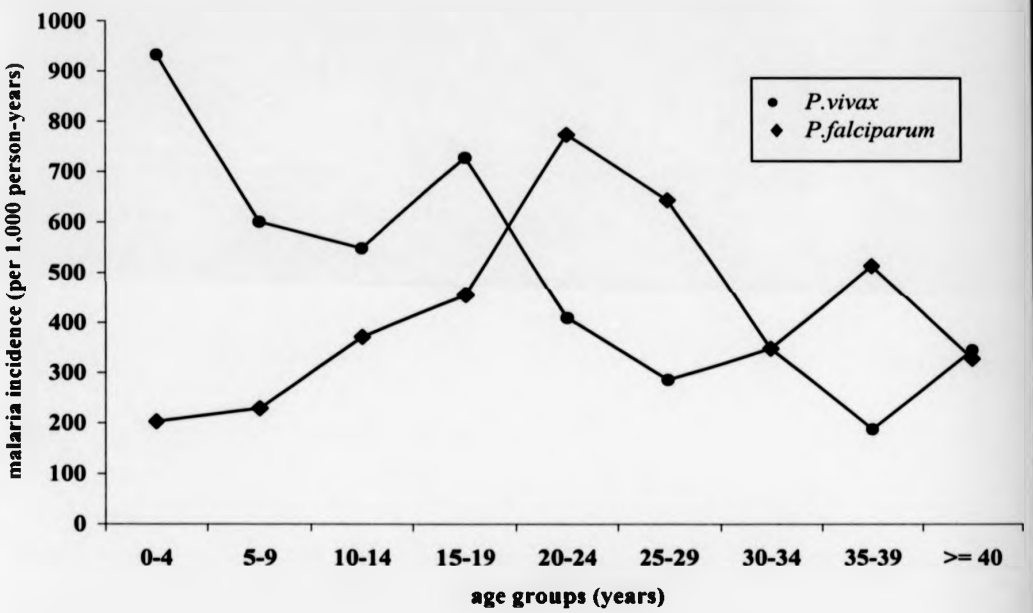
Malaria transmission occurs year-round with two seasonal peaks: May-July and December-January. Symptomatic and severe malaria cases are seen at all ages. Epidemics may arise (8 since 1984). When the Karen settled in the camps, malaria-related morbidity and mortality were high. Forty per cent of the consultations at the dispensary were due to malaria (*P.falciparum:* 75%; *P.vivax*: 20%; mixed infections:

5%; *P.malariae*: very rare) as well as 24% of the deaths registered in the community. Multi-drug resistance of *P.falciparum* necessitated the introduction of mefloquine (Nosten *et al.*, 1987). A malaria control programme was established based on early diagnosis and treatment (Decludt *et al.*, 1991). For 15 years the population has been encouraged to come to the dispensary for any febrile episode. All cases are confirmed by microscopic examination of a blood smear prior to treatment. Overall there were 1,067 malaria cases per thousand refugees in 1984 and 716 in 1994. By 1994, the importance of malaria in overall morbidity and mortality had decreased (15-20% of the consultations; 10% of the deaths) and the ratio *P.falciparum*/*P.vivax* had changed and was closer to 1. Most of the malaria-related deaths were in people coming from surrounding areas (MSF unpublished reports). Thus the development of curative services combined with laboratories is thought to have had an effect on the malaria-related morbidity and mortality in the camps. During the 10 year period, multi-drug resistance had increased. In 1994 a three-day combination of artesunate and mefloquine was introduced for the treatment of uncomplicated falciparum malaria (Nosten *et al.*, 1994b). This combination proved highly efficient and artesunate reduced gametocyte production (Price *et al.*, 1996). Following the introduction of this treatment on a large scale, falciparum malaria transmission was reduced by 70% and the therapeutic combination remained highly efficient, indicating that it may protect mefloquine against further deterioration of the resistance.

In order to describe the epidemiology of malaria in this area more precisely, the SMRU has conducted several cohort studies. Age-specific incidence rates, risk factors for malaria infection, risk factors of developing severe malaria, and parasite threshold for the development of symptoms were defined. Despite some variations between camps and a trend towards reduction with time, the malaria situation in the camps can be estimated from this work (Luxemburger *et al.*, 1996). Malaria incidence rate was around 1.0 (95% CI: 0.9- 1.1) infection per person per year. *P.vivax* causes 54%, *P.falciparum* 36%, and 10% of the infections were mixed. The age-specific incidence rates of these two species were very different, reflecting a different transmission pattern (**fig 3.2**). By the age of 4 years, the children had experienced 4 times more *P.vivax* episodes than *P.falciparum*. But older children and adults still developed symptoms for

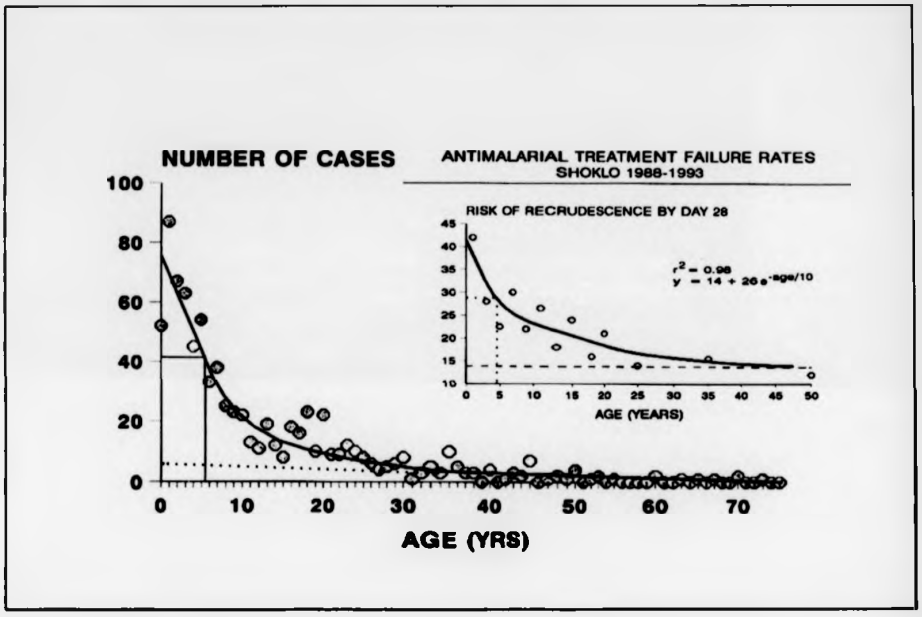
both species. The parasite threshold for developing symptoms was very low (1500/µl for *P.falciparum*; 200 for *P.vivax*), reflecting a lack of immunity.

Fig 3.2. Age-specific *P.vivax* and *P.falciparum* malaria incidence



Nevertheless, the risk of getting severe malaria, the malaria attributable mortality rate, and the risk of having a recrudescence after an antimalarial treatment decreased with age, due to a certain amount of premunition (Luxemburger *et al.*, 1997) (**fig 3.3**). Male, young adults, subjects with a past history of falciparum malaria and those who travel outside the camp had an increased risk of *P.falciparum* episodes.

Fig 3.3. Age distribution of hospitalised patients and age-specific risk of antimalarial treatment failure (from Luxemburger *et al.*, 1997 and ter Kuile *et al.*, 1995).



Early detection and treatment of malaria are clearly the most important part of the malaria control programme. Nevertheless, some other measures have been introduced. The Thai Malaria Zone Office (MZO) runs an in-house spraying programme (DDT every 6 months). Mosquito nets are distributed by the BBC. Since 1996, they are impregnated with permethrin. Only 40% of the families accept the house spraying and its effect has never been evaluated in the area (MZO personal communication). In two trials conducted in the camps, permethrin-impregnated bed nets reduced malaria by 42% in schoolchildren (Luxemburger *et al.*, 1994) and anaemia by 50% in pregnant women, but they had little effect on detectable parasitaemic episodes in the latter group (Dolan *et al.*, 1993). Their efficacy after introduction on a large scale and without supervision of their use was not clearly established (MSF; unpublished data).

3.3.2. Malaria during pregnancy

In this area of low malaria endemicity, malaria during pregnancy is associated with maternal morbidity and mortality, and reduced birthweight. In 1985, there were 5 maternal deaths from malaria, giving an estimation of malaria-related mortality rate in pregnant women of 20 per 1000 (Nosten *et al.*, 1991). The ANC programme was implemented by the SMRU in 1986. All pregnant women were encouraged to attend the ANC and by 1989, 90% of pregnant women were registered. This proportion remained stable in recent surveys.

3.3.2.1. Routine data collected from ANC attendees

At their first visit, a questionnaire enquiring about demographic data, past obstetrical history and past malaria history is applied to the women. Pregnant women are then followed weekly. At each consultation, they are asked to report any symptoms during the previous week, a blood smear is performed and they receive a supplementation to their food ration (eggs and beans). A blood sample for an haematocrit testing is taken fortnightly, and an obstetrical examination (fundal height, foetal heart beat, blood pressure) on a monthly basis. All women with a positive malaria smear are treated. The majority of women are asymptomatic, because malaria is actively detected before they develop fever. In this area, it is well established that most subjects with parasites would develop symptoms in the absence of treatment. This may differ in pregnant women, but it is considered unethical to leave them untreated. Pregnant women are also encouraged to come to the clinics in between two weekly consultations, in case of fever. Iron and folic acid treatments are given to all women who develop anaemia (HCT less than 30%). Haematinics are given as soon as anaemia is detected and continued until delivery. Blood transfusion is given when HCT is below 20% or in the presence of clinical symptoms of severe malaria. Other medical conditions, such as ARI, urinary tract infections (UTI) and pre-eclampsia are treated when needed. All women are encouraged to deliver in the SMRU hospital. At birth, the newborns are weighed and a Dubowitz score is performed within 6-24 hours. Home-visitors report on deliveries that occur at home, and research staff visit the mother and child within 72 hours.

During their entire follow-up in ANC, the women keep a card that they bring back at each contact with the health services. All data collected during the period are reported on this card: demographic data, past obstetrical and malaria history, weekly results of blood smears, fortnightly HCT levels, monthly fundal height, as well as complaints, treatments for malaria or other conditions. This card is completed at delivery with the type and site of delivery, weight and sex of the infant, Dubowitz score, and collected by the SMRU staff. Data are coded and entered into a database.

3.3.2.2. Summary of the main results

Since 1986, more than 11,000 pregnancies have been followed prospectively. A detailed epidemiological description of malaria during pregnancy in this area of low transmission (Nosten *et al.*, 1991) and the first report of the consequences of vivax malaria during pregnancy on the mother and newborn (Nosten *et al.*, in press) have been published. Three successive randomized trials have been conducted in order to assess the efficacy of preventive antimalarial measures in the area: mefloquine prophylaxis (Nosten *et al.*, 1994a), permethrin-impregnated bed nets (Dolan *et al.*, 1993), skin mosquito-repellent with DEET (McGready *et al.*, in preparation).

In summary, the incidence of malaria during pregnancy is about 30%. Both species, *P.falciparum* and *P.vivax* are more common in primigravidae than in multigravidae and may be associated with symptoms. Pregnant women are more likely to develop severe malaria than their non-pregnant counterparts (Luxemburger *et al.*, 1997). Since the implementation of the ANC programme, malaria-attributable mortality has albeit disappeared. Malaria-related anaemia is the most important consequence for the mother. Anaemia is present with both species and increases with the number of malaria episodes. Both species reduce birthweight, but the effects of *P.falciparum* are more marked than that of *P.vivax*. Whereas the reduction in birthweight due to *P.falciparum* is present mainly in the first three pregnancies, that due to *P.vivax* occurs in all gravidae, but is relatively more important in multigravidae. Anaemia has no apparent independent effect on birthweight. Malaria during pregnancy has no effects on the duration of gestation, nor on the outcome. The rate of stillbirth/late abortions is 5-7% and is not influenced by malaria during pregnancy.

3.3.3. Malaria during the first two years of life

The epidemiology of malaria in young children has been less studied, but scattered data suggest a very different picture from that of malaria in infants in highly endemic areas. The parasite rate is close to zero. All falciparum malaria infections are symptomatic and often severe (40% of cases). In 1992, the falciparum malaria incidence rate was estimated to be 70 per 1000 in children under 1 year old and 164 per 1000 in children aged 1 to 2 years. Vivax malaria was acquired at a younger age than *P.falciparum* malaria: median (range) age: 12.5 (5-23) and 14.5 (10-23) months respectively. Case fatality rate of falciparum malaria was higher in infants under 1 year (34 per 1000; 4/117) than in children 1-4 years (12 per 1000; 11/936).

3.3. Infant mortality and morbidity

IMR is surprisingly high in the camps, despite a good access to health structures, a high immunization coverage (90% BCG; 70% complete EPI) and the absence of harmful cultural behavior. The first estimation of IMR was obtained from the follow-up of infants born to mothers recruited for the mefloquine prophylaxis and permethrin-impregnated bed nets: 170 (49/288) and 160 per 1000 live births (32/196) respectively (Nosten *et al.*, 1994a; Dolan *et al.*, 1993). Risk factors for infant deaths were prematurity, low birthweight and maternal anaemia at the time of delivery. The causes of deaths were unusual. Diarrhoea was not the main killer as in most tropical countries. In the first cohort of infants, thiamin deficiency caused 38% (19/49) of the deaths, prematurity, respiratory infections and malaria 8% each, the remainder were unexplained. Thiamin deficiency has been well described in similar rice-eating populations in the region (Thanangkul & Whitaker, 1966; Pongpanich *et al.*, 1974). It is the most common nutritional deficiency in the camps, especially in pregnant women and lactating mothers (McGready, unpublished data). A weekly distribution of vitamin B1 for lactating women has been implemented for 2 years, but has not yet been evaluated.

Morbidity was not described in the two studies mentioned previously, but data gathered from dispensaries activity show different pattern than that described in other developing countries. Respiratory infections account for 20% of the consultations as in most places, but diarrhoea incidence is generally low with periodic epidemics. Malnutrition is rare (< 5%) and usually reflects social problems or underlying health problem such as tuberculosis.

CHAPTER 4

METHODS

4.1. Study site and population

The study was conducted in five camps: Shoklo (8,000 inhabitants), Bonoklo (6,000), Maesalit (3,500), Klaymuta (1,500) and Maela (21,000). These 5 camps have similar general organization and relief programmes, but they differ slightly in their population structure, socio-economic factors and malaria situation. Whereas the two ethnic groups co-exist in most camps, Black Karen are absent in Maesalit and Klaymuta. Maesalit comprises a section inhabited by hilltribe Karen who are less educated than expected in the camps. Klaymuta opened in 1994 and refugees in this place have only recently arrived in Thailand. The inhabitants from Bonoklo have the highest socio-economic level in the camps.

The study started in 3 well-established camps (Shoklo, Maesalit and Bonoklo) where the SMRU had been present for seven years. Epidemiological surveillance of the malaria situation indicated that malaria transmission was usually higher in Shoklo than in the two other camps (MSF reports). ANC was implemented in Klaymuta a few weeks after the camp opened, in July 1994, and the first children included in the study were born in September. Malaria was much higher than in other places as is generally the case on arrival of new refugees. Moreover an epidemic of malaria occurred in August-September 1994. The situation was radically different in Maela. Although this camp is among the oldest in the area, SMRU was not regularly working there. In May 1995, the refugees from Bonoklo were relocated there after their camp had been burnt. Thus the SMRU started to work in Maela in order to follow pregnant women and infants from Bonoklo who were already admitted into studies. Later the refugees from Maesalit and Klaymuta were also relocated there, and the development of SMRU structures followed that of the camp (from 4,500 refugees in December 1994 to 21,000 by July 1995). At the end of the study, Maela was the main base of the SMRU. In Maela, as the camp was growing, malaria transmission inside the camp nearly stopped and the majority of cases were acquired during travel outside the camps. Pregnant women were infected during their movements outside the camps, but their children, who usually stay at home, rarely developed malaria during infancy.

All live born singletons (spontaneous breathing and presence of heart beats) whose mothers were followed in ANC were included into the study if their mothers gave informed consent. Twins, stillbirths, newborn whose mothers did not attend ANC and whose those mothers did not reside into the camps and only stayed during pregnancy were excluded from the study.

4.2. Study design

The study was prospective. A birth cohort was followed weekly from birth to one year of age in order to calculate the IMR, to describe the acquisition of malaria, and to estimate morbidity rates from malaria and other infectious diseases. Monthly measurements of anthropometric markers and HCT levels were use to compare growth and haematological status of infants exposed to different factors *in utero*. The history of malaria and anaemia of their mothers had also been collected prospectively from their admission into the ANC until delivery. Socio-economic markers and parental education were scored by means of a questionnaire applied to the mother as soon as possible after birth.

4.3. Study procedures

4.3.1. Admission

All newborn included into the study were given a unique identification number with a letter for the camp and three digits for the child. If the children moved during the follow-up they were given a new number, but both identifications were entered in the database in order to follow the child's history. This information was used to determine in which camp the mother and her/his child had lived for the longest period and therefore to determine the variable "camp" used for the analysis. In most cases, it was the camp of infant's birth. Similarly, "year" was defined as the year of birth.

As the recruitment for the study lasted for three years, some women delivered twice. Identification number of the first born was reported on the notes of the second in order to recognise siblings. Newborn were examined within 72 hours after delivery whenever possible. Admission was sometimes delayed in children born outside the camp during a visit of their mother to Burma or to another camp, or in case of referral to a Thai hospital and delivery there. Such births were recorded by home-visitors who systematically visited the women who had been absent from the weekly antenatal visits. At this first examination, child's weight (to the nearest 50g), height, head and arm circumferences (0.1cm) were measured and a test conducted to assess the gestational age (Dubowitz score). The use of the Dubowitz score in the Karen population was validated by Dr. Dubowitz in a pilot study conducted before the beginning of this cohort. She trained Karen staff and visited the site once a year. Gross external malformations were recorded.

ANC cards were collected after their completion by the midwife. All information contained in the card was reviewed by the study's supervisor and summarized in the infant's admission form. This summary contained the following information: demographic and obstetrical data of the mother (age, address, gravidity, parity); history of previous children (number alive, number dead, interval since last birth and survival of the child); history of confirmed malaria before pregnancy (obtained by questionnaire); slide-confirmed malaria attacks between conception and first antenatal visit (questionnaire and cross-check with dispensary attendance); results of antenatal examinations: weekly malaria smear, malaria episodes (parasite count, presence or absence of symptoms, treatment), fortnightly HCT levels; symptoms and treatment of suspected thiamin deficiency; diagnosis and treatment of other morbid events; type and site of delivery; complications during the post-partum (**Appendix 1**). When the card was not available (lost, burnt), data were extracted from the log books filled by the midwives at each ANC visit and kept in the SMRU's office.

4.3.2. Socio-economic status (SES)

A questionnaire was applied to the mother as soon as possible after the infant's birth. Interviews were conducted at home and questions were asked in the mother's language. Information was collected on date of arrival in Thailand, past work in Burma, level of education, current work and income, house materials and families properties (**Appendix 2**). Scores for education of each parent and for the current SES (i.e. since arrival in the camp) were calculated as indicated in **tables 4.1 and 4.2**:

Table 4.1: Education score of each parent (0 to 10)

(a) went to school	no (0); yes (1)
(b) school level reached	none or only kindergarden (0) Primary school (1) secondary school (2)
(c) can write	no (0); yes (1)
(d) number of languages spoken	only mother's tongue (1) 2-3 local languages (2) 2-3 languages including foreign language (3)
(e) number of languages written	none (0) only mother's tongue (1) 2-3 local languages (2) 2-3 languages including foreign language (3)

Table 4.2: Score of current economic level (0 to 20).

Family income	
(a) mother has income	no (0); yes (1)
(b) father has income	no (0); yes (1)
(c) family has a garden	no (0); yes (1)
(d) number of chicken	none (0) 1-9 (1) ≥ 10 (2)
(e) number of pigs or goats	none (0) 1 (1) ≥ 2 (2)
(f) number of cows	none (0) 1-2 (1) ≥3 (2)
(g) proportion of households fed by the food ration distributed	<80 % (0) ≥80% (1)
Hygiene and quality of life	
(a) housing material	only leaves (0) leaves and bamboos (1) leaves, bamboos and wood (2)
(b) interviewer impression on economical level	very poor (0) poor (1) average (2)
(c) interviewer impression on the hygiene of house	dirty (0) tidy (1) very tidy (2)
(d) watch	no (0); yes (1)
(e) radio	no (0); yes (1)
(f) furniture	none (0) 1 table (1) ≥ 2, e.g. table + cupboard... (2)

4.3.3. Follow-up

Each infant was seen weekly by a home-visitor living in the neighbourhood. They enquired about illnesses during the previous week and reported absences from the camp (**Appendix 3**). When they found that the child had been sick, they ensured that he/she had been brought to a clinic. As they were living close to the children that they followed, they usually knew the events that happened between two visits. Thus most deaths that occurred at home or hospitalizations in MSF or Thai structures were reported immediately to the study staff.

Mothers were encouraged to bring their sick babies to the SMRU (Shoklo, Maela) or MSF (Bonoklo, Maesalit, Klaymuta) dispensaries for any illnesses. Complaints, clinical signs, and laboratory results were collected on a standardised form (**Appendix 4**). When the child attended the MSF health facilities, information contained on his/her health card was reported to the study notes. In the 3 camps in which the SMRU had no permanent health structures (Bonoklo, Maesalit, Klaymuta), research staff living in the camp registered all events (births, deaths, dispensary attendance, transfer to hospital) that occurred among the studied children in between the weekly visits from the SMRU ANC and Infant Clinic mobile teams.

In this setting, most of the children who died presented to the dispensary at one stage of their final illness and a large proportion of deaths occurred in hospital. Hospital and dispensary records are kept by MSF. A copy of the file was obtained and cause of death was defined after both physicians (from MSF and SMRU) had agreed on a cause. When death occurred at home, the mothers were interviewed by the investigators.

All children were seen at one week of life and then monthly until 1 year of age at the SMRU Infant Clinic. At each visit, anthropometric measurements and HCT levels were performed, children were examined, milestones and vaccinations were recorded (**Appendix 5**). Children were seen systematically by a physician at 6 months of age. During this visit, mothers were also asked if they were breastfeeding and if they had started to give complementary food to the infant (**Appendix 6**).

At the end of the study the follow-up period was determined in days. The children either completed the study (365 days) if they attended the Clinic or were seen by home-visitors at 12 months of age or they were lost (number of days = date of lost – date of birth), or they died (number of days = date of death – date of birth) before 12 months of age. Whenever possible, the exact date of death or withdrawal from the study was recorded, otherwise the date was arbitrary chosen as the 15th of the month.

4.3.4. Case definitions

Investigations were limited in the camps, thus most diagnosis were based on clinical grounds. The majority of consultations were done by locally-trained medical assistants. Case definitions were therefore similar to that used by health agencies working on the border when they train health workers or classify morbid events in monthly activity surveillance.

Single episodes of morbidity: in each individual, consecutive morbid events were all numerated if they were from a different diagnosis, but episodes with similar diagnosis were counted only when there was a time gap of seven days free of symptoms between the two episodes. This delay is rather short and the two episodes may have been due to the same infectious agent, but there was no recognised definitions for single events, and many episodes would have been missed with a longer period.

Symptomatic malaria case: presence of asexual forms of plasmodium species on a blood smear, associated with fever (axillary temperature above 37.5^{0}C) or history of fever during the previous 3 days. This definition was used for infants, but in pregnant women symptoms included fever and/or any of the followings: headache, chills, arthralgia, myalgia.

Asymptomatic malaria infection: presence of asexual forms of plasmodium species on a blood smear without any of the symptoms listed above. Asymptomatic malaria were diagnosed only in pregnant women in whom active detection was performed. Therefore the infection was sometimes detected before the development of symptoms.

Severe malaria case: symptomatic malaria case with one or more of the following signs of severity: history of convulsions, impairment of consciousness, hyperparasitaemia (≥ 4% parasitised red blood cells), severe anaemia (HCT< 20%), intractable vomiting, oligo- or anuria, bleeding, pulmonary oedema, shock.

Acute Respiratory Infections (ARI): recent history of cough and fever. ARI are classified in three levels of severity: (i) mild ARI: respiratory rate < 40/min, presence of runny nose, no impairment of daily life (eating, playing...); (ii) moderate ARI: persistence of the symptoms previously described and productive cough with abnormal pulmonary auscultation; (iii) severe ARI: respiratory rate > 60/min, presence of one or more signs of severity: chest indrawing, alar flare, cyanosis.

Diarrhoea: more than 3 loose stools per day (or increased number of stools in a breastfed infant), with/without fever and/or cramps. There are no routine laboratory diagnoses in the camps. Diarrhoeal episodes are classified as watery, or bloody. They are considered as moderate when oral rehydration salt are sufficient, or severe when the degree of dehydration requires intravenous treatment.

Infantile Beri-Beri: sudden onset of respiratory distress in a young (around 6 weeks-4 months), previously healthy, breastfed infant: respiratory rate > 60/min, enlarged liver, normal pulmonary auscultation, with sometimes cyanosis, moderate fever, hoarse voice and rapid improvement (within few hours) of these signs after a single intramuscular injection of vitamin B1. In Shoklo and Maela camps, the diagnosis was always assessed by one SMRU physician or the trained medical assistant in-charge of the Infant Clinic. In the other camps, the frequency was usually overestimated, as the health workers have been encouraged to prevent this serious event by an injection of vitamin B1 if they have any doubt. SMRU physicians subsequently interviewed the mother and classified the case as probable, unlikely or unknown.

Low Birthweight (LBW): birthweight, as weight recorded in the first 3 days of life, under 2500g. Children with a birthweight of 2500g or more were classified as "Normal" Birthweight (NBW). This classification is independent from the gestational age. In both groups of children, there could be some newborn with appropriate weight-for-age and others small-for-age.

Premature birth: birth before 37 weeks of gestation. Gestational age was estimated by using Dubowitz score, which combines external (superficial) and neurological criteria. The method is reliable when applied from 6 hours to 5 days after birth and provides an estimate of gestational age with 95% confidence limits of ± 2 weeks (Dubowitz & Dubowitz, 1977).

Anaemia during pregnancy: a woman was considered anaemic when her HCT was lower than 30%. This cut-off corresponded to 2 standard deviations (SD) below the mean HCT level in pregnant women in this population. Severe anaemia was defined by a HCT < 25%. There are physiological variations of HCT during the course of pregnancy. HCT levels decrease at the end of the first trimester, and then remain in plateau until 30-34 weeks of gestation. The levels raise again during the last weeks before delivery (Nosten *et al.*, in press). Moreover, there is a certain degree of haemo-dilution during pregnancy that may lead to an overestimation of the prevalence of anaemia. Using different definitions of anaemia at various stage of pregnancy rendered the analysis too complex. We therefore used only one cut-off to determine if a woman had been anaemic (HCT<30%) or severely anaemic at any time during the pregnancy, and if she remained so at the time of delivery.

Infant anaemia: normal values of HCT in infants living in developing countries are not well defined. Reference values have been calculated for developed countries (Saarinen & Siimes, 1978; Lubin, 1987) and take in account the physiological anaemia that develops by two-three months of age (O'Brien & Pearson, 1971). In the study, infant anaemia was defined using lower values, in order to harmonize the definition with that of maternal anaemia (e.g. infant anaemia was defined as HCT < 30% from 6 months onwards whereas the reference values is 33%). Anaemia at birth was defined as HCT<45%, and at two and three months: HCT<28%.

Underweight/stunted/wasted infant: Underweight was defined as a weight-for-age Z score (WAZ) below 2 SD of the mean of the National Center for Health Statistics/ World Health Organization (NCHS/WHO) reference population. Stunting and wasting were defined respectively as a height-for-age Z score (HAZ) and a weight-for-height Z score (WHZ) below 2 SD of the NHCS/WHO reference population.

4.3.5. Treatment

Children with vivax malaria were given 25 mg base/kg of chloroquine phosphate over three days. Infants who developed uncomplicated falciparum malaria were administered a three day combination of oral artesunate (4 mg/kg per day) and mefloquine (15 mg/kg on the second day of treatment followed by 10 mg/kg on the third day (Nosten *et al.*, 1994b). In case of recrudescence (i.e. presence of asexual forms of *P.falciparum* on the blood film, with fever or not, within 63 days after the treatment), a further supervised seven day course of oral artesunate was given to the patients. Children with severe malaria received different treatments according to their clinical features and the health structures in which they were admitted. Oral artesunate (7 days) combined with mefloquine was given if they presented with hyperparasitaemia but no signs of vital organ dysfunction (Luxemburger *et al.*, 1995). Whenever they had any other signs of severe malaria (as defined previously), they received parenteral treatment: intramuscular injection of artemether in the SMRU hospitals or intravenous quinine when they were hospitalized in MSF structures. In both hospitals, ancillary treatments were similar: paracetamol as antipyretic, intrarectal diazepam for the treatment of convulsions, followed by phenobarbitone for the prevention of further convulsions, intravenous glucose for hypoglycaemia, transfusion of whole blood when required. Other conditions than malaria, especially ARI and diarrhoea, were prescribed treatments in accordance with the MSF guidelines, using essential drugs (WHO lists).

4.3.6. Laboratory procedures for malaria diagnosis:

Blood smears were stained with Giemsa and read by microscopic examination (objective X1000). The result was declared negative after examination of 200 fields. The parasite count for positive smears was expressed as the number of parasites per 500 white blood cells or per 1000 red blood cells in cases of high parasitaemia (>2% of parasitised red blood cells). A white blood cell count and HCT (QBC haematology Becton Dickinson) was performed on any positive case. When the smears were read in the MSF laboratory and could not be double-checked by the SMRU staff, parasite counts were classified using a semi-quantitative method (rare; + to ++++). Previous comparisons of the two methods have shown little correlation between this classification and parasite density. But parasite densities that were classified as (++++) using the semi-quantitative method were indeed hyperparasitaemia in a small comparative study. Forty smears classified as (++++) by the Karen MSF laboratory technicians were double-checked and counted. Median parasitaemia on these smears was 7.5% of infected red blood cells, with a lower limit of 3.5% (Proux & Luxemburger; unpublished data). Thus in the study, parasite density was considered as missing when only MSF results were available, but (++++) results were classified as hyperparasitaemia (i.e. severe malaria cases).

4.4. Quality control

At random, physical measurements and examination (temperature, spleen palpation, weight) were performed on the same children by health workers and investigators and differences were discussed in order to underline the errors in the method (the most common being to weigh children with clothes in winter). Laboratory quality control was part of routine checking of SMRU study procedures. Sampled smears were double-checked and inter and intra-laboratory technician variations in reading HCT were evaluated.

4.5 Ethical approval

The study was approved by the Ethical Committees of the Karen Refugee Committee and Mahidol University, Thailand; and the London School of Hygiene and Tropical Medicine, London, UK.

4.6. Data entry

All forms were checked for missing values and coded prior computer-entry by trained data entry clerks using Dbase IV software. Database structures were created with internal checks for impossible values (e.g.: sex male = 1 or female = 2; the value 3 cannot be entered). A print out of the database spreadsheets was then compared against paper notes by teams of home-visitors and the corrections were entered in the computer files. A third data check was performed by the main investigator. Outliers were detected and either corrected or considered as missing values (e.g. height at one visit much shorter than at the previous and further visits). Illogical entries were also corrected. Most in them were dates (e.g. December 94, followed by January 94 instead of 95).

4.7. Sample size

An initial sample size was calculated by using Epitable in Epi-Info software. For the detection of a 50% difference (from 15 to 7.5%) in infant mortality in children born to anaemic mothers when compared to those born to non-anaemic mothers, with 5% of significance level and 80% of power, and assuming from previous studies that the prevalence of maternal anaemia at delivery would be 25% and the proportion lost to follow-up 20%, the recruitment of 1018 children was necessary. After two years of study, 2 major components changed. The IMR was apparently decreasing over time and the estimated proportion of children lost to follow-up was likely to increase after the important population movements that happened in 1995. The sample was therefore re-estimated by using a reduction of infant mortality from 12 to 6% and a drop out of 30%. The final sample size required was 1414 children.

4.8. Analysis

Analysis was performed using SPSS software for basic statistics, logistic regression and survival analysis. Poisson regression was performed using STATA software. The Z scores for growth analysis were calculated using the ANTHRO programme (WHO, 1990).

4.8.1. Morbidity during pregnancy and pregnancy outcomes

All variables on mother's characteristics, mother's morbidity and newborn's weight and gestational age were summarized at birth (i.e. admission to the study). Mother's characteristics and their morbidity during pregnancy were described in order to identify potential confounders for the two main exposures (i.e. malaria and anaemia during pregnancy) for further analysis in the study. Proportions were compared using the Chi-squared test and continuous variables were compared using *t*-tests and anova or Mann-Whitney *U* and Kruskal-Wallis tests, as appropriate. Risk factors for malaria during pregnancy (at any time; presence of one or more malaria episodes or no malaria episodes detected); for anaemia during pregnancy and/or at delivery; and the determinants of LBW or prematurity were identified in univariate analysis using methods for unmatched case-control studies. All factors associated with the outcome in univariate analysis ($p<0.10$) were included in a logistic regression model by forward step addition. Population attributable fractions (PAF) were calculated assuming that the exposure had a causal effect on the outcome. The formula was: PAF= pc (Adjusted OR-1)/Adjusted OR, where pc is the prevalence of the factor among the cases (Bruzzi *et al.*, 1985; Rothman & Greenland, 1998).

4.8.2. Infant mortality

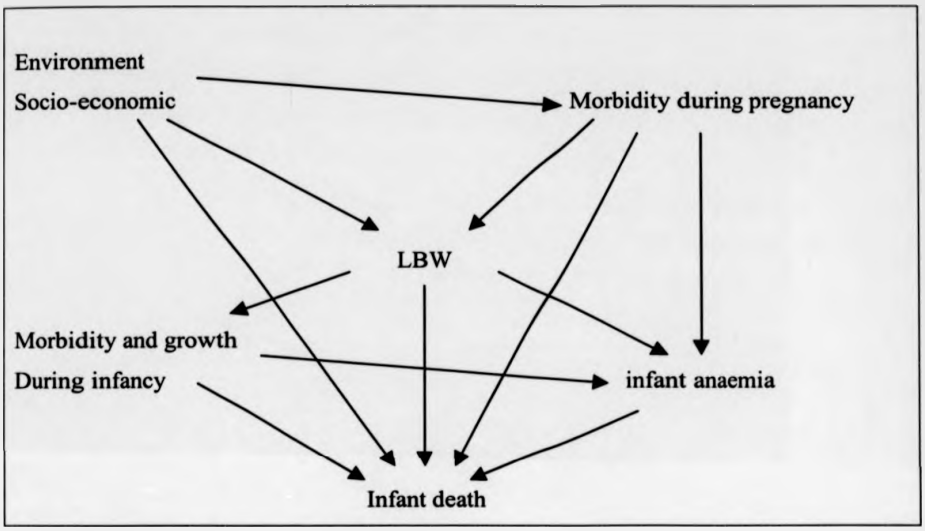
The main outcome of the study was the comparison of IMR in the presence or absence of the exposure factors, mainly maternal anaemia and malaria. Various classifications were used to describe anaemia during pregnancy (HCT<30%): anaemia at any time during the follow-up, the duration of anaemia, development of severe

anaemia (HCT<25%) at any time during pregnancy, persistence of moderate or severe anaemia at delivery. Similarly, some variables were created to describe malaria during pregnancy in order to include the available information on the infected species, on the number of attacks and on the timing of first exposure to a *Plasmodium* species during pregnancy. These variables were created for malaria of any species, and for falciparum and malaria species separately. A variable was created in order to summarize malaria history during pregnancy. There were four groups of pregnant women: those who never had malaria during pregnancy, those who had only falciparum malaria, a third group with only vivax malaria and women who were infected at the same time or successively by the two species.

Apart from malaria and anaemia during pregnancy, the effects of other morbidity during pregnancy (ARI, UTI, hypertension, paresthesia) on infant mortality were analysed and potential confounders such as mother's education, birth order and camp, were identified whenever possible. Unfortunately, information on smoking during pregnancy was missing. Smoking was identified as an important determinant of LBW in another study (McGready *et al.*, 1998), but this was unknown when the follow-up of the cohort started.

There were numerous potential confounding factors and a hierarchy between them which made the issue of how to fit confounders more complex. Each level of the hierarchy could affect those further down, either directly and/or indirectly through an intermediate and some of the potential confounders were secondary outcomes (infant morbidity and growth) (**Fig. 4.1**). Multivariate analysis was therefore performed by step, including at each level an intermediate explanatory variable. Using that method, the first step (i.e. model without intermediate explanatory variable) allowed to identify an effect that could have been underestimated if the intermediate explanatory variable was contained in the model. In the second step, that included the intermediate variable, the effect of that intermediate variable was estimated, as well as the effect from the first exposure factors not mediated by the intermediate variable. Further steps were analysed when appropriate (Victora *et al.*, 1997).

Fig. 4.1: Exposure factors and counfounders in the analysis of infant mortality.



Analysis of infant mortality was done using survival methods. Prior to use survival analysis, another method, Poisson regression showed that rate ratios varied with the infant age. Analysis of survival was therefore done for three different periods: neonatal (1-28 days), 1-3 months (29-92 days), 3-12 months (93-365 days). Kaplan-Meyer plots and log rank tests were performed for each potential factor that could influence infant mortality. A summary of the univariate analysis was done and Hazard Ratios (HR) were presented to see the direction of the effects. All factors identified by this method ($p<0.10$) were included in Cox regression as well as the determinants of LBW and/or prematurity. Nevertheless, variables that were not independent from each others were not included together in final models (e.g. presence or absence of falciparum malaria is contained in presence or absence of malaria from any species; when both were significant in univariate analysis, they were tested one after the other in multivariate analysis and only the best was retained).

Anaemia and malaria during pregnancy could have a direct effect on infant deaths, but they were likely to act via low birthweight. Malaria could also act though anaemia at delivery. Three models were therefore fitted, the first without anaemia at delivery, nor LBW and prematurity. Anaemia at delivery was added in the second model, and LBW and prematurity in the third.

4.8.3. Infant morbidity

Poisson regression was used to identify risk factors for infant morbidity. The outcomes were morbidity from all causes, malaria, ARI and diarrhoeal episodes. Incidence Rates (IR) were calculated for each of these diseases and Incidence Rate Ratios (IRR) were compared in the presence or absence of each factor. Incidence Rates of each disease varied widely between camp and year, but the risk factors identified in Poisson regression were similar in models including camp and year or study and in models not including them. Although this is not shown in summarised results, site and year of study were always taken in account.

4.8.4. Anaemia and growth

Anaemia in infancy was not detected prospectively, but at the time of each monthly visit. Anaemia could develop between 2 visits and remained undetected. It was therefore not possible to calculate the incidence rate of anaemia in infancy. Case-control study methods were used to identify the risk factors of infant anaemia at a given age (1 month, 3 months, 6 months and 12 months). Similarly, impaired growth (stunted, underweight or wasted infants) was analysed at 6 and 12 months. Logistic regression and Odds Ratio (OR) were used for this part of the analysis.

CHAPTER 5

MOTHERS AND LIVE NEWBORN

5.1. Registration and inclusion into the cohort

From June 1993 to August 1996, 1,624 live born children were registered in the study. Eight per cent (129/1624) were excluded from the analysis. Fifty-seven were twins (the number is odd, because 1 twin was stillborn) and 72 singletons did not meet inclusion criteria. Twenty of them were born to mothers not followed in ANC. The other 52 mothers were followed in ANC, but 45 were not residents of the camps and left after delivery, 4 ANC cards were lost, 2 mothers with psychiatric problems were unable to bring their children for follow-up, and 1 mother gave her baby to a foster mother not living in the camps.

Thus, 1,495 children born to 1,448 mothers were included in the cohort. Forty-seven (3%) women delivered twice during the study time. Although the socio-economic environment of the siblings was similar, the exposure factors referring to morbid events during pregnancy was different for each child. Thus the unit of analysis was the pregnancy and not the woman. The second child of these 47 mothers was therefore included into the cohort. Seven (0.5%) babies were adopted (5 within the first week, one at 2 and one at 3 months of age). For these children exposure factors were morbidity during pregnancy of their biological mother and socio-economic level of their foster parents.

5.2. Description of mothers population

5.2.1. Demographic and obstetrical characteristics

Demographic and obstetrical characteristics differed greatly between camps (**table 5.1**). Differences in mothers mean age was partly due to the distribution of ethnic groups in the camps. Black Karen women, who are Muslims, tend to have their first child at a younger age than women from the other ethnic groups (Karen, Burmese, Pa-Oh, Mon), who are Buddhist or Christian. Means (SD) age at first pregnancy were 18.5 (2.9) years for Black Karen and 20.1 (3.2) for the other ethnic groups ($p<0.01$). But the numbers of pregnancies were similar in all ethnic groups. Differences between camps

remained after adjusting by ethnic groups. There were obviously various environmental and socio-economic factors influencing reproductive life of the women.

Women living in well-established camps (Shoklo, Bonoklo, Maesalit) attended ANC earlier in pregnancy than did women from Klaymuta and Maela where the programme was implemented recently, although this improved with time in the latter camp. In all camps, multigravidae came earlier to ANC than primigravidae: 15.6 (8.4) vs 17.8 (9.1) weeks of pregnancy ($p<0.01$). Overall nearly half of the women were registered during the first trimester of pregnancy and were seen regularly until delivery (at 91% of the planned visits. The expected number of ANC attendance was defined as the number of weeks between registration to ANC and delivery).

5.2.2. History of refugee situation and SES

Questionnaires on background and current SES were applied to 81% (1210/1495) of the women. This proportion was lower in Maesalit and Klaymuta where many women moved before the interview. There was also a problem in reaching mothers whose children had died in early life because most of them left the camp immediately after the death in order to find work. Thus women whose child died within the first three months of life were interviewed less often than those for whom the child survived: 48% (32/67) vs 83% (1178/1428); $p<0.01$. Overall, information on SES could be used in the analysis of risk factors of morbidity during pregnancy, and infant deaths from 3 to 12 months and infant morbidity. But the lack of information on SES factors for half of early infant deaths, probably introduced a bias in the analysis of events at birth and in the first three months of life. Two models, with and without SES factors, were therefore applied for the analysis of determinants of LBW and that of risk factors for infant deaths in the first three months. The results of the questionnaires are summarized in **table 5.2.** The majority of the women (93%; 1121/1205) were living in the camps for more than 2 years and a quarter of them (26%; 308) arrived in Thailand during their childhood. Most of those who arrived only after 15 years old were farmers when living in Burma (82%; 689/842). Education of both parents was similar in the camps, but economic scores were different. There were various reasons for that. Bonoklo was a well-established camp with a good access to trade between the two

countries. Klaymuta was located on Burma soil, thus the refugees had still access to their farms. On contrary, people from Maela, who were located there when the security situation worsened, could not go easily out of the camps and many had lost their garden and poultry while moving. Education and economic scores were similar between ethnic groups.

5.2.3. History of previous children

Multiparae (N=1066) had a mean (range) 3 (1-12) children born before the index child. There were no difference between camps and ethnic groups. However, there were more deaths among the previous children born to Karen than to Muslims women: mean (range) 0.8 (0-8) and 0.4 (0-3) respectively ($p<0.01$). Within ethnic groups, mean numbers of dead or live previous children were similar between camps. Among the last born children for whom information was known, 14% (104/741) had died before one year of age.

Interval between births was similar between camps and ethnic groups: median (range) 31 (9-151) months. Only 11% of the women (75/696) delivered within 18 months after the last born child, and 26% (181/696) within 2 years.

Table 5.1: Demographic and obstetrical characteristics of the mothers in the five study sites.

Study site	Sohklo (n=523)	Bonoklo (n=214)	Maesalit (n=101)	Klaymuta (n=56)	Maela (n=601)	All camps (n=1495)	p
Ethnic group: n (%) Karen	462 (88)	176 (82)	101 (100)	55 (98)	498 (83)	1292 (86)	
Black Karen	50 (10)	32 (15)	0 (0)	1 (2)	92 (15)	175 (12)	
Others	11 (2)	6 (3)	0 (0)	0 (0)	11 (2)	28 (2)	<0.01
Mean (SD) age in years:	24.9 (5.9)	25.3 (6.1)	27.0 (6.7)	27.5 (5.7)	25.4 (6.3)	25.4 (6.1)	<0.01
Age distribution : n (%): < 20 y	100 (19)	42 (20)	12 (12)	3 (5)	118 (20)	275 (18)	
≥ 20 y	423 (81)	172 (80)	89 (88)	53 (95)	483 (80)	1220 (82)	0.04
Mean (SD) No of gravida	3.2 (2.3)	3.5 (2.3)	4.2 (3.2)	4.1 (2.3)	3.5 (2.5)	3.5 (2.5)	<0.01
Primigravidae: n (%)	146 (28)	49 (23)	24 (24)	5 (9)	158 (26)	382 (26)	0.03
Mean (SD) gestational age in weeks on admission to ANC	14.6 (8.2)	16.5 (8.6)	16.3 (9.2)	23.5 (8.1)	17.4 (8.7)	16.4 (8.7)	<0.01
Trimester of pregnancy on admission to ANC: n(%): 1st	295 (56)	104 (49)	51 (51)	7 (13)	249 (42)	706 (47)	
2nd	172 (33)	72 (34)	33 (33)	27 (48)	247 (41)	551 (37)	
3rd	56 (11)	37 (17)	17 (17)	22 (39)	102 (17)	234 (16)	<0.01
Attendance to ANC: no weeks seen at ANC /n of weeks between registration and delivery (%)	11,273/ 12,452 (91)	4,371/ 4,647 (94)	2,048/ 2,259 (91)	749/ 844 (89)	11,663/ 12,755 (91)	30,104/ 32,957 (91)	<0.01

Table 5.2: History of refugee situation and current SES of the parents

Study site	Sohklo (n=523)	Bonoklo (n=214)	Maesalit (n=101)	Klaymuta (n=56)	Maela (n=601)	All camps (n=1495)	p
No (%) women interviewed:	430 (82)	150 (70)	46 (46)	29 (52)	555 (92)	1210 (81)	<0.01
Time in refugee camps: n (%)							
< 2 years	26 (6)	4 (3)	2 (4)	7 (4)	45 (8)	84 (7)	
2-5 years	168 (39)	43 (29)	16 (35)	14 (48)	190 (34)	431 (36)	
6-10 years	162 (38)	37 (25)	15 (33)	6 (21)	176 (32)	396 (33)	
> 10 years	71 (17)	65 (43)	13 (28)	2 (7)	143 (26)	294 (24)	<0.01
No (%) arrived during childhood	95 (22)	52 (35)	9 (20)	2 (7)	150 (27)	308 (26)	<0.01
No (%) farmers in Burma	249/324 (77)	76/96 (79)	31/32 (97)	20/24 (83)	313/366 (86)	689/842 (82)	<0.01
No (%) literate mothers	235 (55)	91 (61)	25 (54)	12 (41)	292 (53)	655 (54)	0.29
No (%) literate fathers	323 (75)	115 (77)	30 (65)	19 (66)	408 (74)	895 (74)	0.43
Means (SD) score of family's education	10.2 (4.6)	10.4 (4.6)	9.6 (5.2)	8.8 (4.4)	9.7 (4.3)	10.0 (4.5)	0.21
Means (SD) score of family's income and quality of life	7.0 (2.4)	8.1 (3.3)	6.7 (2.2)	7.8 (3.1)	6.5 (2.1)	6.9 (2.4)	<0.01

5.3. Malaria during pregnancy

5.3.1. Timing of detection and symptoms

Thirty-seven per cent (555/1495) of the women developed malaria during pregnancy. In 128 women (23%), the first slide-confirmed malaria episode was diagnosed during the period between conception and first attendance to ANC (i.e. they were diagnosed and treated in another health structure). In the other 427 women (77%), malaria was detected during the follow-up period in ANC, either at the weekly visit (active detection) or in between two visits, if the women presented to the clinic with fever (passive detection). Overall, 31% (170) of the first detected malaria infection occurred during the first trimester, 40% (222) during the second and 29% (162) during the third part of pregnancy. This distribution was similar for both major plasmodium species *P.falciparum* and *P.vivax*. Five per cent (72) of the women had malaria during the week prior delivery (31 *P.falciparum*, 39 *P.vivax* and 2 mixed with *P.falciparum* and *P.vivax*).

There were 1,096 parasitaemic episodes [1-8 per woman] in the 555 malaria-infected women (491 *P.falciparum*; 570 *P.vivax*; 34 mixed infection with *P.falciparum* and *P.vivax*; 1 malaria from unrecorded species). Thirty-eight per cent (210/555) of the women infected with malaria had only had falciparum malaria episodes [1-5 parasitaemic episodes] during their whole pregnancy, 40% (225) only vivax infections [1-6] and 22% (120) had experienced malaria from each of the two species [1-8].

Symptoms were present in 59% (566/960) of the episodes for which this information was available (136 missing values: 12%). Nearly all malaria episodes detected prior to admission in ANC were symptomatic (96%; 135/140) whereas only 53% (431/820) of the episodes detected during ANC were associated with fever ($p<0.001$). Symptoms were more often present in falciparum than in vivax malaria episodes: 72% (309/433) vs 47% (233/498) ($p<0.001$). Primary episodes of falciparum malaria (i.e. first detected *P.falciparum* parasitaemia) were also more often associated with symptoms than subsequent episodes: 77% (210/274) vs 62% (99/159) ($p=0.002$), whereas the risk to develop symptoms did not change from one vivax episode to the next one ($p=1.0$).

Out of 525 falciparum (or mixed) malaria episodes, 13 (2%) were severe. As only live births were admitted into the study, it is not surprising that none of the mothers died, but during the same period only 1 pregnant woman died with severe malaria.

Table 5.3 summarises the relationships between timing and type of detection in the first *P.falciparum* parasitaemic episodes diagnosed during pregnancy. The presence of symptoms and the co-existence with vivax malaria are also described. The proportion of episodes detected during the period of ANC follow-up (as opposed to history recall) increased with the gestational age at first falciparum malaria (following the increasing chance to be registered in ANC). Similarly, the number of women who had suffered from vivax malaria earlier in pregnancy, and the proportion of those who developed their first episode of falciparum malaria as a mixed infection (*P.falciparum* and *P.vivax*), increased with the stage of pregnancy at which falciparum malaria was diagnosed. Nevertheless, the proportion of first falciparum malaria infections associated with symptoms and signs of severity were the same throughout pregnancy. And overall, the proportion of women who also had *P.vivax* malaria during pregnancy did not change with the stage at which falciparum malaria was first detected.

Table 5.3: Description of detection, symptoms, and co-existence with *P.vivax* according to the stage of pregnancy at which first falciparum malaria episodes occurred.

Stage of pregnancy	1st trimester	2nd trimester	3rd trimester	P
No (%) detected during ANC	45/93 (48)	113/132 (86)	98/105 (93)	<0.001*
No (%) symptomatic	70/85 (82)	87/118 (74)	71/92 (77)	0.35
No (%) severe	3/85 (4)	3/118 (3)	5/92 (5)	0.54
No (%) mixed infection	3/93 (3)	8/132 (6)	12/105 (11)	0.01*
No (%) with *P.vivax* malaria detected earlier in pregnancy	6/93 (7)	16/132 (12)	21/105 (20)	0.005*
No (%) with *P.vivax* at any time during pregnancy	36/93 (39)	52/132 (39)	32/105 (31)	0.31

*: $\chi 2$ for trend.

5.3.2. Risk factors for malaria during pregnancy

Twenty-two per cent (330/1495) of the women had at least one *P.falciparum* malaria episode during pregnancy. Falciparum malaria occurred more often in primigravidae than in multigravidae: 28% (107/382) vs 20% (223/1113) (OR: 1.6; 95%CI: 1.2-2.1) (**fig 5.1**). The infection was also more common in recent refugees (< 5 years in the camps) than in those who were living in Thailand for more than 5 years: 26% (133/515) and 18% (125/690) respectively (OR: 1.6; 95%CI: 1.2-2.1). Malaria was less common when the education of the husband was high than when he had a low or average score: 18% (91/497) vs 24% (160/676) (OR: 0.7; 95%CI: 0.5-1.0; p=0.03). The proportion of women with *P.falciparum* (Pf) during pregnancy also varied from one camp to the other and was seasonal, but the general tendency was to decrease over time (**fig 5.2**). In a multivariate analysis, only first gravida (Adjusted OR: 1.7; 95%CI: 1.3-2.3) and low-average father education (Adjusted OR: 1.4; 95%CI: 1.1-1.9) remained independently associated with infection with falciparum malaria during pregnancy. These two risk factors were identified in the model that included camp and year in order to take in account the differences in transmission level, and in the other model that did not adjust for these variables.

The proportion of women who suffered from *P.vivax* malaria during pregnancy was 23% (345/1495), with important variations from one camp to another and over time and season. Infection with vivax malaria increased in Shoklo and Maesalit, whereas it decreased slightly in the other camps (**fig 5.3**). Overall, the proportion of women infected with *P.vivax* during pregnancy tended to become more important than that with *P.falciparum* (**fig 5.4**). The risk to have vivax malaria was similar in all gravidae (**fig 5.1**). Past history of slide-confirmed malaria (OR: 1.4; 95%CI: 1.1-1.8), Karen group (OR: 1.5; 95%CI: 1.0-2.3) and number of consultations in ANC (OR per unit of 1 visit: 1.02; 95%CI: 1.00-1.03) were associated with vivax malaria during pregnancy in univariate analysis. On the contrary the risk of having vivax infection decreased as the time spent in the camps increased (OR per unit of 1 year: 0.89; 95%CI: 0.87-0.93). In multivariate analysis, a past history of malaria (Adjusted OR: 1.5; 95%CI: 1.1-2.0), and the number of ANC consultations (Adjusted OR per unit of 1 visit: 1.02; 95%CI: 1.00-1.04) remained independently associated with the risk of having vivax malaria during pregnancy, whereas the time spent in the camp (Adjusted OR per unit of 1 year: 0.90;

95%CI: 0.87-0.93) protected against *P.vivax* (Pv) infection. The past history of malaria may reflect previous vivax infection and therefore a higher risk of relapses. It could also be related to behaviour. The association between the number of ANC consultations and *P.vivax* infection was not surprising. As infection with this species sometimes remains asymptomatic, it is likely that a higher number of consultations, and therefore of active detection, increased the chance to diagnose it.

Fig 5.1: Proportion of malaria-infected pregnant women according to gravidity.

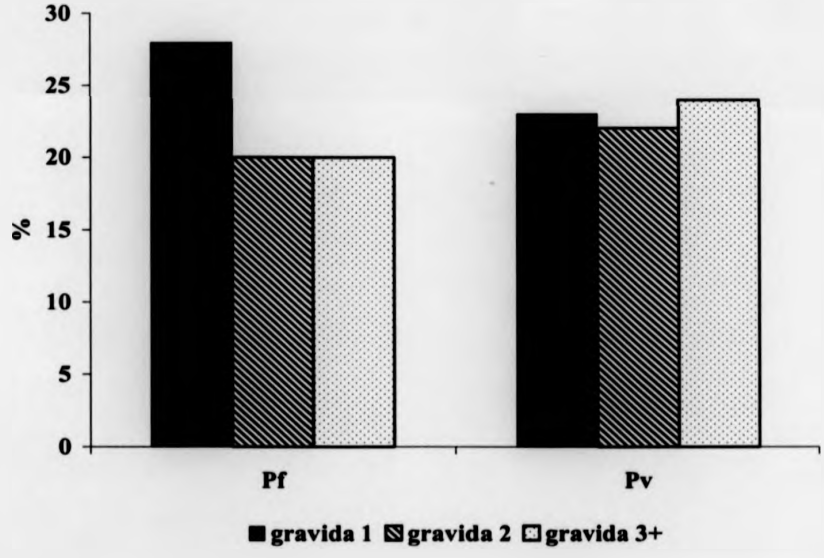


fig 5.2: Falciparum malaria prevalence during pregnancy per site and year

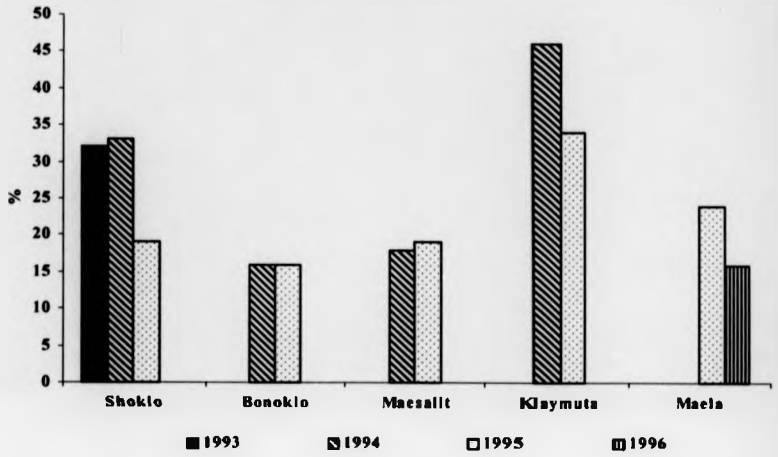


fig 5.3: Vivax malaria prevalence during pregnancy per site and year

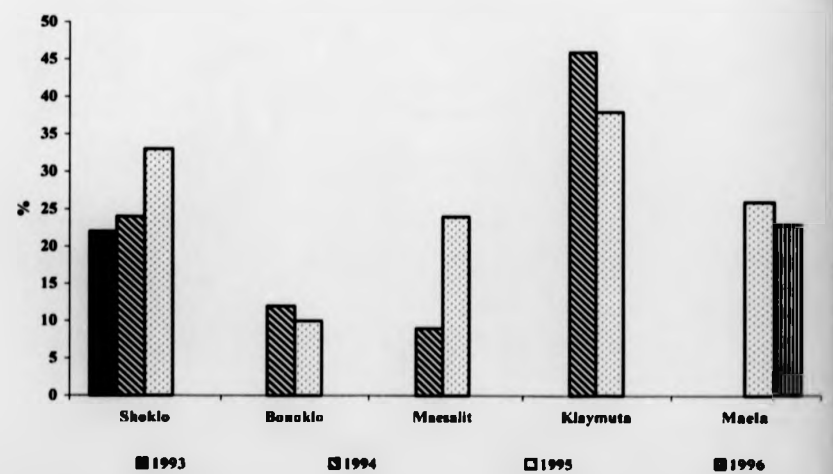
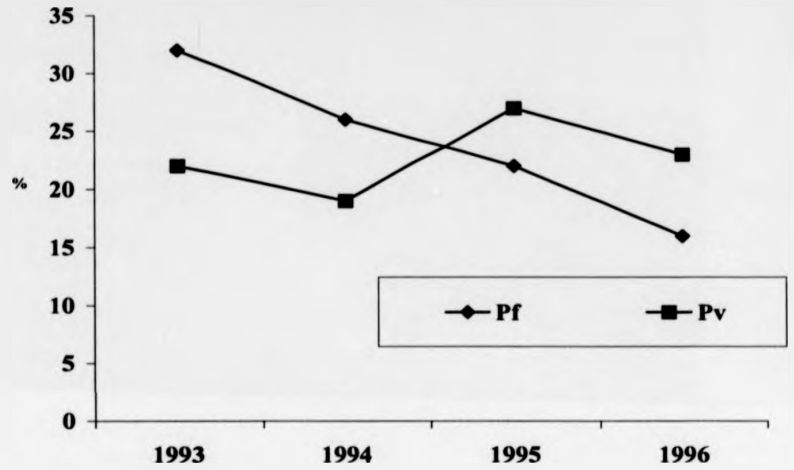


fig 5.4: Time-trend malaria prevalence rate during pregnancy



5.4. Anaemia during pregnancy

Unlike malaria that could be detected throughout pregnancy because reported history of malaria is reliable in this area, there was no way to know the haematological status of the women prior to their registration to ANC. Thus the data collected in this study focused on anaemia during the second and third trimester of pregnancy. The duration of anaemia was defined as the number of weeks between the first HCT level below 30% and the first HCT level that reached again 30% or more. In women who were anaemic on admission, the duration of anaemia was underestimated (from admission to the first HCT level above 30%, as the number of weeks with anaemia before the first ANC consultation could not be defined). During the follow-up period in ANC, anaemia (HCT < 30%) was extremely common. A quarter (25%; 373/1466) of women were anaemic on admission and 69% (759/1093) of the women not anaemic when they registered for ANC developed anaemia later in pregnancy. Thus overall 77% (1132/1466) of the women developed anaemia at some stage of pregnancy, for a mean

(SD) duration of 7.7 (5.9) weeks. When a woman started haematinic treatment, it was given until delivery and thus the mean (SD) duration of iron and folic treatment was 11.2 (8.7) weeks. At delivery 24% (353/1444) of the women remained anaemic. Severe anaemia (HCT < 25%) developed in 17% (247/1445) of the pregnant women and 1% (21/1494) needed transfusion (haematocrit < 20% or clinical signs of severe anaemia).

Risk factors for anaemia during pregnancy are summarized in **tables 5.4 and 5.5**. Camp, year and number of ANC consultations were added as covariates in the final models of multivariate analysis. Falciparum malaria during pregnancy was the strongest risk factor for moderate and/or severe anaemia. A quarter (26%) of anaemic women had falciparum malaria. Thus the PAF of anaemia due to *P.falciparum* malaria was 18% (95%CI: 13-21). Ethnic group and education were also independently associated with anaemia during pregnancy. Multigravidae were more often anaemic than primigravidae, but the risk to develop severe anaemia was not associated with gravidity. In the model without "camp" and "year", vivax malaria during pregnancy was associated with anaemia (Adjusted OR: 1.58; 95%CI: 1.05-2.36), but not with severe anaemia.

Table 5.4: Risk factors for anaemia (HCT< 30%) during pregnancy.

Risk factors	Prevalence N (%)	Univariate analysis OR (95%CI)	Multivariate analysis OR (95%CI)
Mother's age: < 20 years	185/268 (69)	1.00	
≥ 20 years	949/1200 (79)	1.70 (1.25-2.30)	NS
Gravidity: Multigravidae	883/1093 (81)	2.08 (1.58-2.72)	1.76 (1.24-2.50)
Primigravidae	251/375 (67)	1.00	
Ethnic group: Karen	1018/1296 (79)	1.77 (1.23-2.53)	1.65 (1.07-2.53)
Black Karen	116/172 (67)	1.00	
Woman's education: Low	438/534 (82)	1.45 (1.08-1.94)	NS
Average-high	498/656 (76)	1.00	
Husband's education: Low-average	543/668 (81)	1.45 (1.08-1.95)	1.64 (1.20-2.56)
High	365/487 (75)	1.00	

Family's economic level: Low	273/333 (82)	2.19 (1.22-3.91)	NS
Average	519/663 (78)	1.94 (1.15-3.24)	
High	54/83 (65)	1.00	
P.falciparum malaria during pregnancy: Yes	296/329 (90)	3.22 (2.20-4.73)	3.10 (1.93-5.00)
No	838/1139 (74)	1.00	
P.vivax malaria during pregnancy: Yes	292/341 (86)	2.02 (1.45-2.81)	NS
No	842/1127 (75)	1.00	
ARI during pregnancy: Yes	335/410 (82)	1.45 (1.09-1.93)	NS
No	799/1058 (76)	1.00	

Table 5.5: Risk factors for severe anaemia (HCT< 25%) during pregnancy.

Risk factors	**Prevalence N (%)**	**Univariate analysis OR (95%CI)**	**Multivariate analysis OR (95%CI)**
Ethnic group: Karen	230/1277 (18)	1.95 (1.13-3.41)	1.82 (1.01-3.29)
Black Karen	17/168 (10)	1.00	
Woman's education: Low	109/528 (21)	1.59 (1.17-2.15)	1.54 (1.11-2.14)
Average-High	91/646 (14)	1.00	
Husband's education: Low	69/317 (22)	1.57 (1.13-2.17)	1.44 (1.02-2.04)
Aver.-High	124/822 (15)	1.00	
P.falciparum malaria during pregnancy: Yes	113/322 (35)	3.99 (2.98-5.34)	3.63 (2.60-5.08)
No	134/1123 (12)	1.00	
P.vivax during pregnancy: Yes	69/333 (21)	1.37 (1.01-1.87)	NS
No	178/1112 (16)	1.00	

At the time of delivery, falciparum malaria during pregnancy and low education remained risk factors for moderate and/or severe anaemia. As the prevalence of falciparum malaria was 26% in women anaemic at delivery, the PAF of anaemia due to *P.falciparum* malaria was 12% (95%CI: 5-17). Multigravidae were more often anaemic at delivery but not severely (**Table 5.6**). UTI during pregnancy was associated with severe anaemia at delivery (**Table 5.7**)

Table 5.6: Risk factors for anaemia (HCT< 30%) at delivery.

Risk factors	**Prevalence N (%)**	**Univariate analysis OR (95%CI)**	**Multivariate analysis OR (95%CI)**
Gravidity: Multigravidae	289/1078 (27)	1.73 (1.27-2.36)	1.75 (1.21-2.52)
Primigravidae	64/366 (18)	1.00	
Ethnic group: Karen	321/1274 (25)	1.45 (0.95-2.22)	NS
Black Karen	32/170 (19)	1.00	
Woman's education: Low	152/526 (29)	1.61 (1.22-2.13)	1.59 (1.19-2.12)
Average-High	130/646 (20)	1.00	
Husband's education: Low	94/317 (30)	1.45 (1.07-1.96)	NS
Aver.-High	185/821 (23)	1.00	
Family's economic level:			
Low	94/330 (29)	1.35 (0.99-1.83)	NS
Average-High	167/732 (23)	1.00	
P.falciparum malaria during pregnancy: Yes	105/319 (33)	1.74 (1.32-2.28)	1.67 (1.19-2.34)
No	248/1125 (22)	1.00	
ARI during pregnancy: Yes	114/405 (28)	1.31 (1.01-1.70)	NS
No	239/1039 (23)	1.00	

Table 5.7: Risk factors for severe anaemia (HCT< 25%) at delivery.

Risk factors	Prevalence N (%)	Univariate analysis OR (95%CI)	Multivariate analysis OR (95%CI)
Woman's education: Low	22/526 (4)	2.30 (1.13-4.70)	NS
Average-High	12/645 (2)	1.00	
Husband's education: Low	16/317 (5)	2.37 (1.19-4.71)	2.37 (1.17-4.80)
Aver.-High	18/820 (2)	1.00	
P.falciparum malaria during pregnancy: Yes	19/319 (6)	2.90 (1.57-5.37)	2.55 (1.25-5.22)
No	24/1123 (2)	1.00	
UTI during pregnancy: Yes	7/103 (7)	2.64 (1.14-6.09)	4.12 (1.58-10.79)
No	36/1339 (3)	1.00	

5.5. Other morbidity during pregnancy

Non-malarious morbid events were recorded only during the ANC period and there were no means to detect episodes that could have occurred before mother's follow-up.

5.5.1. Febrile episodes with negative malaria smear

Nearly half (42%; 620/1495) of the women had at least one febrile episode with a negative malaria smear during ANC. Prevalence rates of ARI and UTI, the two most common diseases, were 28% (414) and 7% (104) respectively. Fifty-two women (3.5%) presented with a febrile non-malaria infection within the week preceding delivery (37 ARI; 5 urinary tract infections UTI; 5 diarrhoeal diseases; 2 self-limited fever; 1 otitis).

Overall, there were 1,037 morbid events in 620 women: 651 ARI; 126 UTI; 115 self-limited undiagnosed fever; 85 diarrhoeal episodes; 28 infected skin diseases or

abscesses; 16 upper respiratory tract infection and 14 other infections. One woman was diagnosed with tuberculosis and one with paragonimus.

5.5.2. Hypertension

Three per cent (38/1495) of the women were treated for hypertension. Three women remained with hypertension during the post-partum period.

5.5.3. Thiamin deficiency

Half of the mothers (54%; 804/1495) had paresthesia and were treated with vitamin B1 for a mean (SD) duration of 11.2 (8.7) weeks. Two women presented with severe dry Beri-Beri and one developed cardiac failure.

5.6. Live newborn

5.6.1. Description

Half (51%; 761/1495) of the babies were born in hospital, including 690 in SMRU's obstetrical department and 71 in Thai hospitals. But most (91%; 1355) were seen within the first 3 days of life; 90% (1346) were weighed and Dubowitz score was performed in 84% (1263). Mean (SD) birthweight was 2872 (490) g; mean (SD) height 48 (3) cm; mean (SD) head circumference 33 (2) cm; and mean (SD) gestational age 38.5 (1.6) weeks.

Eighty per cent (1201) of the newborn were weighed and tested by using Dubowitz score within the first three days of life. Sixteen per cent (190) of them were LBW and 11% (135) were premature. Distribution of birthweight and gestational age is given in **table 5.8**. Nearly all (94%; 43/46) the children weighing less than 2000 g were premature. On contrary, among the infants weighing between 2000 and 2499 g, the majority (68%; 98/144) were born at term and 32% (46/144) were premature. Five per

cent (47/1011) of children with a birthweight of 2500g or more were reported as premature, but all were born after 34 weeks of gestation.

Partial information was also available for another 229 children. Either they had birthweight or Dubowitz score alone, or they had been weighed within the first week of life and a reliable mother's history of last menstruation had been collected together with fundal height at previous ANC consultations. Overall 1430 (96%) were could be categorised. Nine per cent (125) were pre-term LBW (<2500g and <37weeks); 8% (121) were full-term LBW (<2500g and ≥37weeks) and 83% (1184) were regarded as "normal" or appropriate birthweight (NBW) (≥ 2500 g, regardless of gestational age).

Table 5.8: Distribution of birthweight and gestational age in 1201 children seen at birth; n (%).

Birthweight	Gestational age			Total
	<34w	34-36.9w	>=37w	
<2000g	17 (37) (74)	26 (57) (23)	3 (6) (.3)	46 (100) (4)
2000-2499g	6 (4) (26)	40 (28) (35)	98 (68) (9)	144 (100) (12)
2500-2999g	0	37 (8) (33)	433 (92) (41)	470 (100) (39)
3000-3499g	0	10 (2) (9)	408 (98) (38)	418 (100) (35)
>=3500g	0	0	123 (100) (12)	123 (100) (10)
Total	23 (2) (100)	113 (9) (100)	1065 (89) (100)	1201 (100) (100)

5.6.2. Determinants of LBW

Several factors were associated with LBW in univariate analysis (**table 5.8**). Malaria during pregnancy was associated with LBW, but also with anaemia at delivery. Thus anaemia at delivery could be on the pathway of the association between malaria (especially falciparum malaria) and LBW. Similarly, recent arrival and education were previously shown to be associated with falciparum malaria and anaemia during pregnancy. But, as seen previously, this information was biased toward children who survived the first three months of life, and should be used with caution. Multivariate analysis was therefore performed in three steps. A first model was applied, without "anaemia" and "SES factors". A second model contained "maternal anaemia" (as anaemia at delivery or severe anaemia during pregnancy or severe anaemia at delivery) and the third model included SES factors (recent arrival, camp, husband's education). Results were identical in the three models. First gravida, premature birth and malaria during pregnancy were risk factors for LBW (**Table 5.10**). Half (49%) of the women who delivered a LBW infant had falciparum malaria during pregnancy, giving an estimated PAF of LBW due to malaria: 23% (95%CI: 14-30).When the analysis was repeated using "falciparum malaria" and "vivax malaria" separately rather than "malaria", each species were associated with LBW.

Table 5.9. Factors associated with LBW in univariate analysis

	Prevalence LBW N (%)	OR (95%CI)
Camp: Shoklo	88/445 (20)	1.34 (0.95-1.88)
Bonoklo	22/203 (11)	0.66 (0.39-1.11)
Maesalit	9/90 (10)	0.60 (0.27-1.30)
Klaymuta	19/55 (35)	2.87 (1.50-5.43)
Maela	86/553 (16)	1.00
Year of birth: 1993	20/98 (20)	1.35 (0.75-2.44)
1994	68/427 (16)	0.91 (0.63-1.32)
1995	77/447 (17)	1.11 (0.75-1.64)
1996	59/374 (16)	1.00
Gravidity: Primigravidae	90/346 (26)	2.27 (1.66-3.10)
Multigravidae	134/1000 (13)	1.00

Ethnic group: Karen	34/169 (20)	1.31 (0.87-1.97)
Black Karen	190/1177 (16)	1.00
Age: < 20 years	61/250 (24)	1.85 (1.31-2.61)
≥ 20 years	163/1096 (15)	1.00
Trimester of pregnancy at registration to ANC:		
1st trimester	81/641 (14)	1.00
2nd	98/493 (20)	1.50 (1.08-2.08)
3rd	34/210 (16)	1.18 (0.75-1.85)
Infant sex: Male	106/685 (16)	0.84 (0.63-1.12)
Female	118/661 (18)	1.00
Premature infant: Yes	88/135 (65)	17.70 (11.53-27.21)
No	102/1066 (10)	1.00
Malaria during pregnancy: Yes	109/496 (22)	1.80 (1.35-2.41)
No	115/850 (14)	1.00
P.falciparum malaria during pregnancy: Yes	68/293 (23)	1.74 (1.25-2.42)
No	156/1053 (15)	1.00
P.vivax malaria during pregnancy: Yes	65/310 (21)	1.46 (1.05-2.04)
No	159/1036 (15)	1.00
Anaemia (HCT<30%) during pregnancy: Yes	176/1021 (17)	1.20 (0.84-1.71)
No	45/304 (15)	1.00
Severe anaemia (HCT<25%) during pregnancy: Yes	47/221 (21)	1.45 (1.01-2.07)
No	171/1086 (16)	1.00
Anaemia (HCT<30%) at delivery: Yes	67/319 (21)	1.48 (1.06-2.06)
No	151/990 (15)	1.00
Severe anaemia (HCT<25%) at delivery: Yes	14/39 (36)	2.92 (1.49-5.72)
No	204/1268 (16)	1.00
Hypertension during pregnancy: Yes	10/33 (30)	2.23 (0.98-5.00)
No	214/1313 (16)	1.00
ARI during pregnancy: Yes	65/378 (17)	1.06 (0.77-1.45)
No	159/968 (16)	1.00
UTI during pregnancy: Yes	21/93 (23)	1.51 (0.91-2.51)
No	203/1253 (16)	1.00

Time spent in the camp: < 5 years	94/473 (20)	1.82 (1.29-2.57)
≥ 5 years	74/617 (12)	1.00
Woman's education: Low	86/497 (17)	1.47 (0.96-2.27)
Average	44/291 (15)	1.25 (0.76-2.05)
High	38/305 (13)	1.00
Husband's education: Low	56/295 (19)	1.67 (1.09-2.56)
Average	53/318 (17)	1.43 (0.93-2.19)
High	55/448 (12)	1.00
Family's economic level: Low	58/305 (19)	2.05 (0.89-4.90)
Average	82/606 (14)	1.37 (0.61-3.20)
High	8/78 (10)	1.00

Table 5.10. Factors associated with LBW in multivariate analysis

Models	Adjusted OR (95%CI)
First step: without "maternal anaemia" and "SES factors"	
Primigravidae	2.27 (1.53-3.37)
Gestational age (continuous variable)	0.36 (0.31-0.42)
Malaria during pregnancy	1.97 (1.34-2.90)
Second step: with "maternal anaemia" but no "SES factors"	
Primigravidae	2.29 (1.53-3.41)
Gestational age (continuous variable)	0.36 (0.31-0.43)
Malaria during pregnancy	1.94 (1.31-2.87)
Third step: with "maternal anaemia" and "SES factors"	
Primigravidae	2.37 (1.52-3.70)
Gestational age (continuous variable)	0.38 (0.32-0.45)
Malaria during pregnancy	1.67 (1.08-2.58)

5.6.3. Determinants of prematurity

Malaria from *P.falciparum* or *P.vivax* and anaemia during pregnancy did not reduce the duration of gestation. The mean (SD) number of weeks of gestation was 38.5 (1.7) in malaria-infected women and 38.5 (1.4) in women without malaria during pregnancy (p=0.33). The duration of gestation were also similar in anaemic and non-anaemic women: 38.5 (1.6) and 38.5 (1.6) weeks respectively (p=0.40).

Premature labour was more common in primi- (17%; 57/335) and secondi- (13%; 32/238) than in third or higher gravidae (8%; 57/690) [OR (95%CI) primi- vs gravidae 3+: 2.28 (1.51-3.44); OR (95%CI) secondi- vs gravidae 3+: 1.73 (1.06-2.80)]. The risk of delivering prematurely was higher in young mothers (<20 years) than in older ones: 20% (47/236) vs 10% (99/1027) (OR: 2.33; 95%CI: 1.56-3.47), and varies between camp and year. In univariate analysis, *P.falciparum* malaria during pregnancy was associated with a risk of delivering prematurely: 15% (41/267) vs 11% (105/996) in women without falciparum malaria during pregnancy (OR: 1.54; 95%CI: 1.02-2.31). Premature birth was also associated with febrile disease within the week preceding delivery: 28% (22/78) vs 10% (123/1183) in non-febrile women (OR: 3.39; 95%CI: 1.93-5.90). Diagnosis of the 78 febrile diseases in the week preceding delivery were: symptomatic falciparum (23), vivax (17) and mixed (1) malaria, ARI (33) and UTI (4). The risk of premature delivery was present for each condition, probably reflecting an effect of fever. Asymptomatic malaria infection was not associated with premature delivery. A multivariate analysis was done that included the factors identified in univariate analysis and adjusted for the number of ANC consultations. Only febrile episodes preceding delivery (Adjusted OR: 3.00; 95%CI: 1.72-5.25) and mothers under 20 years old (Adjusted OR: 2.19; 95%CI: 1.48-3.26) remained independently associated with the risk of delivering prematurely. Fifteen percent of the women who delivered prematurely had an infection within the previous week. The PAF of premature birth due to infection close to term was: 10% (95%CI: 6-12).

5.6.4. Effects of malaria during pregnancy on anthropometric markers of the newborn.

All anthropometric markers were affected in children born to mothers with malaria, indicating a chronic symetric growth retardation. **(table 5.11)**.

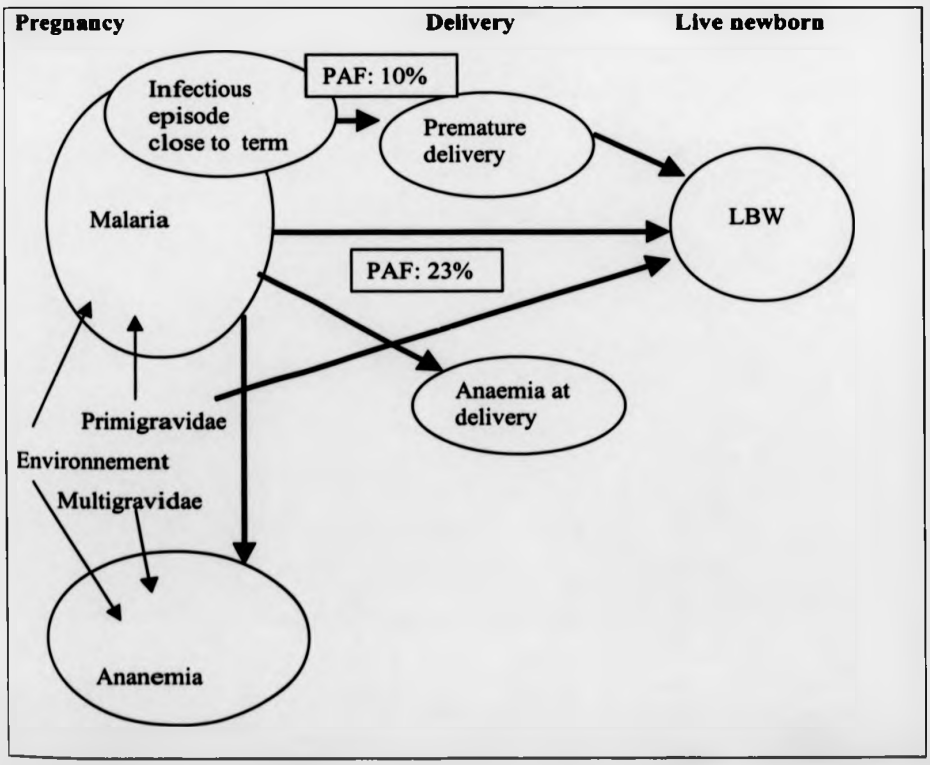
Table 5.11: Anthropometric markers of the newborn according to maternal malaria during pregnancy.

Malaria during pregnancy	Mean (SD) height in cm	p	Mean (SD) head circumference in cm	p	Mean (SD) birthweight in g	p
No malaria	48.4 (2.8)		33.0 (1.6)		2940 (455)	
Falciparum malaria	47.7 (2.3)	0.01	32.6 (2.0)	0.02	2755 (502)	<0.01
Vivax malaria	47.7 (3.3)	<0.01	32.5 (1.9)	<0.01	2813 (515)	<0.01

5.7. Summary of the results contained in this chapter

Figure 5.5 summarises the results contained in this chapter. Both vivax and falciparum malaria during pregnancy reduced birthweight, and the effect was independent of socio-economic factors. Twenty-three per cent of LBW were attributable to malaria during pregnancy. Malaria did not affect the duration of gestation and the reduction of all anthropometric markers was compatible with a prolonged foetal growth retardation. Nevertheless, symptomatic malaria episodes that occurred close to term were risk factors for premature birth, as well as ARI and UTI during that period. Infections close to term were responsible for 10% of premature deliveries. Maternal anaemia did not have an independent effect on birthweight.

Fig 5.5: Effects of malaria and anaemia during pregnancy on live newborn



CHAPTER 6

INFANT MORTALITY

6.1. Follow-up of the infants

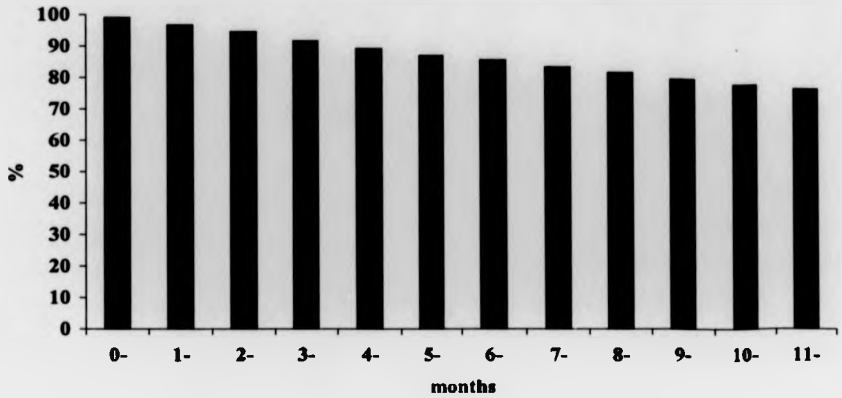
Eighteen months after the beginning of the study, the situation in the Karen Refugee camps gradually deteriorated. During the 1995 dry season, dissident Karen groups supported by the Burmese army attacked the camps in order to destabilize the situation and to pressure the refugees to go back to Burma. Bonoklo was burnt (April 1995) and insecurity increased in Maesalit and Klaymuta. The inhabitants of these 3 camps were relocated to Maela in May and July 1995. Some refugees from Shoklo camp arrived in Maela in January 1996 after an attack, but Shoklo camp was not burnt and most people stayed in the site. Another attack occurred in Maela at the end of January 1997 and the camp was evacuated for one week. As a result, there were important population movements. Overall, 24% (366/1495) of the infants in the cohort were affected by these migrations. Some of them (212; 14%) went back to Burma or were lost during the crisis periods (152 between January and April 1995; 34 in July 1995; 16 in January 1996 and 10 in February 1997). Another 10% (154) of children moved from their birth place to Maela during infancy, but were still followed in the study.

Overall 368 (25%) of the infants were lost to follow-up. More than half of them (58%; 212/368) left during periods of insecurity, the others moved, or followed their mothers working outside the camps; only 1 mother refused to pursue the follow-up after the older brother of the index child died in the Research Unit's hospital. Children were lost regularly over infancy (**fig 6.1**). **Table 6.1** summarises the study compliance in each camp.

Table 6.1: Admission, movements and lost to follow-up in the 5 camps: n (%)

	period of registration	No admitted	No moved to another camp	No lost to follow-up
Shoklo	June 93-December 95	523	31(6)	118 (23)
Bonoklo	January 94- April 95	214	79 (37)	92 (43)
Maesalit	January 94- July 95	101	13(13)	47 (47)
Klaymuta	September 94- July 95	56	30 (54)	25 (45)
Maela	May 95-August 96	601	3 (0.5)	86 (14)
Total	June 93-August 96	1495	154 (10)	368 (25)

Fig 6.1: Proportion of children remaining in the study per age

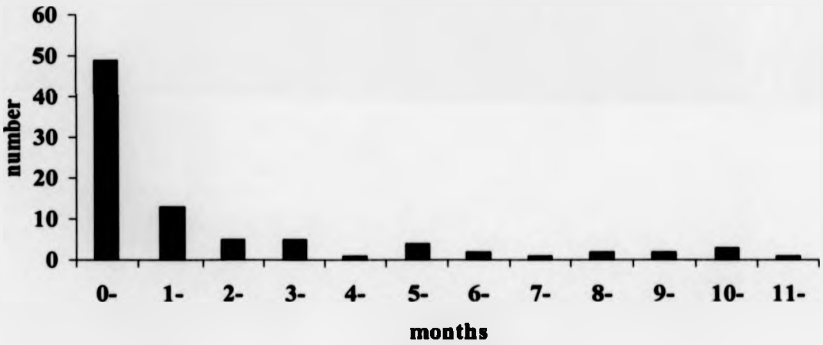


Malaria during pregnancy was similar in mothers whose children were lost and in those who completed follow-up. But pregnant women whose children were lost during follow-up were less likely to be anaemic during pregnancy (73%; 262/360) than those whose children were followed (79%; 872/1108) (p=0.02). Similarly mothers of lost children had less ARI during pregnancy (22%; 82/368) than mothers of children who were followed (30%; 333/1127) (p<0.01). But the former were seen later in pregnancy: 22% (82/368) registered to ANC during the third trimester of pregnancy whereas only 14% (152/1123) did so in the group of successively followed children (p<0.001). Recent refugees (<2 years) were also more often lost than those who were living in Thailand for a longer time: 12% (21/183) vs 6% (63/1022) (p=0.02). In a logistic regression in which all these factors were included, as well as camp and year of study, the only risks to be lost during follow-up were a history of recent arrival to Thailand (Adjusted OR: 2.43; 95%CI: 1.36-4.33) and attendance to ANC late in pregnancy (Adjusted OR: 1.92; 95%CI: 1.14-3.22).

6.2. Crude Infant Mortality

Out of 1,127 children who completed the follow-up, 88 died before 1 year of age, giving a crude infant mortality rate of 78.1 per 1,000 live births. Forty-nine (56%) died during the neonatal period and 39 (44%) during the post-neonatal period. Seventy-six per cent (67) of deaths occurred during the first 3 months of life. **Fig 6.2.** represents the age distribution at the time of death.

fig 6.2. Age distribution of infant deaths



6.3. Causes of infant deaths

Fifty-nine per cent (58/88) of the infants who died were hospitalised or seen at the dispensaries during their final illness. Among children who died at home, 21 mothers reported a history that permitted a classification of the cause of deaths. Thus overall, 90% (79/88) of the deaths could enter in a broad classification. The causes of deaths are listed in **table 6.2**. Among infectious diseases, ARI was the leading cause of deaths (12), followed by diarrhoea (7) and sepsis (7), malaria (2), neonatal tetanus (1) and congenital herpes (1).

Table 6.2: main causes of infant deaths

Causes	n (%)
Infectious diseases	32 (36.4)
Premature	23 (26.1)
Impaired growth	7 (8.0)
Abnormalities	6 (6.8)
Infantile Beri-Beri	6 (6.8)
Complication of difficult labour	3 (3.4)
Others	2 (2.3)
Unknown	9 (10.2)

6.3.1. Neonatal deaths

The majority (63%; 31/49) of the children who died in the neonatal period did not survive the first week of life. The leading cause of death in the neonatal period was "prematurity" (23), but a quarter (12) of the children died from infectious diseases (6 sepsis, 2 diarrhoeal diseases, 1 congenital herpes, 1 tetanus, 1 congenital malaria and 1 ARI). Six infants were grossly abnormal, 3 died from consequences of complicated labour, 1 child presented with severe hemolytic disease, 1 whose mother suffered from hypertensive disease prior to the pregnancy was grossly growth-retarded and died within 24 hours. Causes of deaths were unknown for 3 other children (**fig 6.3**).

6.3.2. Post-neonatal deaths

In the post-neonatal period, half of the children (52%; 20/39) died from infectious diseases. Among them, 11 infants died from ARI, 5 from diarrhoea, 2 from meningitis, 1 from sepsis and 1 from malaria. Six infants died from suspected thiamin deficiency. Five presented with impaired growth from birth and eventually died from undefined cause. One adopted child suffered from lactose intolerance and could not be breast-fed by a wet mother. Another one fell down a well. The causes of death were unknown for the 6 remaining infants (fig 6.4).

Fig 6.3: Causes of neonatal deaths

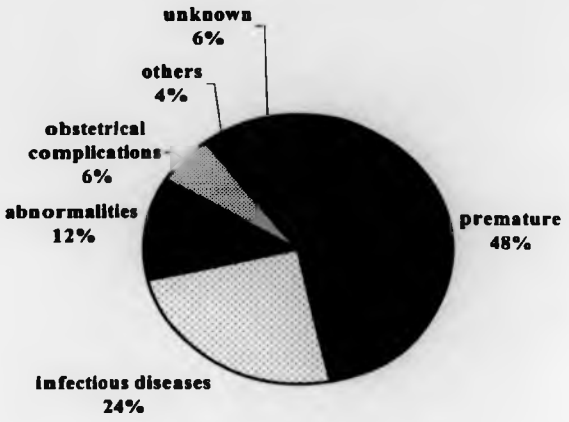
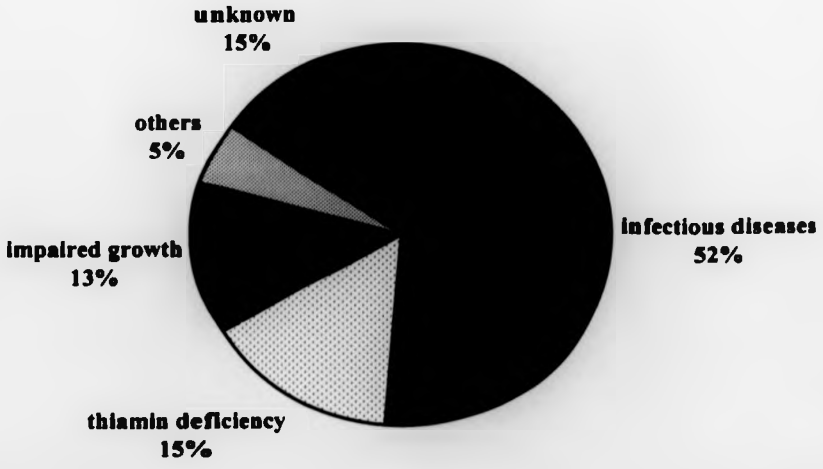


Fig 6.4: Causes of post-neonatal deaths



6.4. Risk factors for infant deaths

6.4.1. Birthweight and gestational age

Analysis was restricted to the 1201 newborn with known birthweight and gestational age, and for each period, to the children who completed follow-up. In the first 3 months of life, premature infants were more likely to die than full-term infants. Out of 1179 babies for whom survival at 1 month of age was known 31 had died (26 per 1,000). The risk of neonatal deaths was 19.1 (95%CI: 9.0-40.5) times higher in premature (164 per 1,000%; 22/134) than in infants born at term (9 per 1,000; 9/1045). Among children who had survived the neonatal period, those premature remained at higher risk of deaths from 1 to 3 months of age (37 per 1,000; 4/107) than term infants (11 per 1,000; 11/979): Relative Risk (RR): 3.3 (95%CI: 1.1-10.3). In the period from 3 to 12 months of age, mortality rates were similar in premature infants who had survived the first 3 months (12 per 1,000; 1/83) and in term children (20 per 1,000; 15/785), but numbers were small (RR=0.6; 95%CI: 0.1-4.7).

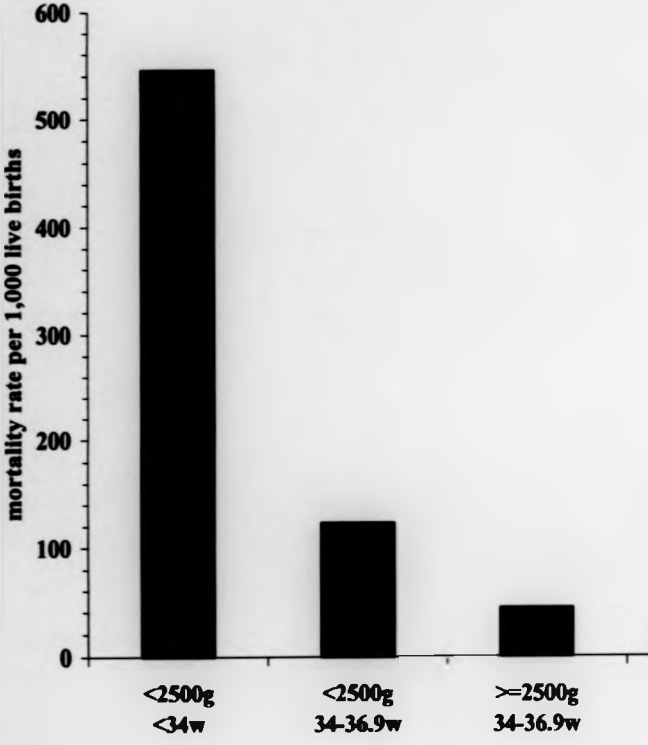
Similarly LBW infants were at higher risk to die in the neonatal period (112 per 1,000; 21/187) than children weighing more than 2500g (10 per 1,000; 10/992) (RR= 11.1; 95%CI: 5.3-23.3). The risk remained at 3.0 (1.0-8.6) from 1 to 3 months of age: mortality 32 (5/156) and 11 (10/930) per 1,000 respectively. From 3 to 12 months of age, mortality was 24 per 1,000 (3/126) in LBW infants and 18 per 1,000 (13/742) in children weighing more than 2500g at birth (RR=1.4; 95%CI: 0.4-4.7).

When looking at both factors, weight in itself seemed a less important risk factor for deaths in infancy than did prematurity. **Fig 6.5** shows neonatal mortality rates in different groups of children classified by their birthweight and gestational age. Within each class of gestational age, mortality rates were similar in children weighing less than 2000g and those weighing 2000 to 2499g. Thus the 2 groups were combined. Similarly, all children weighing more than 2500g within a gestational age class were combined. Children moderately premature (34-36.9 weeks) were more likely to die than full term infants within the same weight group. Among LBW children, mortality rates were 123 per 1000 (8/65) in moderately premature and 10 per 1000 (1/100) in full-term infants: RR=12.3 (95%CI: 1.6-96.1). When newborn were considered to be born with a

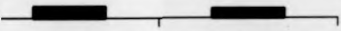
"normal" birthweight (i.e ≥ 2500g), those premature remained more likely to die during the neonatal period than did full-term infants: 43 (2/47) vs 8 per 1,000 (8/945) (RR=5.1; 95%CI: 1.1-23.2). On the contrary, within children born between 34 and 36.9 weeks, LBW infants had a higher (but not significantly so) neonatal mortality than NBW newborn: 123 (8/65) and 43 (2/47) per 1,000 respectively (RR=2.9; 95%CI: 0.6-13.0). Among children born at term, mortality rates were similar in LBW and NBW infants: 10 (1/100) and 8 (8/945) per 1,000 (RR=1.2; 95%CI: 0.2-9.4). In the post-neonatal period, all infants born at term had the same chance of survival, irrespective of their birthweight.

Overall, infant mortality rate was 362 per 1,000 (25/69) in premature LBW infants, 50 per 1,000 (2/40) in premature NBW infants, 48/1000 (4/83) in LBW infants born at term, and 43 per 1,000 (31/722) in NBW infants born at term.

Fig 6.5: neonatal mortality rates according to gestation



al age and birthweight



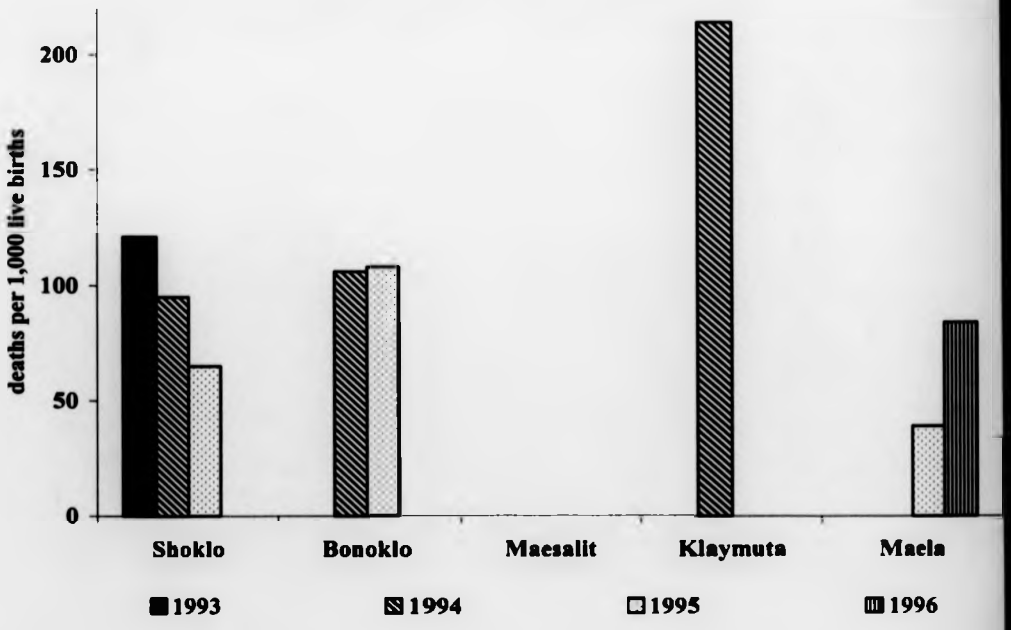
<2500g
>=37w

>=2500g
>=37w

6.4.2. Study site and period

Infant mortality differed from 1 camp to the other and over time (**fig 6.6**). These differences occurred mainly in the post-neonatal period. Neonatal mortality rates did not differ between camps and did not change over time. There were no deaths in Maesalit, or among the children born in Klaymuta in 1995. In Shoklo, the reduction in infant mortality was due to a marked decrease in post-neonatal deaths. From 1993 to 1995, neonatal mortality rates remained stable (36 to 33 per 1,000) whereas post-neonatal deaths decreased from 84 to 23 per 1,000. On the contrary, in Maela, the apparent increase in infant mortality was due to a higher, but not significant, neonatal mortality rate (15 to 48 per 1,000). Post-neonatal mortality rate was stable (23 and 28 per 1,000) in this camp.

fig 6.6: Infant mortality rates per camp and year



6.4.3. Risk factors for neonatal deaths.

Potential risk factors for neonatal deaths are listed in **Table 6.3** in which HR are indicated in order to indicate the direction and the magnitude of the effect. *P.falciparum* malaria during pregnancy was associated with neonatal death, but the number of episodes or the gestational age at first detection of *P.falciparum* did not influence neonatal infant survival. When symptomatic falciparum malaria occurred close to term, the risk of neonatal death was important, but children whose mothers were infected earlier in pregnancy or remained asymptomatic were not more likely to die than those born to non-infected mothers. Similarly vivax malaria was associated with increased risk of neonatal death if it occurred within the week of delivery and was associated with symptoms. The risk of neonatal death was also higher in children whose mothers presented with non-malarious infection within the week prior delivery than in babies born to non-infected mothers. Maternal anaemia at delivery and complicated labour were also identified as potential risk factors of neonatal death in univariate analysis. Confounding factors were mother's age, gravidity, number of ANC consultations and birth in winter. There were no SES factors associated with neonatal deaths, but only 24 (49%) of the mothers those newborn died in the neonatal period, were interviewed.

Table 6.3. Potential risk factors for neonatal deaths; univariate analysis.

	Hazard Ratio (95%CI)
P.falciparum malaria during pregnancy: Yes	**2.07 (1.16-3.70)**
No	1.00
P.falciparum malaria during pregnancy:	
- yes, including 1 symptomatic episode during the week prior delivery	**6.39 (2.25-18.10)**
- at least 1 episode, but not within 1 week prior delivery	1.74 (0.92-3.27)
- no	1.00
Number of *P.falciparum* episodes during pregnancy:¶	1.14 (0.85-1.53)
Gestational age at first *P.falciparum* malaria during pregnancy:	
- 1st trimester	1.00
- 2nd	1.43 (0.43-4.76)
- 3rd	1.34 (0.38-4.75)

P.vivax malaria during pregnancy: Yes	1.21 (0.64-2.29)
No	1.00
P.vivax malaria during pregnancy:	
- yes, including 1 episode during the week prior delivery	**6.88 (2.45-19.32)**
- at least 1 episode, but not within 1 week prior delivery	0.90 (0.43-1.87)
- no	1.00
Number of *P.vivax* episodes during pregnancy:[1]	**1.26 (1.01-1.56)**
Gestational age at first *P.vivax* malaria during pregnancy:	
- 1st trimester	1.00
- 2nd	0.65 (0.16-2.60)
- 3rd	0.87 (0.23-3.25)
Non-malarious infection (ARI or UTI) within 1 week prior delivery: Yes	3.00 (0.93-9.73)*
No	1.00
Infection (malaria or ARI or UTI) within 1 week prior delivery: Yes	**5.30 (2.71-10.38)**
No	1.00
Infection within 1 week prior delivery:	
- Falciparum malaria (or mixed)	2.09 (0.47-9.36)
- Vivax malaria	3.01 (0.67-13.44)
- ARI or UTI	1.00
Maternal anaemia during pregnancy: Yes	1.18 (0.33-4.18)
No	1.00
Severe maternal anaemia during pregnancy: Yes	0.75 (0.17-3.31)
No	1.00
Maternal anaemia at delivery: Yes	**1.81 (1.01-3.24)**
No	1.00
Severe maternal anaemia at delivery: Yes	2.33 (0.31-17.72)
No	1.00
Duration of anaemia during pregnancy: none	1.00
< 6 weeks	0.78 (0.35-1.74)
≥ 6 weeks	1.28 (0.63-2.60)
ARI during pregnancy (except last week): Yes	1.50 (0.83-2.71)
No	1.00
Hypertension during pregnancy: Yes	0.80 (0.11-5.79)
No	1.00

Complicated delivery: Yes		**2.63 (1.34-5.14)**
No		1.00
Mother's age: < 20 years		**2.19 (1.21-3.99)**
≥ 20 years		1.00
Gravidity: Primigravidae		**2.04 (1.15-3.60)**
Multigravidae		1.00
Number of ANC consultations:¶		**0.96 (0.93-0.99)**
Ethnic group: Karen		3.16 (0.77-13.03)
Black Karen		1.00
Infant's sex: Male		1.37 (0.78-2.43)
Female		1.00
Birth in winter: Yes		**1.91 (1.08-3.39)**
No		1.00
Recent arrival (< 2years) in Thailand:	-Yes	3.34 (0.37-29.85)
	- No	1.00
Composite score of mother's education:	- low	1.23 (0.11-13.58)
	- average	2.03 (0.18-22.39)
	- high	1.00
Composite score of father's education:	- low	0.51 (0.05-4.89)
	- average	0.47 (0.05-4.55)
	- high	1.00

Bold: $p<0.05$
*: $p \geq 0.05$ and <0.10
¶: continuous variable: HR per unit.

Multivariate analysis was performed in three steps. The first model included the factors identified in univariate analysis and the determinants of LBW and prematurity (**Table 7.4**), but not anaemia at delivery, LBW and prematurity. When some factors were closely related, they were entered in the model separately and only one was retained. A similar method was employed when one factor was included in another one. (e.g. the variable "*P.falciparum* overall" was not retained, nor "*P.falciparum* classification separating the episode within last week of pregnancy", because

information was contained in "infection last week"). In the second model, "anaemia at delivery", that could be on the causal pathway between malaria and neonatal death, was included. LBW and prematurity, combined in four categories, were added in the third step. The children were classified as preterm-LBW, pretem-NBW, term-LBW and term-NBW.

Table 6.4: Potential risk factors and confounders included in multivariate analysis

Factors identified in univariate analysis - *P.falciparum* malaria during pregnancy - *P.falciparum* malaria during pregnancy (last week separated) - *P.vivax* malaria during pregnancy (last week separated) - Number of *P.vivax* episodes during pregnancy - Infection (malaria or ARI or UTI) within 1 week prior delivery - Maternal anaemia at delivery - Complicated delivery - Mother's age - Gravidity - Number of ANC consultations - Birth in winter
Determinants of LBW - *P.falciparum* malaria during pregnancy - *P.vivax* malaria during pregnancy - Gravidity **Determinant of prematurity** - Mother's age - Infection (malaria or ARI or UTI) within 1 week prior delivery

In the multivariate analysis, malaria during pregnancy was not associated with neonatal death. Febrile episode (due to malaria, ARI or UTI) within the week prior delivery was the most important risk factor for neonatal death and remained in the model when premature birth and LBW were accounted for (**table 6.5**). Thus the effect of malaria on neonatal mortality was all mediated through LBW, whereas fever in the week prior birth had additional independent effect as well as inducing premature birth.

Table 6.5: Risk factors for neonatal deaths; multivariate analysis

Risk factor	Adjusted HR (95%CI)	PAF (95%CI)
Model not including maternal anaemia at delivery, LBW and prematurity		
Mother under 20 years old	1.98 (1.08-3.61)	16 % (2-24)
Complicated delivery	2.25 (1.14-4.44)	12 % (3-17)
Infection within the week prior delivery	4.82 (2.46-9.48)	18 % (13-20)
Model including maternal anaemia at delivery, but not LBW and prematurity		
Mother under 20 years old	1.94 (1.06-3.54)	16% (2-23)
Complicated delivery	2.25 (1.14-4.45)	12% (3-17)
Infection within the week prior delivery	4.01 (2.00-8.05)	17% (11-20)
Number of ANC consultations (continuous)	0.97 (0.94-1.00)	NA
Model including maternal anaemia at delivery, LBW and prematurity		
Infection within the week prior delivery	4.27 (1.98-9.24)	17% (11-20)
Premature birth	9.79 (3.80-25.22)	65% (53-69)
LBW	2.91 (1.15-7.33)	46% (9-60)

6.4.4. Risk factors for deaths from 1 to 3 months of age.

The number of deaths occurring from 1 to 3 months of age was lower than in the neonatal period. Thus analysis that compared various exposure levels (such as gestational age at first malaria episode divided in 3 groups corresponding to trimester) could not be done. Numbers in each group would have been too small. Infection within the week prior delivery was the only risk factor for infant death from 1 to 3 months of age identified in univariate analysis, and in a multivariate model including LBW, prematurity and their determinants (i.e. malaria during pregnancy, mother's age and gravidity) (AHR: 3.98; 95%CI: 1.15-13.74). PAF for this factor was 13% (95%CI: 2-15).

6.4.5. Risk factors for deaths from 3 to 12 months of age.

In univariate analysis, malaria and anaemia during pregnancy were not associated with infant deaths from 3 to 12 months of age. LBW and prematurity did not influence late infant survival either. Infant who were anaemic (HCT<28%) at 3 months of age were more likely to die later in infancy than non-anaemic children. Recent arrival (<2 years) in Thailand was also a risk factor of dying between 3 to 12 months of age (**Table 6.6**). As LBW was associated with infant anaemia (see chapter 8.4.2), and that infant anaemia might be on the causal pathway of the effect of LBW, two models of multivariate analysis were used. One model included "recent arrival", "infant anaemia", "camp" and "year". The other model also contained "LBW". None of these factors remained independently associated with late infant deaths in multivariate analysis.

Table 6.6: Potential risk factors for infant deaths from 3 to 12 months of age; univariate analysis.

	Hazard Ratio (95%CI)
P.falciparum malaria during pregnancy: Yes	0.91 (0.31-2.72)
No	1.00
P.vivax malaria during pregnancy: Yes	1.35 (0.53-3.49)
No	1.00
Infection (malaria or ARI or UTI) within 1 week prior delivery:	
Yes	1.11 (0.15-8.26)
No	1.00
Maternal anaemia during pregnancy: Yes	2.54 (0.59-10.94)
No	1.00
Maternal anaemia at delivery: Yes	0.84 (0.28-2.52)
No	1.00
Mother's age: < 20 years	0.76 (0.23-2.59)
≥ 20 years	1.00
Gravidity: Primigravidae	0.96 (0.35-2.61)
Multigravidae	1.00
Ethnic group: Karen	1.28 (0.30-5.50)
Black Karen	1.00
Infant's sex: Male	1.03 (0.44-2.43)
Female	1.00
Premature infant: Yes	0.56 (0.07-4.21)
No	1.00
LBW: Yes	1.29 (0.37-4.49)
No	1.00
Infant anaemia (HCT<28%) at 3 months of age: Yes	**5.62 (1.59-19.93)**
No	1.00
Recent arrival (< 2years) in Thailand: -Yes	**4.11 (1.15-14.72)**
- No	1.00
Composite score of mother's education: - low/ average	5.00 (0.65-38.25)
- high	1.00

Composite score of father's education:	- low/ average - high	1.32 (0.44-3.92) 1.00
composite score of economic level:	- low/ average - high	0.93 (0.12-7.19) 1.00

6.5. Deaths from birth to three months of age in children born to malaria-infected mothers

In the cohort, 4 neonatal deaths (at 1, 2, 6 and 12 days of age) occurred in premature newborns whose mothers suffered from symptomatic malaria (2 *P.falciparum* and 2 *P.vivax*) during the week preceding delivery. One child, born at term, but who weighed less than 2500g died at day 14 (his mother suffered from vivax malaria near delivery). Three other children, born at term and weighing more than 2500g, but whose mothers had symptomatic malaria within the last week of pregnancy died within the neonatal period: 1 from congenital malaria (8 days) and 1 from sepsis (15 days) (both born to a mother infected with *P.falciparum*). The third child was abnormal and thus the direct relationship with maternal malaria is unlikely. Two children whose mothers had falciparum malaria within the week preceding delivery, died between 1 and 3 months. One was born prematurely and never put on weight (1840g at birth; 1850g at 1 month age; he died at 58 days of age from a final episode of diarrhoea). The second infant was born at term and died at 84 days of life after a 2-3 days history of fever and jaundice. Thus 13% (9/67) of the deaths in the first three months of age may have been the direct consequence of symptomatic malaria infection in the third trimester of pregnancy. Among the other neonatal deaths, there were 13 LBW newborn whose mothers had suffered from falciparum or vivax malaria during pregnancy. Although this is probably a gross over-estimation, these children may have died from the effect of malaria during pregnancy. Assuming a causal effect of malaria on neonatal mortality through LBW, 27% (13/49) of the neonatal deaths could be attributed to malaria during pregnancy. Overall, malaria could be responsible for 41% (20/49) of the neonatal deaths and of 11% (2/18) of the deaths that occurred between one and three months of life.

6.6. Summary of the results contained in this chapter

Infectious diseases (mainly ARI, diarrhoea and sepsis) and prematurity were the leading causes of infant deaths. Maternal infection within the week prior delivery (PAF: 17%), premature birth (PAF: 65%) and LBW (PAF: 46%) were identified as risk factors for neonatal deaths. These three factors accounted for 80% (39/49) of the neonatal deaths in the cohort. The effect of malaria during pregnancy was mediated through LBW, whereas maternal infection in the week prior birth had additional independent effect as well as inducing premature birth. Thirteen per cent of the deaths that occurred between one and three months of age were attributable to maternal infection within the week prior birth. There were no independent exposure factors association with infant deaths that occurred between three and twelve months of age. Maternal anaemia was not associated with infant death, at any period of infancy.

CHAPTER 7

INFANT MORBIDITY

7.1. Morbidity from all causes

7.1.1. Description

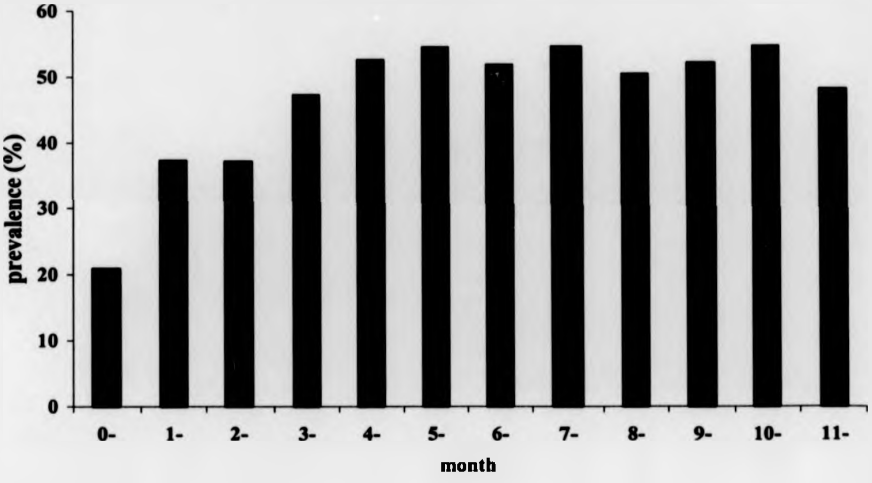
During their first year of life, the infants presented to the dispensary or hospital on average 5.5 times (6776 consultations / 1228 child-year) (95%CI: 5.3-5.6). The main diagnoses are summarized in **table 7.1**. Some children presented with two diagnoses at the same consultation (such as ARI and diarrhoea), but only the most important cause (complaints from the mother or decision of the medic) was used for this classification. Malaria was always counted as the main diagnosis. More than half of the consultations (53%; 3580/6776) were due to respiratory infections (53% mild viral upper respiratory tract infections and 47% moderate or severe ARI). The second leading cause of consultation was diarrhoea. Malaria represented only 3% of the consultations.

Table 7.1: Main diagnoses in children seen at the health structures

Diagnoses	N	%
Mild ARI (common cold)	1896	28.0
ARI (bronchitis, pneumonia...)	1684	24.9
Diarrhoeal episodes	1286	19.0
Eyes Nose Throat (ENT)	511	7.5
Self-limited undiagnosed fever	451	6.7
Malaria all species	205	3.0
Skin diseases (mainly scabies, impetigo...)	139	2.1
Suspicion of thiamin deficiency	82	1.2
Chicken pox	24	0.4
Measles	21	0.3
Others	454	6.7
Unknown	23	0.3

The prevalence of morbid events increased with age in the first 3 months of life and then remained in plateau **(fig 7.1)**. The seasonal and annual variations were more difficult to assess. There were not enough children in each camp at each time point to be able to analyze monthly incidence by camp.

fig 7.1: Age-specific prevalence of dispensary attendance. Morbidity from all causes



Hospitalization was required in 3% (174/6776) of the morbid episodes. Diagnoses of severe events included 54 ARI, 47 diarrhoea, 19 falciparum malaria, 17 suspicion of thiamin deficiency, 7 meningitis, 6 sepsis, 4 undiagnosed fever, 4 abscesses, 2 measles, 2 malnutrition, 1 severe anaemia, 1 vivax malaria and 9 other conditions.

7.1.2. Risk factors for morbidity from all causes during infancy

Poisson regression was used to identify morbid events during pregnancy that could be related to morbidity from all causes during infancy. Potential risk factors and confounders identified in univariate analysis are summarized in **table 7.2.** Falciparum malaria during pregnancy was associated with infant morbidity, only when the first episode occurred during the first trimester. Maternal anaemia was also associated with infant morbidity, and the association was stronger when the duration of anaemia increased. ARI and numbness during pregnancy were also associated with infant morbidity.

Table 7.2. Potential risk factors for morbidity from all causes during infancy (univariate analysis).

Factors	OPD consultations during infancy (0-12 months)	
	Incidence Rate (episodes/child-year)	IRR (95%CI)
Maternal and birth characteristics		
Mother age: <20	5.8 (1267/220)	1.05 (0.99-1.12)
≥ 20 years	5.5 (5509/1007)	1.00
Mother primigravidae	5.4 (1640/304)	0.97 (0.92-1.03)
Multigravidae	5.6 (5136/924)	1.00
Attendance to ANC in		
1st trimester of pregnancy	5.6 (3371/598)	1.00
2nd	5.5 (2469/452)	0.97 (0.92-1.02)
3rd	5.3 (912/173)	0.93 (0.87-1.01)*
Birth order: first born	5.5 (1869/339)	1.00
Second	5.3 (1319/249)	0.96 (0.90-1.03)
Third	4.9 (998/205)	**0.88 (0.82-0.96)**
fourth or above	6.0 (2586/433)	**1.08 (1.02-1.15)**
Ethnic group: Karen	5.5 (5954/1081)	1.00
Black Karen	5.6 (822/146)	1.02 (0.95-1.10)
Sex: male	5.7 (3599/633)	**1.06 (1.01-1.12)**
Female	5.3 (3177/594)	1.00

Complicated delivery: Yes	6.1 (756/124)	**1.12 (1.03-1.21)**
No	5.5 (6020/1103)	1.00
Weight/ gestational age:		
preterm-LBW	4.8 (372/78)	**0.86 (0.77-0.95)**
term-LBW	6.0 (583/98)	1.08 (0.99-1.17)*
NBW	5.6 (5519/993)	1.00
Season of birth: winter	5.6 (1617/288)	1.00
dry season	5.3 (1550/294)	0.94 (0.88-1.01)*
rainy season	5.6 (2660/475)	1.00 (0.94-1.06)
post-rainy season	5.6 (949/170)	1.00 (0.92-1.08)
Malaria during pregnancy		
Malaria during pregnancy: Yes (any species)	5.6 (2457/437)	1.03 (0.98-1.08)
No	5.5 (4319/791)	1.00
Detection of 1st or only malaria infection during:		
1st trimester of pregnancy	6.1 (855/140)	**1.12 (1.04-1.21)**
2nd	5.4 (961/177)	0.99 (0.92-1.07)
3rd	5.3 (635/119)	0.97 (0.90-1.06)
absence of malaria	5.5 (4319/791)	1.00
Malaria species during pregnancy		
absence of malaria	5.5 (4319/791)	1.00
only *P.falciparum*	5.5 (861/157)	1.00 (0.93-1.08)*
only *P.vivax*	5.6 (1014/182)	1.02 (0.95-1.09)
both species	6.0 (582/97)	**1.09 (1.00-1.19)**
P.falciparum malaria during pregnancy: Yes	5.7 (1443/255)	1.03 (0.97-1.09)
No	5.5 (5333/973)	1.00
Detection of 1st or only *P.falciparum* malaria during:		
1st trimester of pregnancy	6.6 (509/77)	**1.20 (1.10-1.31)**
2nd	5.5 (554/101)	1.00 (0.92-1.09)
3rd	5.0 (380/76)	0.91 (0.82-1.01)*
no falciparum malaria	5.5 (5333/973)	1.00
Number of *P.falciparum* malaria episodes during pregnancy:		
none	5.5 (5333/973)	1.00
one	5.8 (920/160)	1.05 (0.98-1.13)
two or more	5.5 (523/95)	1.0 (0.92-1.10)

P.vivax malaria during pregnancy:		
Yes	5.7 (1596/280)	1.04 (0.99-1.10)
No	5.5 (5180/948)	1.00
Detection of 1st or only *P.vivax* malaria during:		
1st trimester of pregnancy	5.5 (385/70)	1.00 (0.91-1.11)
2nd	5.6 (623/111)	1.03 (0.95-1.12)
3rd	5.9 (582/98)	1.09 (1.00-1.19)*
no vivax malaria	5.5 (5180/948)	1.00
Number of *P.vivax* malaria episodes during pregnancy:		
none	5.5 (5180/948)	1.00
one	5.6 (991/177)	1.02 (0.96-1.10)
two or more	5.9 (605/102)	1.08 (0.99-1.18)*
Anaemia during pregnancy		
Anaemia at any time: Yes	5.6 (5267/938)	**1.09 (1.03-1.16)**
No	5.2 (1376/267)	1.00
Anaemia at delivery: Yes	5.4 (1534/286)	0.97 (0.91-1.02)
No	5.6 (5001/900)	1.00
Lengh of anaemia during pregnancy		
None	5.1 (1415/275)	1.00
< 6 weeks	5.5 (2312/419)	**1.07 (1.00-1.15)**
≥ 6 weeks	5.7 (2916/512)	**1.11 (1.04-1.18)**
Non-malarious maternal morbidity		
Hypertension during pregnancy: Yes	6.2 (192/31)	1.13 (0.97-1.30)
No	5.5 (6584/1197)	1.00
ARI during pregnancy: Yes	6.5 (2243/345)	**1.27 (1.20-1.33)**
No	5.1 (4533/882)	1.00
UTI during pregnancy: Yes	5.5 (455/83)	0.99 (0.91-1.09)
No	5.5 (6321/1145)	1.00
Numbness during pregnancy: Yes	5.8 (3821/661)	**1.11 (1.06-1.16)**
No	5.2 (2942/564)	1.00

Socio-economic markers:		
Mother's age at arrival in Thailand:		
< 15 years	5.0 (1371/273)	1.00
≥ 15 years	6.0 (4859/813)	**1.19 (1.12-1.26)**
Time elapsed since mother's arrival in Thailand: < 2 years	6.6 (455/69)	**1.28 (1.15-1.42)**
2-5 years	6.0 (2300/384)	**1.16 (1.08-1.24)**
5-10 years	5.7 (2100/366)	**1.11 (1.04-1.19)**
> 10 years	5.2 (1375/267)	1.00
Composite score of mother's education: low	6.1 (2966/487)	**1.18 (1.11-1.25)**
average	5.7 (1714/299)	**1.11 (1.04-1.19)**
high	5.2 (1564/303)	1.00
Composite score of father's education: low	6.4 (1875/293)	**1.20 (1.13-1.28)**
average	5.8 (1820/316)	**1.08 (1.02-1.15)**
high	5.3 (2382/447)	1.00
Composite score of economic level low	6.6 (1976/300)	**1.60 (1.42-1.80)**
average	5.7 (3469/610)	**1.38 (1.23-1.55)**
high	4.1 (315/77)	1.00
Site of birth: Shoklo	4.8 (2094/434)	1.00
Bonoklo	3.4 (525/154)	**0.71 (0.64-0.78)**
Maesalit	5.1 (411/80)	1.06 (0.95-1.18)
Klaymuta	3.8 (137/36)	**0.79 (0.66-0.94)**
Maela	6.9 (3609/523)	**1.43 (1.36-1.51)**
Year of birth: 1993	6.6 (638/97)	1.00
1994	4.0 (1559/390)	**0.61 (0.56-0.67)**
1995	6.2 (2455/397)	0.94 (0.86-1.03)
1996	6.2 (2124/343)	0.95 (0.87-1.03)

bold: $p<0.05$
*: $p\geq0.05$ and $p<0.10$

In multivariate analysis, falciparum malaria detected in the first trimester of pregnancy, ARI and numbness during pregnancy remained associated with infant morbidity. Male and full-term LBW infants were also associated with higher morbidity, whereas premature LBW children presented with less morbid events. Morbidity decreased in the third child of a family and increased again for higher birth order. Low father's education, low or average economic level and mother recently arrived in Thailand (as she was adult) were also associated with morbidity during the first year of life (**Table 7.3**).

Table 7.3: Risk factors for morbidity from all causes during infancy; multivariate analysis.

	Adjusted IRR (95% CI)
Detection of 1st or only *P.falciparum* during the first trimester of pregnancy	1.14 (1.03 - 1.26)
ARI during pregnancy	1.14 (1.07 - 1.21)
Numbness during pregnancy	1.06 (1.00 - 1.13)
Third child	0.88 (0.80 - 0.96)
Male gender	1.06 (1.00 - 1.12)
Term LBW	1.09 (1.00 – 1.20)
Premature LBW	0.87 (0.77 – 0.99)
Mother arriving in Thailand in adulthood (>15 years old)	1.16 (1.08 - 1.25)
Low father's education:	1.09 (1.02 - 1.17)
Low economical level:	1.31 (1.15 - 1.49)
Average economical level	1.21 (1.07 - 1.37)

7.2. Malaria during infancy

7.2.1. Morbidity and mortality from malaria.

There were 205 malaria cases in 132 infants. The majority (70%; 143) of the malaria cases were due to *P.vivax*, 26% (53) to *P.falciparum* and 4% (9) were mixed infections with *P.vivax* and *P.falciparum*. Fifty-five *P.vivax* malaria cases were possibly relapses and 2 falciparum malaria episodes were considered as treatment failures. Thus only 148 cases were "new" infections (88 *P.vivax*, 51 *P.falciparum* and 9 mixed), giving a malaria incidence rate during infancy of 121 (148/1228) per 1,000 child-year (95%CI: 103-142). Falciparum malaria incidence rate was 49 per 1,000 child-year (51 *P.falciparum* plus 9 mixed infections/1228 child-years) (95%CI: 38-63). Vivax malaria incidence rate was 76 per 1,000 child-year (88 *P.vivax* plus 5 mixed infections in which *P.vivax* was encountered for the first time/1228 child-years) (95%CI: 62-93).

Out of the 132 children who developed malaria during infancy, 74 (56%) had only vivax malaria during infancy, and 22 of them had subsequent relapses during the follow-up (1 to 5 times) (total 110 vivax malaria cases). Thirty-nine infants (29% of those with malaria) suffered from *P.falciparum* malaria only during infancy. Four infants (3%) had uncomplicated mixed infections, 2 of them followed by vivax relapses. The remaining 15 children (12%) had alternatively *P.vivax* and *P.falciparum* during infancy. *P.vivax* was the first infecting species in 12 infants, *P.falciparum* in 2 and the remaining child had 1 mixed infection followed by 1 falciparum malaria infection.

Out of the 143 pure vivax malaria cases, 1 required hospitalization for severe anaemia (HCT=15%) but the child was already anaemic (HCT=25%) at his previous routine visit three weeks before.

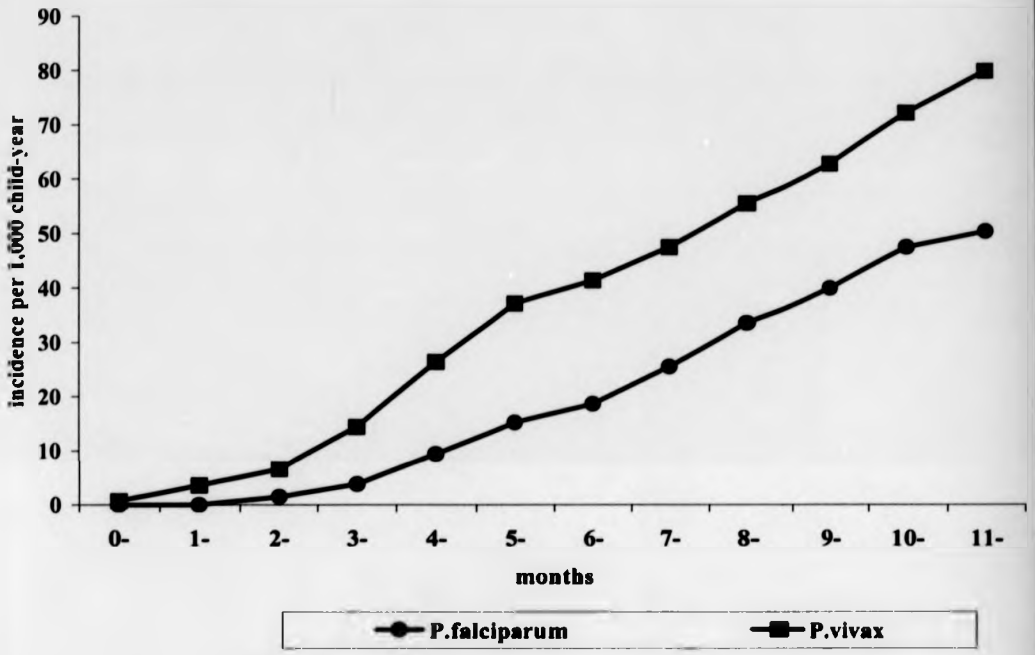
The risk of developing severe malaria was 31% (19/62 cases of *P.falciparum* malaria alone or mixed with *P.vivax*) (95%CI: 19-42%) and the CFR was 32 per 1,000 (2/62) (95%CI: 9-112). Thus malaria-attributable mortality was 1.6 (2/1228) per 1,000 child-year (95%CI: 0.4-6.5). One child developed severe falciparum malaria at 8 days of life and died. The mother was infected with falciparum malaria at delivery and the child was likely to have congenital malaria. The second child died at 8 months. He

presented to the hospital with acute severe anaemia (HCT= 3%). He had been living in the forest with the mother during the 2 previous weeks and was febrile and unwell for 3 days when he arrived at the hospital. Twelve of the 17 other patients with severe malaria were hospitalized in the SMRU structures where clinical and laboratory features were recorded. All but one had hyperparasitaemia (median (range): 6.4 (4.0-20.4) % infected red blood cells), two of them combined with severe anaemia (HCT= 13 and 17.5%). One infant presented with severe malaria-attributable anaemia (no HCT available, but clinical signs of cardiac failure). None of the patients with severe malaria were comatose. The risk of developing severe malaria was similar in children for whom it was the first malaria episode (34%; 14/41), in those with mixed infection first (20%; 1/5) and in those who had vivax malaria prior their falciparum infection (33%; 4/12) (p=0.89).

7.2.2. Acquisition of malaria during infancy

Children with congenital malaria (N=3; 1 severe case and 2 asymptomatic infections) were excluded from this analysis. Malaria was rare in the first three months of life and then increased regularly. Overall the incidence of malaria from 3 to 12 months of age was 13 per 1,000 child-month (137/10,529) whereas it was 3 per 1,000 child-month (10/4,163) in the first 3 months of life (IRR: 4.9; 95%CI: 2.7-9.1). Vivax malaria was acquired earlier in life than *P.falciparum*. Cumulative incidence rates for each species are represented in **fig 7.2**. The median age (range) at first *P.vivax* malaria was 6.3 (0.9-11.9) months and that of first *P.falciparum* malaria was 7.9 (2.3-11.5) months, but this was not significant (p=0.29). As reported previously, most (80%; 12/15) of the children who had both species during infancy first developed *P.vivax* malaria. Malaria during pregnancy did not influence the age of the first malaria attack during infancy. Children born to mothers with malaria during pregnancy acquired their first malaria at a median (range) age of 6.8 (1.5-11.6) months and those whose mothers had no malaria during pregnancy developed their first malaria attack at 7.0 (0.9-11.9) months of age (p=0.87). There were also no differences in the age at first malaria during infancy, when the timing or the species of malaria during pregnancy were taken in account.

Fig 7.2: Cumulative malaria incidence rates in the first year of life



7.2.3. Relationships between malaria during pregnancy and malaria during infancy

In univariate analysis (**table 7.4**), falciparum malaria during pregnancy was weakly associated with increased malaria during infancy. But the stage at which the first *P.falciparum* malaria occurred during pregnancy seemed essential in this association. Only the infections detected in the first trimester of pregnancy were associated with increased malaria in infancy. Children whose mothers had a last or only falciparum malaria at the end of their pregnancy (within the last 2 months) were not protected against malaria in infancy. Prolonged maternal anaemia was also associated with increased malaria in infancy. Surprisingly children born to mothers who had non-malarious morbidity during pregnancy had less malaria in infancy than infants whose mothers did not present with numbness, ARI or UTI. Karen infants were more likely to

acquire malaria during infancy than Black Karen. Camp and year of study were important confounding factors. Overall, malaria morbidity decreased over time. In Maela, transmission was reduced and malaria incidence in this camp was much lower than in the other sites.

In multivariate analysis, a first episode of *P.falciparum* malaria during the first trimester of pregnancy remained strongly associated with malaria in infancy. Karen ethnicity and low father's education were other independent risk factors for malaria in infancy (**Table 7.5**).

Table 7.4. Potential risk factors for malaria (all species) during infancy (univariate analysis).

	Malaria incidence rate during infancy (per 1,000 child-year)	
	Incidence rate (episodes/child-year)	Rate ratio (95%CI)
Maternal and birth characteristics		
Mother age: <20	105 (23/220)	0.85 (0.54 -1.32)
≥ 20 years	123 (124/1007)	1.00
Mother primigravidae	99 (30/304)	0.78 (0.52-1.16)
Multigravidae	127 (117/924)	1.00
Attendance to ANC in		
1st trimester of pregnancy	110 (66/598)	1.00
2nd	124 (56/452)	1.12 (0.79-1.60)
3rd	144 (25/173)	1.31 (0.83-2.07)
Birth order: First born	115 (39/339)	1.00
Second	125 (31/249)	1.08 (0.68-1.73)
Third	146 (30/205)	1.27 (0.79-2.05)
Fourth or above	109 (47/433)	0.94 (0.62-1.44)
Ethnic group: Karen	120 (141/1081)	**3.17 (1.40-7.18)**
Black Karen	41 (6/146)	1.00
Sex: Male	122 (77/633)	1.03 (0.75-1.43)
Female	118 (70/594)	1.00
weight/ gestational age: preterm-LBW	64 (5/78)	0.53 (0.22-1.30)
term-LBW	113 (11/98)	0.93 (0.50-1.72)
normal BW	121 (120/993)	1.00

season of birth: winter	121 (35/288)	1.00
dry season	102 (30/294)	0.84 (0.52-1.37)
rainy season	128 (61/475)	1.04 (0.63-1.71)
post-rainy season	123 (21/170)	1.02 (0.59-1.75)
Malaria during pregnancy		
Malaria species during pregnancy		
absence of malaria	106 (84/791)	1.00
only *P.falciparum*	172 (27/157)	**1.62 (1.05-2.50)**
only *P.vivax*	126 (23/182)	1.19 (0.75-1.89)
both species	133 (13/97)	1.26 (0.70-2.25)
P.falciparum malaria during pregnancy:		
Yes	157 (40/255)	1.43 (0.99-2.05)*
No	110 (107/973)	1.00
Detection of 1st or only *P.falciparum* malaria during:		
1st trimester of pregnancy	208 (16/77)	**1.89 (1.12-3.20)**
2nd	119 (12/101)	1.08 (0.59-1.96)
3rd	158 (12/76)	1.43 (0.79-2.60)
no falciparum malaria	110 (107/973)	1.00
Last or only *P.falciparum* malaria during the last 2 months of pregnancy: Yes	167 (12/72)	1.43 (0.72-2.58)
No	117 (135/1156)	1.00
Number of *P.falciparum* malaria episodes during pregnancy: none	110 (107/973)	1.00
one	150 (24/160)	1.37 (0.88-2.13)
two or more	168 (16/95)	1.53 (0.91-2.59)
P.vivax malaria during pregnancy:		
Yes	129 (36/280)	1.10 (0.75-1.60)
No	117 (111/948)	1.00
Detection of 1st or only *P.vivax* malaria during: 1st trimester of pregnancy	43 (3/70)	0.37 (0.12-1.15)*
2nd	135 (15/111)	1.15 (0.67-1.98)
3rd	184 (18/98)	1.57 (0.95-2.58)
no vivax malaria	117 (111/948)	1.00
Number of *P.vivax* malaria episodes during pregnancy: none	117 (111/948)	1.00
one	141 (25/177)	1.21 (0.78-1.86)
two or more	108 (11/102)	0.92 (0.50-1.71)

Anaemia during pregnancy		
Anaemia at any time Yes	131 (123/938)	1.52 (0.98-2.38)*
No	86 (23/267)	1.00
Anaemia at delivery: Yes	143 (41/286)	1.29 (0.90-1.86)
No	111 (100/900)	1.00
Lengh of anaemia during pregnancy		
none	84 (23/275)	1.00
< 6 weeks	124 (52/419)	1.48 (0.91-2.42)
≥ 6 weeks	139 (71/512)	**1.66 (1.04-2.65)**
Non-malarious maternal morbidity		
Hypertension during pregnancy: Yes	97 (3/31)	0.80 (0.26-2.52)
No	120 (144/1197)	1.00
ARI during pregnancy: Yes	93 (32/345)	0.71 (0.48-1.05)*
No	130 (115/882)	1.00
UTI during pregnancy: Yes	48 (4/83)	0.39 (0.14-1.04)*
No	125 (143/1145)	1.00
Numbness during pregnancy: Yes	98 (65/661)	**0.68 (0.49-0.95)**
No	144 (81/564)	1.00
Socio-economic markers:		
Mother's age at arrival in Thailand:		
< 15 years	92 (25/273)	1.00
≥ 15 years	126 (102/813)	1.37 (0.88-2.12)
Time elapsed since mother's arrival in Thailand: < 2 years	145 (10/69)	1.61 (0.77-3.37)
2-5 years	128 (49/384)	1.42 (0.87-2.31)
5-10 years	120 (44/366)	1.34 (0.81-2.20)
> 10 years	90 (24/267)	1.00
Composite score of mother's education:		
low	119 (58/487)	0.93 (0.62-1.39)
average	100 (30/299)	0.78 (0.48-1.25)
high	129 (39/303)	1.00
Composite score of father's education:		
low	164 (48/293)	**1.74 (1.15-2.64)**
average	114 (36/316)	1.21 (0.78-1.89)
high	94 (42/447)	1.00

Composite score of economic level		
low	137 (41/300)	2.09 (0.83-5.29)
average	112 (68/610)	1.72 (0.69-4.26)
high	65 (5/77)	1.00
Site of birth: Shoklo	177 (77/434)	1.00
Bonoklo	182 (28/154)	1.03 (0.67-1.58)
Maesalit	249 (20/80)	1.40 (0.86-2.29)
Klaymuta	305 (11/36)	1.72 (0.91-3.23)*
Maela	21 (11/523)	**0.12 (0.06-0.22)**
Year of birth: 1993	402 (39/97)	1.00
1994	169 (66/390)	**0.42 (0.28-0.63)**
1995	91 (36/397)	**0.23 (0.14-0.35)**
1996	18 (6/343)	**0.04 (0.02-0.10)**

Bold: $p<0.05$
*: $p \geq 0.05$ and <0.10

Table 7.5. Risk factors for malaria during infancy (multivariate analysis).

	Adjusted incidence rate ratio (95% CI)
- *P.falciparum* during first trimester of pregnancy	2.75 (1.48-5.10)
- Karen ethnic group	2.93 (1.18-7.25)
- Low father's education:	1.60 (1.04-2.47)

Following these results, further analysis was performed in order to identify the risk factors for developing falciparum malaria during the first trimester of gestation rather than later in pregnancy. Pregnant women were more likely to be infected with *P.falciparum* during the first trimester when they delivered in winter than in other seasons ($p=0.08$), but this was not significant. Birth in winter corresponded to conception between February and April, immediately before the malaria peak (May-July). But the main risk factor of having a first falciparum malaria episode during the first trimester rather than later in pregnancy was a maternal history of slide-confirmed malaria before the pregnancy (Mantel-Haenszel OR after adjusting for season: 2.35;

95%CI: 1.17-4.73). Nevertheless, a history of maternal malaria before pregnancy was not associated with infant malaria. Malaria incidence rates during infancy were 167 per 1,000 child-year (12/72) in children born to mothers with a pre-pregnancy history of malaria compared to 117 (135/1156) per 1,000 in infants whose mothers did not declare malaria before pregnancy (IRR: 1.43; 95%CI: 0.72-2.58). When history of maternal malaria was accounted for in the Poisson regression, *P.falciparum* during the first trimester of pregnancy was an even stronger risk factor for malaria during infancy (Adjusted IRR: 3.24; 95%CI: 1.67-6.30). The higher risk for the Karen ethnic group remained unchanged (Adjusted IRR: 2.80; 95%CI: 1.01-7.73).

In order to avoid potential interactions between *P.vivax* and *P.falciparum*, the analysis was repeated in the sub-group of infants those mothers had only *P.falciparum* malaria or did not suffer from malaria during pregnancy. Results were identical. Children born to mothers who had their first or unique falciparum malaria during the first trimester of pregnancy were 2.71 (1.46-5.04) times more likely to develop malaria during infancy than those whose mothers had no malaria or were infected later in pregnancy. The Karen group remained at higher risk of malaria than Black Karen (Adjusted IRR: 2.44; 95%CI: 0.98-6.08; p=0.056), although it was not significant anymore.

When looking at the acquisition of different species during infancy, first falciparum malaria during the first trimester of pregnancy remained a risk factor for *P.vivax* malaria (Adjusted IRR: 2.46; 95%CI: 1.17-5.16), whereas it did not reach significance for *P.falciparum* malaria (Adjusted IRR: 2.92; 95%CI: 0.96-8.87; p=0.059). Infants born to multigravidae (Adjusted IRR: 2.92; 95%CI: 1.12-7.61) and those whose mothers attended ANC only during the third trimester of pregnancy (Adjusted IRR: 2.96; 95%CI: 1.34-6.54) were also at risk of having *P.falciparum* malaria during infancy.

7.3. Morbidity from respiratory infections and diarrhoea

7.3.1 Respiratory infections (excluding mild diseases)

Children had an average of 1.37 (95%CI: 1.30-1.43) episodes of ARI per child-year (1677/1228). Three per cent (54/1677) of the patients with ARI were referred to hospital and 12 died, giving a CFR of 7.2 per 1,000 (95%CI: 3.1-11.2) and mortality attributable to ARI of 9.8 per 1,000 child-year (95%CI: 5.5-17.2).

ARI during pregnancy, male gender, arrival of the mother in Thailand at age 15 or more, low education and low economic level were identified as risk factors of having ARI during infancy (**Table 7.6**).

Table 7.6: Risk factors for ARI during infancy

	Incidence rate (No/child-year)	IRR (95%CI)	Adjusted IRR (95%CI)
ARI during pregnancy: Yes	1.76 (608/345)	**1.45 (1.31-1.61)**	**1.30 (1.14-1.49)**
No	1.21 (1069/882)	1.00	
Numbness during pregnancy:			
Yes	1.44 (949/661)	**1.12 (1.02-1.24)**	NS
No	1.28 (723/564)	1.00	
Birth order: first born	1.21 (412/339)	1.00	
Second	1.28 (320/249)	1.06 (0.91-1.22)	
Third	1.24 (253/205)	1.02 (0.87-1.19)	
fourth or above	1.60 (692/433)	**1.32 (1.16-1.49)**	NS
Sex: Male	1.49 (941/633)	**1.20 (1.09-1.32)**	**1.21 (1.08-1.35)**
Female	1.24 (736/594)	1.00	
Mother's age at arrival in Thailand: < 15 years	1.12 (306/273)	1.00	
≥ 15 years	1.46 (1184/813)	**1.30 (1.14-1.48)**	**1.34 (1.16-1.53)**
Mother's education: low	1.53 (744/487)	**1.40 (1.23-1.60)**	**1.18 (1.02-1.37)**
Average	1.40 (418/299)	**1.28 (1.11-1.48)**	1.16 (0.99-1.36)
high	1.09 (330/303)	1.00	

Father's education:	low	1.51 (443/293)	**1.21 (1.07-1.37)**	NS
	Average	1.41 (447/316)	1.13 (1.00-1.28)*	NS
	high	1.25 (561/447)	1.00	
Economic level:	low	1.57 (471/300)	**1.69 (1.32-2.17)**	**1.33 (1.03-1.71)**
	Average	1.38 (842/610)	**1.49 (1.17-1.90)**	**1.31 (1.00-1.71)**
	high	0.93 (71/77)	1.00	

Bold: p<0.05
*: p≥ 0.05 and <0.10

7.3.2. Diarrhoeal diseases

There were 1271 episodes of diarrhoea: 1.04 per child-year (95%CI: 0.98-1.09). Forty-seven (4%) children were hospitalized and 7 died: CFR: 5.5 per 1,000 (95%CI: 1.4-9.6); mortality attributable to diarrhoea: 5.7 per 1,000 child-year (95%CI: 2.7-12.0). LBW infants, premature as well as term-LBW ones, were at higher risk of diarrhoea during infancy than NBW. ARI and UTI during pregnancy were also identified as risk factors for diarrhoea in infancy (**Table 7.7**).

Table 7.7: Risk factors for diarrhoea during infancy

	Incidence rate (No/child-year)	IRR (95%CI)	Adjusted IRR (95%CI)
Weight/gestational age:			
- preterm LBW	0.72 (56/78)	**0.69 (0.53-0.90)**	**1.34 (1.01-1.78)**
- term LBW	1.24 (121/98)	1.19 (0.98-1.43)*	**1.58 (1.14-2.20)**
- NBW	1.04 (1037/993)	1.00	
ARI during pregnancy: Yes	1.30 (449/345)	**1.40 (1.24-1.57)**	**1.24 (1.09-1.41)**
No	0.93 (822/882)	1.00	
UTI during pregnancy: Yes	1.31 (109/83)	**1.29 (1.05-1.58)**	**1.27 (1.03-1.56)**
No	1.01 (1162/1145)	1.00	

Bold: p<0.05
*: p≥ 0.05 and <0.10

7.4. Anaemia during infancy

7.4.1. Monthly visits

Between 74 and 83% of the children who were still followed (i.e. alive and who did not withdrawn from the study) were seen at monthly visits. At each visit, some results were lost, or rejected as unreliable, and some HCT tubes were broken. Thus the numbers of measurements effectively done were lower than the number of children seen. Nevertheless, 72-81% of the infants were weighed and measured at each point, and 63-77% had an HCT level done. Attendance was higher at 6 months and 1 year of age because a special effort was made to see a maximum of children at these ages (**Table 7.8**). Children who were absent at one point were seen at a following visit. 1,318 children were followed at least 3 months (i.e. they should have attended a minimum of 2 visits). Ninety-five per cent of them were weighed (N=1253) and measured (N=1254) more than twice (median: 8 times; range: 0-11) during infancy and 98% (N=1292) had at least two HCT measurements (median: 9 times; range: 0-11).

Table 7.8. Attendance for monthly visits and proportion of anthropometric and haematocrit measurements effectively done.

Age in months	No children remaining in study	No visits (%)	No weight (%)	No height (%)	No hct (%)
1	1410	1135 (80)	1046 (74)	1039 (74)	882 (63)
2	1364	1106 (81)	1029 (75)	1023 (75)	949 (70)
3	1318	1060 (80)	995 (75)	994 (75)	940 (71)
4	1273	1018 (80)	955 (75)	954 (75)	915 (72)
5	1243	992 (80)	926 (74)	929 (75)	888 (71)
6	1208	998 (83)	942 (78)	941 (78)	883 (73)
7	1179	894 (76)	853 (72)	853 (72)	798 (68)
8	1151	900 (78)	853 (74)	853 (74)	820 (71)
9	1119	854 (76)	813 (73)	811 (72)	782 (70)
10	1084	810 (75)	782 (72)	779 (72)	751 (69)
11	1061	784 (74)	767 (72)	766 (72)	716 (67)
12	1039	855 (82)	841 (81)	838 (81)	796 (77)

7.4.2. HCT profile and anaemia during infancy

When the study started, samples for HCT level were not taken routinely at birth and later on, most mothers refused that blood was drawn from their newborn. It was therefore not possible to use the HCT levels obtained at birth. The HCT profile of Karen infants from 1 to 12 months of age is shown in **fig 7.3** (line without markers) together with that of a reference population (line with circle markers) of infants from developed countries (Saarinen *et al.*, 1978). Karen infants did not recover from physiological anaemia at 2-3 months of age. Whereas a plateau was reached at 6 months at around 36-37% HCT in the reference population, HCT in the infants from the cohort continued to decrease from 6 to 12 months (33.3 to 32.0%). As a consequence, the prevalence of anaemia increased with age (**fig 7.4**).

Fig 7.3: haematocrit levels in Karen infants and in a reference population of infants from developed countries.

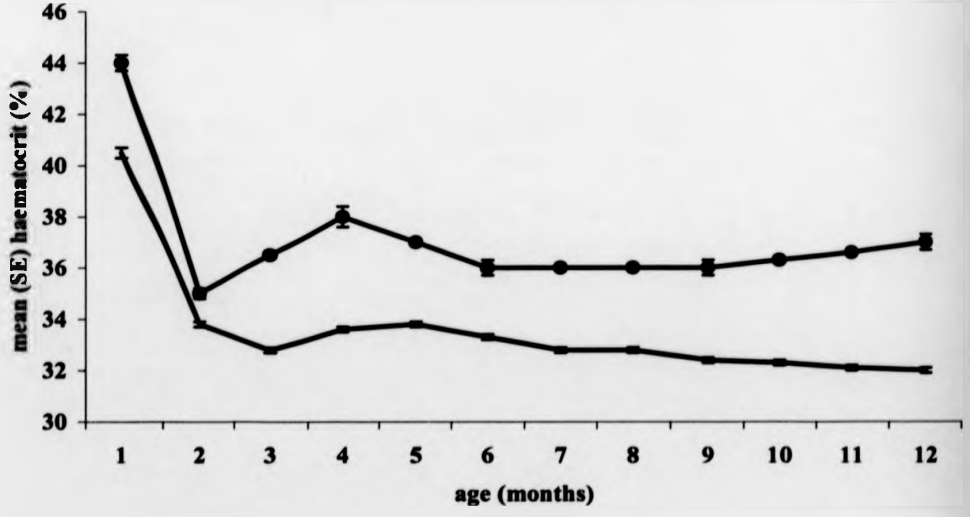


Fig 7.4: Prevalence of anaemia in Karen infants

7.4.3. Risk factors for anaemia in infancy

7.4.3.1. Risk factors for anaemia at one month of age

Only 2.7% (24/882) of the children were anaemic at 1 month of age. Malaria during pregnancy was not associated with anaemia in 1 month-old infants. Babies born during the rainy season were more likely to be anaemic at one month (4.4%; 16/367) than children born during other periods of the year (1.6%; 8/515) (OR=2.89; 95%CI: 1.15-7.45). Children born to mothers who were anaemic during pregnancy were less likely to have anaemia at 1 month of age than those whose mothers were not anaemic: 2.1% (14/662) vs 4.9 (10/206) (OR: 0.42: 95%CI: 0.18-0.97). The risk of infant anaemia decreased as the duration of maternal anaemia increased: 2.6% (8/304) and 1.7 (6/351) for less and more than 6 weeks of anaemia ($\chi 2$ for trend: 4.19; p=0.04). In a multivariate analysis in which the duration of iron supplementation and the number of consultations at ANC were added as covariates, to be born in rainy season remained the only risk factor for anaemia at 1 month of age (Adjusted OR: 2.83; 95%CI: 1.19-6.72).

7.4.3.2. Risk factors for anaemia at three months of age

Malaria and anaemia during pregnancy were not associated with anaemia in 3 month old infants. Similarly, infant anaemia at this age, was not influenced by morbid events and hospitalisation during the first three months of life. Only LBW and prematurity were associated with anaemia at 3 months of age. LBW remained the only identified risk factor for anaemia at 3 months of age after adjustment for gestational age (**Table 7.9**). The proportion of anaemic infants born with LBW was 32% (11/34), giving the PAF of anaemia due to LBW: 20% (95%CI: 2-27).

Table 7.9: Risk factors for anaemia in 3 month old infants

	Prevalence of anaemia % (n)	OR (95%CI)	Adjusted OR (95%CI)
Maternal and newborn characteristics			
Mother primigravidae	6 (14/237)	1.64 (0.84-3.19)	Not in model
Multigravidae	4 (26/703)	1.00	
Attendance to ANC in			
1st trimester of pregnancy	4 (19/462)	1.00	Not in model
2nd	4 (13/355)	0.89 (0.41-1.91)	
3rd	7 (8/120)	1.67 (0.65-4.15)	
Ethnic group: Karen	5 (37/825)	1.75 (0.54-9.03)	Not in model
Black Karen	3 (3/115)	1.00	
Infant sex: Male	4 (20/470)	1.00 (0.53-1.88)	Not in model
Female	4 (20/470)	1.00	
LBW: Yes	10 (11/116)	**3.26 (1.54-6.88)**	**2.58 (1.07-6.21)**
No	3 (23/738)	1.00	
Premature infant: Yes	10 (7/73)	**2.73 (1.15-6.49)**	NS
No	4 (28/749)	1.00	
Maternal morbidity during pregnancy			
Malaria: Yes	5 (15/328)	1.13 (0.59-2.17)	Not in model
No	4 (25/612)	1.00	

Anaemia at any time: Yes	5 (34/716)	1.99 (0.77-5.17)	Not in model
No	2 (5/205)	1.00	
Anaemia at delivery: Yes	6 (13/210)	1.93 (0.96-3.88)*	NS
No	3 (23/695)	1.00	
Severe anaemia at any time: Yes	6 (9/146)	1.72 (0.79-3.73)	Not in model
No	4 (28/761)	1.00	
Infection within the week prior to delivery: Yes	5 (2/43)	1.10 (0.26-4.71)	Not in model
No	4 (38/894)	1.00	
ARI: Yes	4 (12/273)	1.05 (0.53-2.10)	Not in model
No	4 (28/667)	1.00	
UTI: Yes	3 (2/63)	0.72 (0.17-3.07)	Not in model
No	4 (38/877)	1.00	
Numbness: Yes	5 (25/518)	1.37 (0.71-2.64)	Not in model
No	4 (15/421)	1.00	
Hypertension: Yes	8 (2/25)	2.01 (0.46-8.82)	Not in model
No	4 (38/915)	1.00	
Infant morbidity during the first three months of life			
At least one attendance to dispensary: Yes	4 (17/388)	1.05 (0.53-2.09)	Not in model
No	4 (23/552)	1.00	
Malaria: Yes	0 (0/3)	0.00 (0.00-57.82)	Not in model
No	4 (40/937)	1.00	
ARI: Yes	4 (7/162)	1.02 (0.37-2.40)	Not in model
No	4 (33/778)	1.00	
Diarrhoea: Yes	5 (6/117)	1.25 (0.42-3.12)	Not in model
No	4 (34/823)	1.00	
Hospitalised: Yes	4 (1/28)	0.83 (0.02-5.31)	Not in model
No	4 (39/912)	1.00	
Socio-economic and environmental factors			
Birth in rainy season: Yes	4 (14/385)	0.77 (0.40-1.49)	Not in model
No	5 (26/555)	1.00	

Time spend in Thailand (mother):			
< 10 years	5 (29/620)	1.42 (0.61-3.30)	Not in model
≥ 10 years	3 (7/210)	1.00	
Mother's education: Low	4 (16/378)	0.96 (0.47-1.97)	Not in model
Average/high	4 (20/455)	1.00	
Father's education: Low	4 (9/226)	0.89 (0.38-2.02)	Not in model
Average/high	4 (26/582)	1.00	
Economic level: Low/ Average	4 (29/655)	1.00	Not in model
High	8 (5/60)	2.05 (0.60-5.66)	

Bold: $p<0.05$
*: $p \geq 0.05$ and <0.10

7.4.3.3. Risk factors for anaemia at six months of age

Malaria in the first 6 months of life was associated with infant anaemia at 6 months of age. LBW remained another important risk factors for infant anemia. At this age, socio-economic and environmental factors (time spent in Thailand by the mother, low father's education, birth outside the rainy season) were also associated with infant anaemia. Infant nutrition was not associated with infant anaemia. Nearly all (98%; 840/856) infants were still breast-fed, but 26% (207/794) received complementary food (usually rice) before 6 months of age. Exclusively breast-fed infants and those for whom weaning food had been introduced had similar prevalence of anaemia (**Table 7.10**). Environmental factors were more prevalent in anaemic infants than LBW and malaria. Thus PAF of anaemia due to SES and environmental factors were more important than PAF due to LBW or infant malaria (**Table 7.11**).

Table 7.10: Risk factors for anaemia in 6 month old infants

	Prevalence of anaemia % (n)	OR (95%CI)	Adjusted OR (95%CI)
Maternal and newborn characteristics			
Mother primigravidae	15 (33/219)	**1.79 (1.13-2.82)**	NS
Multigravidae	9 (60/664)	1.00	
Ethnic group: Karen	11 (82/781)	0.97 (0.48-2.00)	Not in the model
Black Karen	11 (11/102)	1.00	
Infant sex: Male	12 (55/461)	1.37 (0.89-2.11)	Not in the model
Female	9 (38/422)	1.00	
LBW: Yes	17 (19/109)	**2.10 (1.20-3.67)**	**2.09 (1.16-3.77)**
No	9 (63/690)	1.00	
Premature infant: Yes	16 (13/84)	1.71 (0.90-3.26)	Not in the model
No	10 (66/683)	1.00	
Maternal morbidity during pregnancy			
Malaria: Yes	13 (39/313)	1.36 (0.88-2.11)	Not in the model
No	10 (54/570)	1.00	
Anaemia at any time: Yes	10 (67/679)	0.72 (0.84-1.18)	Not in the model
No	13 (25/190)	1.00	
Anaemia at delivery: Yes	10 (21/205)	0.96 (0.57-1.60)	Not in the model
No	11 (69/648)	1.00	
ARI: Yes	8 (22/268)	0.69 (0.42-1.13)	Not in the model
No	12 (71/615)	1.00	
UTI: Yes	11 (6/55)	1.04 (0.43-2.51)	Not in the model
No	11 (87/828)	1.00	
Infant morbidity during the first six months of life			
Number of morbid events: none	8 (13/157)	1.00	Not in the model
1 to 3	10 (46/461)	1.23 (0.62-2.47)	
≥ 4	13 (34/265)	1.63 (0.80-3.38)	

Malaria: Yes	29 (10/36)	**3.54 (1.65-7.60)**	**2.74 (1.08-6.98)**
No	10 (83/847)	1.00	
ARI: Yes	11 (40/353)	1.15 (0.73-1.81)	Not in the model
No	10 (53/530)	1.00	
Diarrhoea: Yes	11 (12/107)	1.08 (0.54-2.14)	Not in the model
No	10 (81/776)	1.00	
Hospitalised: Yes	16 (8/51)	1.64 (0.69-3.76)	Not in the model
No	10 (85/832)	1.00	
Socio-economic and environmental factors			
Exclusive breast-feeding during the first 6 months: Yes	12 (22/188)	1.09 (0.65-1.83)	Not in the model
No	11 (58/534)	1.00	
Birth in rainy season: Yes	7 (26/364)	1.00	
No	13 (67/519)	**1.93 (1.17-3.19)**	**1.88 (1.10-3.21)**
Time spend in Thailand (mother):			
< 10 years	13 (77/603)	**2.80 (1.42-5.52)**	**2.32 (1.12-4.83)**
≥ 10 years	5 (10/201)	1.00	
Mother's education: Low/Aver.	12 (68/575)	1.41 (0.81-2.46)	Not in the model
High	9 (20/230)	1.00	
Father's education: Low	16 (35/213)	**2.04 (1.28-3.24)**	**1.95 (1.18-3.21)**
Average/high	9 (50/568)	1.00	
Economic level: Low	12 (27/233)	1.14 (0.43-3.54)	Not in the model
Average	11 (46/436)	1.02 (0.41-3.07)	
High	10 (6/58)	1.00	

Table 7.11. Risk factors and PAF for anaemia at 6 months of age.

Exposure factors	Adjusted OR (95%CI)	Prevalence of exposure factors among anaemic infants: % (n)	PAF (95%CI)
LBW	2.09 (1.16-3.77)	23% (19/82)	12% (3-17)
Infant malaria in the previous 6 months of life	2.74 (1.08-6.98)	11% (10/93)	7% (1-9)
Born in winter, dry or post-rainy season	1.88 (1.10-3.21)	72% (67/93)	34% (7-50)
<10 years in Thailand	2.32 (1.12-4.83)	89% (77/87)	50% (9-70)
Low father's education	1.95 (1.18-3.21)	41% (35/85)	20% (6-28)

7.4.3.4. Risk factors for anaemia at one year of age

Nineteen per cent (153/796) of the children for whom the HCT level was known were anaemic at one year of age. **Table 7.12** summarizes the results of the univariate analysis performed in order to identify the risk factors for anaemia at 1 year. Maternal anaemia at delivery was associated with infant anaemia. The risk of being anaemic at one year tended to increase with the number of morbid events during infancy: 12% in children who were not ill, or had only one episode; 20% in children with 2-12 episodes (i.e. less than once a month); 25% in those who had on average more than one episode per month ($\chi 2$ for trend=3.80; p=0.05). Children born to primigravidae, LBW, male gender, recent arrival in Thailand, low parental education were also identified in univariate analysis.

Table 7.12: Risk factors for anaemia at 1 year of age (univariate analysis).

	Prevalence of anaemia: % (n)	OR (95%CI)
Maternal and newborn characteristics		
Gravidity: Primigravidae	27 (51/187)	**1.86 (1.27-2.74)**
Multigravidae	17 (102/609)	1.00
Ethnic group: Karen	20 (143/705)	**2.06 (1.01-4.34)**
Black Karen	11 (10/91)	1.00
Infant sex: Male	24 (95/404)	**1.77 (1.23-2.54)**
Female	15 (58/392)	1.00
LBW: Yes	34 (34/100)	**2.67 (1.68-4.25)**
No	16 (100/618)	1.00
Premature infant: Yes	27 (18/66)	1.65 (0.93-2.94)
No	19 (117/631)	1.00
Maternal morbidity during pregnancy		
Malaria: Yes	19 (50/270)	0.93 (0.64-1.36)
No	20 (103/526)	1.00
Anaemia at any time: Yes	20 (125/617)	1.36 (0.86-2.16)
No	16 (26/165)	1.00
Anaemia at delivery: Yes	25 (47/188)	**1.63 (1.10-2.41)**
No	17 (99/582)	1.00
Lengh of anaemia during pregnancy		
None	17 (28/170)	1.00
< 6 weeks	16 (43/271)	0.96 (0.55-1.66)
≥ 6 weeks	24 (80/341)	1.55 (0.94-2.58)*
ARI during pregnancy: Yes	15 (39/257)	0.67 (0.45-0.99)*
No	21 (114/539)	1.00
UTI during pregnancy: Yes	11 (6/55)	0.50 (0.21-1.18)
No	20 (147/741)	1.00
Infant morbidity since birth:		
Number of morbid events: 0-1	12 (11/89)	1.00
2-6	20 (75/374)	1.78 (0.87-3.73)
7-12	19 (52/272)	1.68 (0.80-3.60)
≥ 13	25 (15/61)	2.31 (0.91-5.95)*

Nu of malaria attacks: none	19 (135/725)	1.00
1-2	23 (14/61)	1.30 (0.66-2.52)
≥ 3	40 (4/10)	1.75 (0.39-6.17)
Nu of ARI: none	22 (66/202)	1.00
1	15 (29/199)	0.61 (0.37-1.01)*
2-6	20 (54/272)	0.89 (0.58-1.35)
≥ 7	17 (4/23)	0.75 (0.18-2.38)
Nu of diarrhoeal episodes: 0-1	18 (99/551)	1.00
2-3	20 (38/186)	1.17 (0.76-1.81)
≥ 4	27 (16/59)	1.70 (0.88-3.26)
Nu of hospitalisations: none	19 (134/723)	1.00
1	24 (16/66)	1.41 (0.74-2.63)
2-3	43 (3/7)	3.30 (0.48-19.69)
Socio-economic and environmental factors		
Exclusive breast-feeding during the first 6 months: Yes	21 (31/151)	1.09 (0.69-1.72)
No	19 (87/453)	1.00
Birth in rainy season: Yes	19 (60/317)	0.97 (0.68-1.39)
No	19 (93/479)	1.00
Recent arrival (< 2 years): Yes	39 (17/44)	**2.86 (1.44-5.63)**
No	18 (130/720)	1.00
Mother's education: Low	23 (75/333)	**1.63 (1.02-2.62)**
Average	19 (40/209)	1.33 (0.78-2.26)
High	15 (34/225)	1.00
Father's education: Low	24 (50/205)	**1.52 (1.03-2.24)**
Average/high	18 (94/537)	1.00
Economic level: Low/Average	21 (135/650)	2.01 (0.83-5.88)
High	12 (6/52)	1.00

Bold: $p<0.05$
*: $p \geq 0.05$ and <0.10

In multivariate analysis, LBW remained an independent risk factor for infant anaemia, together with ethnic group, gender, being first born and recent arrival (**Table 7.13**). Maternal anaemia at delivery and infant morbidity had no independent effect on infant anaemia.

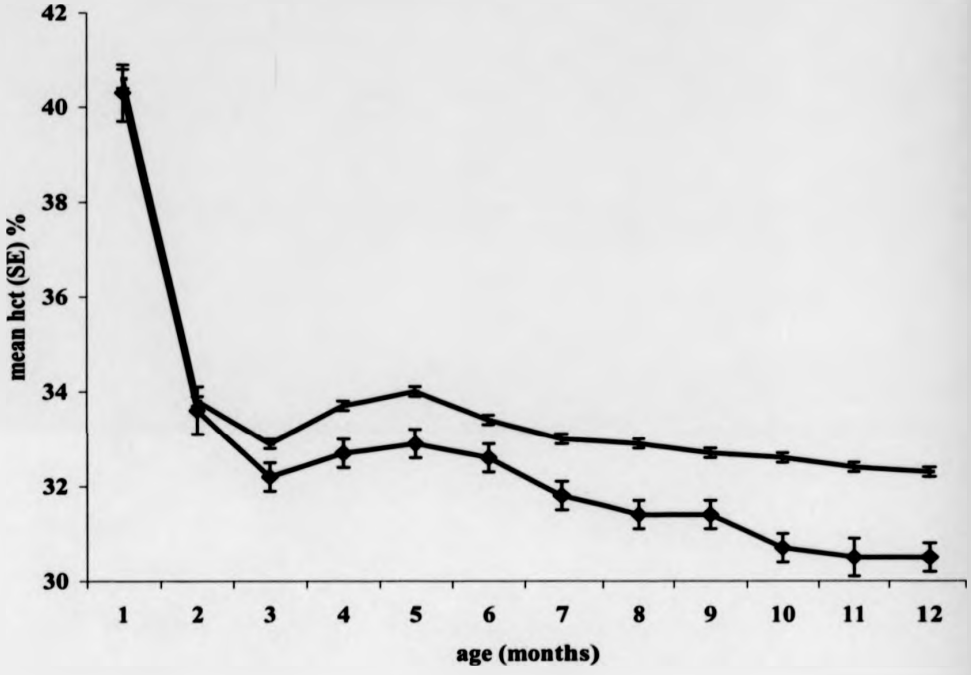
Table 7.13. Risk factors and PAF for anaemia at one year of age.

Exposure factors	Adjusted OR (95%CI)	Prevalence of exposure factors among anaemic infants: % (n)	PAF (95%CI)
LBW	3.19 (1.91-5.36)	25% (34/134)	17% (12-21)
Primigravidae	1.97 (1.26-3.08)	33% (51/153)	16% (7-22)
Karen	2.39 (1.55-3.69)	94% (143/153)	54% (6-76)
Male infant	2.39 (1.55-3.69)	62% (95/153)	36% (22-45)
Recent (<2 years) arrival	3.73 (1.85-7.53)	12% (17/147)	8% (5-10)

7.4.3.5. Anaemia in LBW infants

From 3 months onwards LBW was an important risk factor for infant anaemia. HCT profiles of LBW (diamond markers) and NBW infants (no markers) are represented in **fig 7.5**. HCT levels were comparable at 1-2 months of age. Physiological anaemia at 3 months of age dipped more in LBW infants than in NBW, and from 5 months onwards, HCT decreased more markedly in the former than the latter.

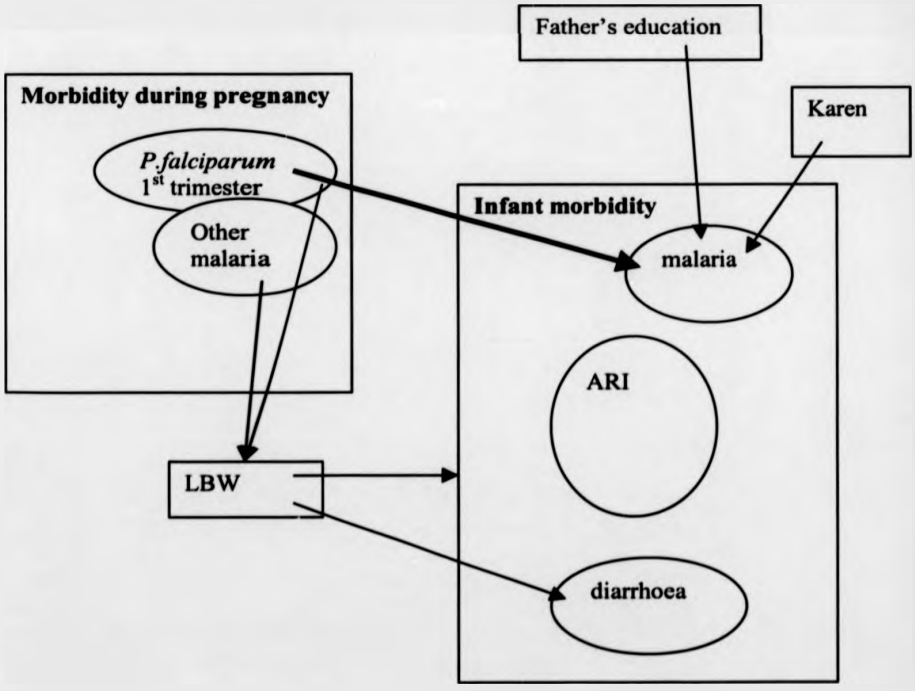
fig 7.5: haematocrit levels during infancy in LBW and NBW children



7.5. Summary of the results contained in this chapter

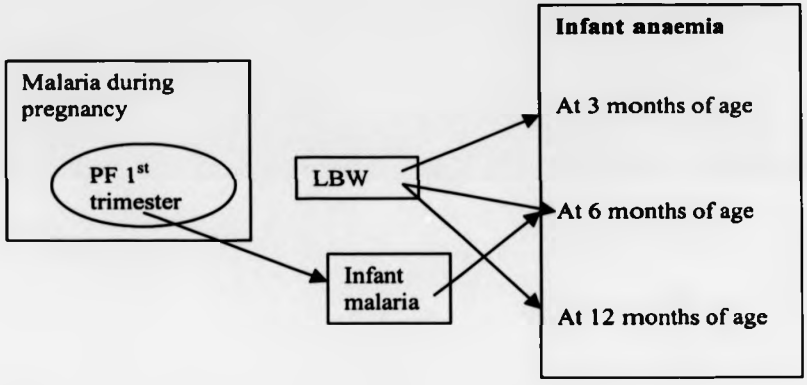
Morbidity during pregnancy influenced infant morbidity, but a number of SES and environmental factors were also important. Falciparum malaria during the first trimester of pregnancy was associated with an increased risk of acquiring malaria during infancy. Apart from this direct effect, malaria during pregnancy influenced infant morbidity through LBW which was associated with an increased overall morbidity and diarrhoeal episodes during infancy (**Fig 7.6**).

Figure 7.6: Effects of malaria during pregnancy on infant morbidity.



The effects of malaria during pregnancy on infant anaemia were all mediated through LBW which was responsible for 12-20% of anaemia throughout infancy. Falciparum malaria in early pregnancy was also indirectly associated with anaemia at 6 months of age, through an increased risk of infant malaria that was responsible for 7% of anaemia at that age (**fig 7.7**).

Figure 7.7.: Effects of malaria during pregnancy on infant anaemia



CHAPTER 8

INFANT GROWTH

8.1 Description

Attendance for monthly visits was described in **paragraph 7.4.1**. Mean WAZ and HAZ scores were constantly under the mean of the NCHS/WHO reference population. Weight increased rapidly during the first 3 months of life and, as a result the infants nearly catch-up with the reference population. But then weight increase flattened, which is a common feature of infant growth pattern. There was less tendency to catch-up for height than for weight in the first 3 months. HAZ remained lower than that of the reference population and regularly decreased (**fig 8.1**; WAZ solid line; HAZ dotted line). Mean WHZ remained close to that of reference population: -0.7 in boys and –0.6 in girls at 1 year of age.

Acute malnutrition was not a common problem in this population and the prevalence of wasted (WHZ<-2 SD) children remained under 5% (range: 0.5-5.3%) throughout infancy. Age-specific prevalence rates of stunted and underweight infants followed the same profile. Prevalences of stunted and underweight infants were comparable at birth and at one year of age. Nevertheless, as a result of faster increase in weight than height during the first 3 months of life, there were only 2% (24/995) of infants with (WAZ<-2 SD) at 3 months of age, whereas 12% (119/992) were stunted (**fig 8.2**).

Fig 8.1: Mean (SEM) WAZ and HAZ during infancy

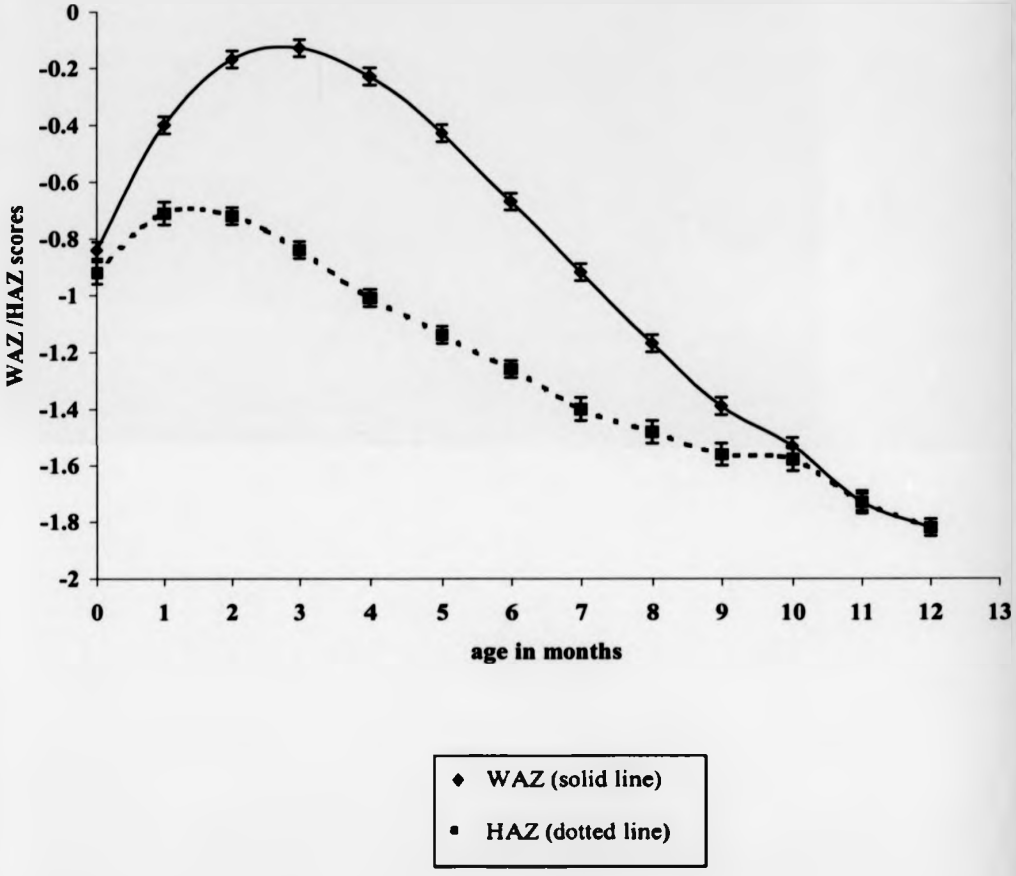
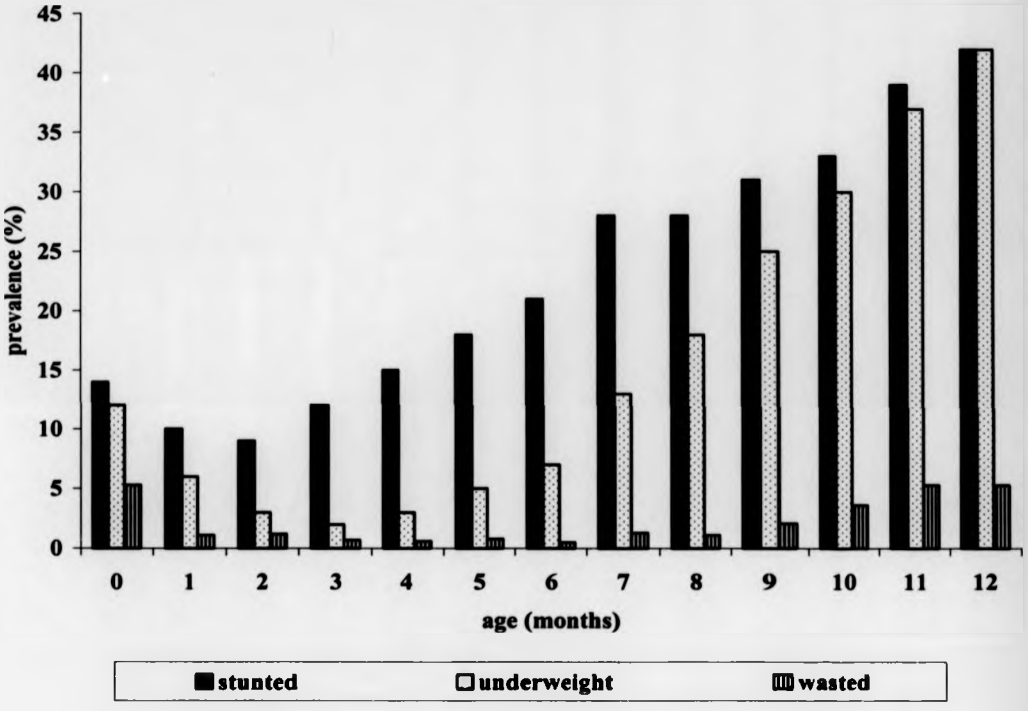


Fig 8.2: Prevalence of malnourished infants



8.2. Risk factors for infant malnutrition

8.2.1. Risk factors for impaired growth at 6 months of age.

Only 3 (0.3%) of the 939 infants who were weighed and measured at 6 months of age were wasted. Numbers were too small to be able to identify risk factors for wasting.

Seven per cent (63/942) of the 6-months old infants were underweight. LBW and prematurity were strongly associated with being underweight in univariate analysis. Black Karen children were also more likely to be underweight than Karen infants. Hospitalisation during the first 6 months of life was more common in underweight infants than in those who had an appropriate weight for age, but this was not significant. Exclusive breast feeding protected children from being underweight, when compared to feeding combining breast milk with complementary food (usually rice), but again this was not significant. In multivariate analysis, LBW and premature birth were the only independent risk factors associated with being underweight at the age of 6 months (**Table 8.1**). The PAF of being underweight due to LBW was 36% (95%CI: 21-43), and that of premature birth was 29% (95%CI: 15-35).

Table 8.1: Risk factors of being underweight at 6 months of age.

	Prevalence of underweight infants: %(n)	OR (95%CI)	Adjusted OR (95%CI)
Maternal and newborn characteristics			
Mother primigravidae	6 (14/233)	0.86 (0.47-1.59)	Not in the model
Multigravidae	7 (49/709)	1.00	
Ethnic group: Karen	6 (51/845)	1.00	
Black Karen	12 (12/97)	**2.20 (1.13-4.28)**	NS
Infant sex: Male	7 (34/489)	1.09 (0.65-1.82)	Not in the model
Female	6 (29/453)	1.00	
LBW: Yes	25 (29/118)	**7.56 (4.34-13.18)**	**3.77 (1.77-8.07)**
No	4 (30/726)	1.00	

Premature infant: Yes No	24 (21/86) 4 (31/728)	**7.26 (3.95-13.36)** 1.00	**3.61 (1.62-8.07)**
Maternal morbidity during pregnancy			
Malaria: Yes No	6 (21/344) 7 (42/598)	0.86 (0.50-1.48) 1.00	Not in the model
Anaemia at any time: Yes No	7 (49/741) 5 (10/187)	1.25 (0.62-2.52) 1.00	Not in the model
Anaemia at delivery: Yes No	8 (18/215) 6 (41/696)	1.46 (0.82-2.60) 1.00	Not in the model
ARI: Yes No	5 (14/275) 7 (49/667)	0.68 (0.37-1.25) 1.00	Not in the model
UTI: Yes No	8 (5/60) 7 (58/882)	1.29 (0.50-3.35) 1.00	Not in the model
Infant morbidity during the first six months of life			
Number of morbid events: none 1 to 3 ≥ 4	6 (7/122) 6 (34/533) 8 (22/287)	1.00 1.12 (0.47-3.07) 1.36 (0.54-3.89)	Not in the model
Malaria: Yes No	4 (2/49) 7 (61/893)	0.58 (0.07-2.31) 1.00	Not in the model
ARI: Yes No	8 (32/394) 6 (31/548)	1.47 (0.86-2.53) 1.00	Not in the model
Diarrhoea: Yes No	7 (23/309) 6 (40/633)	1.18 (0.67-2.06) 1.00	Not in the model
Hospitalised: Yes No	13 (8/60) 6 (55/882)	2.31 (0.96-5.36)* 1.00	NS
Socio-economic and environmental factors			
Camp: Shoklo Bonoklo Maesalit Klaymuta Maela	7 (24/333) 14 (9/65) 7 (5/71) 14 (2/14) 5 (23/459)	1.00 2.07 (0.84-4.98) 0.98 (0.28-2.74) 2.15 (0.22-10.50) 0.68 (0.36-1.27)	Not in the model

Year of birth: 1993	8 (7/89)	1.00	Not in the model
1994	9 (21/248)	1.08 (0.42-2.92)	
1995	7 (20/304)	0.82 (0.32-2.23)	
1996	5 (15/301)	0.61 (0.23-1.73)	
Exclusive breast-feeding during the first 6 months: Yes	4 (8/207)	0.50 (0.23-1.07)*	NS
No	8 (44/587)	1.00	
Birth in rainy season: Yes	5 (19/380)	0.62 (0.34-1.11)	Not in the model
No	8 (44/562)	1.00	
Time spend in Thailand (mother):			
< 10 years	7 (44/645)	1.56 (0.76-3.27)	Not in the model
≥ 10 years	5 (11/206)	1.00	
Mother's education: Low	8 (29/372)	1.82 (0.85-3.96)	Not in the model
Average	6 (15/234)	1.48 (0.62-3.52)	
High	4 (11/248)	1.00	
Father's education: Low	8 (19/231)	1.61 (0.79-3.26)	Not in the model
Average	6 (14/240)	1.11 (0.52-2.38)	
High	5 (19/360)	1.00	
Economic level: Low	8 (21/249)	1.08 (0.34-4.53)	Not in the model
Average	5 (25/482)	0.64 (0.21-2.65)	
High	8 (4/51)	1.00	

Bold: $p<0.05$
*: $p \geq 0.05$ and <0.10

The prevalence of stunted 6-months old infants was 21% (196/940). LBW and prematurity were again identified as risk factors, but stunting was also associated with low parental education, and was more common in children born in 1996 (i.e. after relocation of the refugees and sudden changes in their food ration). Morbidity during pregnancy or during the first 6 months of life was not associated with stunting, nor was the type of infant food. In multivariate analysis LBW, prematurity and birth in 1996, were independent risk factors for stunting (**Table 8.2**), but 31% (95%CI: 15-38) of stunting was due to being born in 1996, whereas LBW accounted for 28% (95%CI: 24-30), and premature birth for 12% (95%CI: 4-16) of stunted infants.

Table 8.2: Risk factors for stunting at 6 months of age

	Prevalence of stunted infants: % (n)	OR (95%CI)	Adjusted OR (95%CI)
Maternal and newborn characteristics			
Mother primigravidae	23 (54/234)	1.19 (0.84-1.70)	Not in the model
Multigravidae	20 (142/706)	1.00	
Ethnic group: Karen	21 (175/843)	0.95 (0.55-1.63)	Not in the model
Black Karen	22 (21/97)	1.00	
Infant sex: Male	23 (110/486)	1.25 (0.91-1.72)	Not in the model
Female	19 (86/454)		
LBW: Yes	51 (60/117)	**5.47 (3.62-8.27)**	**5.52 (3.30-9.24)**
No	16 (117/725)	1.00	
Premature infant: Yes	42 (36/85)	**3.16 (1.98-5.05)**	**2.34 (1.25-4.40)**
No	19 (137/726)	1.00	
Maternal morbidity during pregnancy			
Malaria: Yes	22 (75/344)	1.10 (0.79-1.51)	Not in the model
No	20 (121/596)	1.00	
Anaemia at any time: Yes	20 (150/738)	0.92 (0.62-1.35)	Not in the model
No	22 (41/188)	1.00	
Anaemia at delivery: Yes	19 (41/214)	0.87 (0.59-1.28)	Not in the model
No	21 (149/695)	1.00	
ARI: Yes	21 (57/274)	1.00 (0.70-1.41)	Not in the model
No	21 (139/666)	1.00	
UTI: Yes	25 (15/60)	1.29 (0.70-2.36)	Not in the model
No	21 (181/880)	1.00	
Infant morbidity during the first six months of life			
Number of morbid events: none	21 (25/122)	1.00	Not in the model
1 to 3	20 (104/531)	0.95 (0.57-1.59)	
≥ 4	23 (67/287)	1.18 (0.68-2.05)	

Malaria: Yes	16 (8/49)	0.73 (0.31-1.65)	Not in the model
No	21 (188/891)	1.00	
ARI: Yes	23 (90/393)	1.24 (0.89-1.72)	Not in the model
No	19 (106/547)	1.00	
Diarrhoea: Yes	20 (128/631)	0.99 (0.70-1.39)	Not in the model
No	20 (68/332)	1.00	
Hospitalised: Yes	30 (18/60)	1.69 (0.91-3.11)	Not in the model
No	20 (178/88)	1.00	
Socio-economic and environmental factors			
Birth in rainy season: Yes	22 (46/207)	1.00 (0.69-1.47)	Not in the model
No	22 (130/586)	1.00	
Camp: Shoklo	19 (64/331)	1.00	
Bonoklo	12 (8/65)	0.59 (0.24-1.35)	NS
Maesalit	13 (9/71)	0.61 (0.27-1.34)	
Klaymuta	13 (2/15)	0.64 (0.07-2.95)	
Maela	25 (113/458)	1.37 (0.95-1.96)*	
Year of birth: 1993	15 (13/89)	1.00	
1994	17 (42/248)	1.19 (0.58-2.48)	
1995	19 (56/302)	1.33 (0.66-2.71)	
1996	28 (85/301)	**2.30 (1.17-4.60)**	**3.61 (1.55-8.43)**
Exclusive breast-feeding during the first 6 months: Yes	21 (79/379)	1.00 (0.72-1.39)	Not in the model
No	21 (117/561)	1.00	
Time spend in Thailand (mother):			
< 10 years	22 (142/644)	1.17 (0.77-1.76)	Not in the model
≥ 10 years	20 (40/205)	1.00	
Mother's education: Low	26 (95/371)	**1.69 (1.10-2.59)**	NS
Average	19 (45/233)	1.17 (0.72-1.92)	
High	17 (42/238)	1.00	
Father's education: Low	26 (59/231)	**1.52 (1.00-2.31)**	NS
Average	21 (50/239)	1.17 (0.76-1.81)	
High	18 (66/359)	1.00	
Economic level: Low	25 (62/249)	1.21 (0.56-2.67)	Not in the model
Average	19 (92/480)	0.86 (0.41-1.86)	
High	22 (11/51)	1.00	

Bold: p<0.05
*: p≥ 0.05 and <0.10

8.2.2. Risk factors for impaired growth at one year of age.

At one year of age, only 44% (368/834) of the infants had reached an appropriate weight and height, and had a WHZ score above –2SD. Five per cent (44) of the infants were wasted (including 12 who were also stunted), 28% (231) were underweight and stunted, 13% (108) stunted but not underweight, and 10% (83) underweight but not stunted.

Black Karen infants were more likely to be wasted at 1 year of age than Karen children: 11% (10/93) vs 5% (34/741) (OR: 2.51; 95%CI: 1.19-5.26). Ten per cent (7/70) of the premature infants were wasted compared to 4% (29/665) of the children born at term (OR: 2.44; 95%CI: 1.03-5.79). Wasting was also associated with infant morbidity (OR: 1.07; 95%CI: 1.00-1.14 per morbid event). These 3 risk factors, Black Karen (Adjusted OR: 2.87; 1.29-6.41), premature birth (Adjusted OR: 2.77; 95%CI: 1.14-6.70) and number of morbid episodes (Adjusted OR: 1.07; 95%CI: 1.00-1.15), remained associated with wasting in the multivariate model. The prevalence of premature births among wasted infants was 19% (7/36), giving a PAF of wasting due to premature birth: 12% (95%CI: 2-17).

Univariate analysis was performed in order to identify which of the exposure factors already tested for anaemia (see **chapter 7.4.2.4**) were associated with underweight and/or stunting at one year of age. The results are summarised in **tables 8.3 and 8.4**. In multivariate analysis, LBW and low father's education remained associated with both underweight and stunting. Male infants and children born in 1995 and 1996 were also at risk of being stunted at 1 year. Twenty-three per cent of underweight (72/320) and/or stunted (72/318) infants were born with LBW. The PAF of underweight due to LBW was 14% (95%CI: 8-17), and PAF of stunting due to LBW was: 16% (95%CI: 12-19).

Table 8.3: Risk factors of being underweight at 1 year of age.

	Prevalence of underweight infants % (n)	OR (95%CI)	Adjusted OR (95%CI)
Severe maternal anaemia (HCT<25%) at delivery: Yes	67 (14/21)	**2.75 (1.10-6.88)**	NS
No	42 (334/793)	1.00	
Birth order: First	35 (71/203)	1.00	
Second	39 (62/160)	1.18 (0.75-1.85)	
Third or above	47 (224/478)	**1.64 (1.15-2.34)**	NS
LBW: Yes	66 (72/110)	**3.06 (2.01-4.68)**	**2.57 (1.51-4.38)**
No	38 (248/649)	1.00	
Premature: Yes	61 (43/70)	**2.36 (1.42-3.91)**	NS
No	40 (270/670)	1.00	
Sex: Male	47 (199/427)	**1.41 (1.07-1.86)**	NS
Female	38 (158/414)	1.00	
Nu of diarrhoeal episodes: 0-1	40 (230/581)	1.00	
≥ 2	49 (127/260)	**1.46 (1.07-1.98)**	NS
Exclusive breast-feeding during the first 6 months: Yes	36 (58/160)	0.71 (0.49-1.03)*	NS
No	45 (218/490)	1.00	
Father's education: Low	50 (110/220)	**1.57 (1.13-2.18)**	**1.80 (1.14-2.83)**
Average/High	39 (219/563)	1.00	
Mother's education: Low	48 (170/357)	**1.82 (1.27-2.60)**	NS
Average	43 (94/221)	1.48 (0.99-2.21)*	
High	33 (77/231)	1.00	

Bold: $p<0.05$

*: $p \geq 0.05$ and <0.10

Table 8.4: Risk factors of being stunted at 1 year of age.

		Prevalence of stunted infants % (n)	OR (95%CI)	Adjusted OR (95%CI)
LBW:	Yes	66 (72/200)	3.15 (2.10-4.94)	**3.54 (2.21-5.67)**
	No	38 (246/644)	1.00	
Premature:	Yes	59 (42/71)	2.13 (1.26-3.61)	NS
	No	41 (269/665)	1.00	
Sex:	Male	52 (112/217)	1.72 (1.24-2.38)	**1.63 (1.17-2.26)**
	Female	38 (215/561)	1.00	
Mother's gravidity:	Primigravidae	36 (72/200)	1.00	
	Multigravidae	44 (279/635)	1.39 (0.99-1.50)	NS
Father's education:	Low	47 (200/427)	1.50 (1.13-2.00)	**1.70 (1.15-2.53)**
	Average/High	37 (151/408)	1.00	
Born in:	1993	35 (24/68)	1.00	
	1994	36 (62/174)	1.01 (0.54-1.90)	NS
	1995	44 (135/304)	1.46 (0.82-2.62)	**1.97 (1.02-3.82)**
	1996	45 (130/285)	1.50 (0.84-2.69)	**2.12 (1.10-4.09)**

8.2.3. Growth of LBW infants

Fig 8.3 and 8.4. represent the growth curves of LBW compared to that of NBW infants. LBW infants never catch up weight nor height during infancy.

fig 8.3: Weight growth curves of NBW and LBW infants

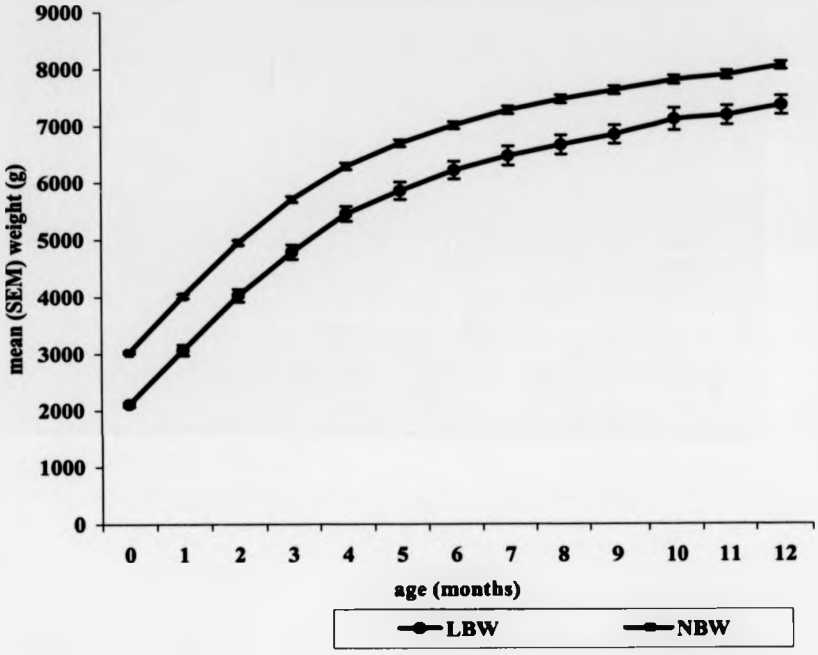
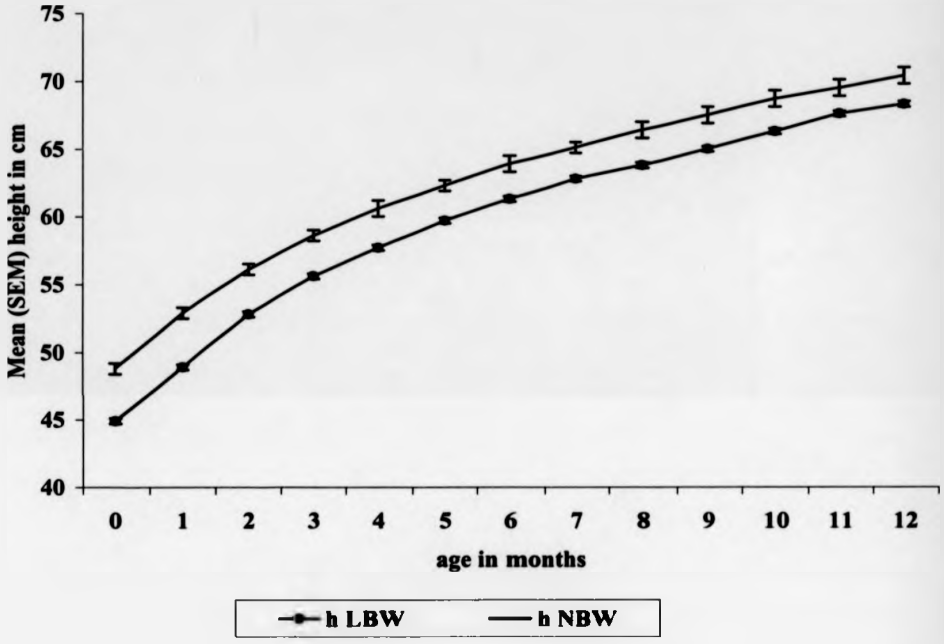


Fig 8.4: Height growth curves of NBW and LBW infants



8.3. Summary of the results contained in this chapter

Malaria during pregnancy had an indirect effect on infant growth through LBW that was responsible for 14% to 36% of impaired growth during infancy (underweight and/or stunted). Premature birth was more responsible for being underweight (29%) than stunting (12%) at 6 months of age, and was not independently associated any more with impaired growth at one year of age. Nevertheless, prematurity was responsible for 12% of wasting at one year of age.

CHAPTER 9

DISCUSSION

9.1. Study population

The epidemiological study described in this thesis encountered some problems in its realisation. The first was the important population movements that occurred in the middle of the study and that led to a much higher proportion of infants lost to follow-up than expected. Nevertheless, the reasons for leaving the cohort were not associated with the exposure factors that were tested or with the outcomes. Withdrawal from the study was mainly due to security reasons and occurred in periods of crisis. In each period, infants from all ages were lost. Maternal characteristics identified as risk factors of being lost during infancy were: recent arrival in Thailand and registration for ANC late in pregnancy. Refugees who had just arrived in Thailand, were probably more inclined to leave and go back to Burma when problems occurred in the camps. Late attendance to ANC was probably also a marker of unstable population. Women who attend ANC at the end of their pregnancy are often working outside and moving readily. These groups of refugees who are not living permanently and for long time in the camps may also differ in their risk of developing malaria. But this was not evident in the cohort.

Although a quarter of the infants admitted in the cohort, were affected by the population movements, outcomes were reliably collected. Karen midwives and home-visitors were living within the community. They were therefore displaced at the same time and were able to locate the children easily. Thus they reported immediately on children's departure to Burma. When the populations from 3 camps were displaced to Maela, the Karen home-visitors from these sites were able to call the children to the SMRU's clinic in Maela, as soon as they arrived. They were given a new code and follow-up was pursued. Thus overall, the proportion of infants lost to follow-up was high (25%) but the quality of the follow-up was good. This is demonstrated by the high proportion (80%) of children that was seen each month at the clinics and the reality of longitudinal follow-up (95% of them seen at least twice between 1 and 12 months of age and a median number of 8 visits in the year).

The second major issue was the dramatic decrease of the two main outcomes, infant mortality and malaria during infancy that occurred during the data collection. There is no doubt that this fact may have reduced the power of the study. An attempt was done to recalculate the sample size needed for infant mortality and a sufficient

number of newborns was recruited. There was no solution for the reduction in malaria incidence during infancy. At the end of the study, only Maela camp was stable but malaria incidence there was extremely low. The situation would not have been modified if more children had been recruited there. Thus it was not possible to study in detail the relationships between malaria during pregnancy and malaria in infancy, in particular numbers became too small when infant malaria incidence rates were calculated by age and by *Plasmodium* species.

In order to have reliable information on morbidity during pregnancy, only children born to mothers who attended ANC were recruited. This may have introduced a selection bias, as the women who were not coming to the clinics may well be a different group. Nevertheless, 90% of the pregnant women in the camps attend ANC and this proportion remained fairly constant over the years. The inconvenience of missing 10% of the pregnant women was greatly compensated by the quality of information collected on women who were followed prospectively during pregnancy, half of them from the first trimester of gestation. Moreover in this area, all patients with *P.falciparum* malaria and most with *P.vivax* malaria develop symptoms (Luxemburger *et al.*, 1996). Thus a history of slide-confirmed malaria episode is a good proxy for malaria infection. This might differ in pregnant women who sequester the parasites in the placenta and may have some undetectable malaria infections. But the extent of the phenomenon in this population is unknown. Malaria during pregnancy was detected by recall of slide-confirmed episodes until registration for ANC and presence of asexual parasites in peripheral blood throughout the follow-up. Thus this study was the first reported cohort of mothers and infants for whom malaria history was known from conception to the first year of life.

Pregnant women in this study were a sub-group of 11,000 pregnancies followed in all camps in the area. They were remarkably similar in age, gravidity, malaria and anaemia history to the entire population. Malaria and anaemia during pregnancy had the same consequences on the newborn in both this cohort and the larger database (Nosten *et al.*, 1991; Nosten *et al.*, in press). Thus the infants in the cohort could be considered as a good representative sub-group of the children born to all pregnant women in the area.

9.2. Consequences of maternal malaria and anaemia on the newborn infant.

The proportion of LBW (16%) in the cohort was comparable to that of neighbouring Thai and Burmese populations (de Onis *et al.*, 1998). Nearly all (91%) of the children weighing less than 2000 g were premature, whereas the majority (68%) of those weighing between 2000 and 2499g were born at term and therefore growth-retarded. Some premature babies were probably also growth-retarded but this was more difficult to assess. Overall, around 10% of the newborns were premature (including some weighing more than 2500 g) and 8% were term-LBW. This situation was in accordance with the general assumption that the excess of LBW in developing countries is mainly due to IUGR, whereas the proportion of premature births is fairly stable worldwide (Villar & Belizan, 1982). Thus in developing countries the aim of prenatal care is to reduce the excess of moderate LBW (2000-2499g) due to infections and/or nutritional deficiencies during pregnancy.

Malaria during pregnancy was associated with LBW. Numerous studies have already shown this association. Most were conducted in areas of high malaria transmission (reviewed by Brabin, 1991; Menendez, 1995). But the association had also been demonstrated at the study site, an area of low malaria transmission (Nosten *et al.*, 1991), and with vivax malaria as well as *P.falciparum* infection (Nosten *et al.*, in press). In this cohort, information on SES was collected and did not modify the results. Malaria during pregnancy was an independent determinant of LBW and accounted for 23% of them. Adjustment for SES had not been done in previous published reports, and this was mentioned as a possible confounding factor in the association between malaria during pregnancy and LBW (Kramer, 1987). In this study, both malaria species were determinant of LBW, after adjusting for SES and environmental factors. All anthropometric markers were reduced in children born to malaria-infected mothers, suggesting that the infants suffered from chronic and symetric growth retardation (Meuris *et al.*, 1993).

Malaria during pregnancy did not reduce the duration of gestation, unless a symptomatic or severe episode occurred during the last trimester of pregnancy. These findings were similar to that of the few studies in which gestational age was assessed. Most reported that malaria during pregnancy was mainly related to IUGR (Reinhardt *et*

al., 1978b; Watkinson *et al.*, 1983; Dolan *et al.*, 1993; Meuris *et al.*, 1993; Nosten *et al.*, 1994a; Nosten *et al.*, in press). Taha *et al.*(1993a) and D'Allessandro *et al* (1996) reported an association between malaria and premature delivery, but both were probably describing the effects of symptomatic malaria. Symptomatic and severe malaria has been known for long time to be associated with high rate of prematurity (Wickramasuriya, 1937; Le Van Hung, 1951; Endeshaw, 1991; Nair & Nair, 1993). In this study, symptomatic malaria from both species, led to premature deliveries, but to a similar extent to other febrile infections (ARI and UTI). This event (i.e. malaria close to term) was rare, and only an estimated 5% of the premature birth could be attributable to symptomatic malaria.

Maternal anaemia during pregnancy was associated with LBW in univariate analysis, and the risk was more important when maternal anaemia was severe. But maternal anaemia had no independent effect on birthweight in multivariate analysis. In a malaria-endemic area of Papua New Guinea, Brabin & Piper (1997) demonstrated that malaria was a more important risk factor than anaemia for LBW. Moreover, in their study, only severe anaemia had an independent effect on LBW. In the current work, maternal anaemia was relatively moderate. Only 17% of the women developed severe anaemia, and most of them (91%; 226/247) had an HCT between 20 and 25%. Thus the lack of association between maternal anaemia and LBW in this study, was possibly due to the moderate level of anaemia.

In summary, malaria during pregnancy was responsible for a substantial number of growth-retarded newborn and for a small proportion of premature births. Maternal anaemia had no independent effect on the newborn.

9.3. Infant mortality

Infant mortality remained higher than in surveys conducted in Thailand (Lumbiganon *et al.*, 1990) and Vietnam (Swenson *et al.*, 1995), but was similar to Java, Indonesia (Handayani *et al.*, 1983) and lower than Bangladesh (Chen *et al.*, 1980; Islam *et al.*, 1982; Bhatia *et al.*, 1989). But the more important fact was that IMR was halved since the last studies conducted in this population (Dolan *et al.*, 1993; Nosten *et al.*, 1994a). This was mainly due to a decrease of post-neonatal mortality and an important factor was the reduction of deaths due to thiamin deficiency (from 38 to 7%). Apart from this local specificity of deaths from thiamin deficiency, the causes were very similar to that of most developing countries. The majority of deaths were due to infectious diseases and "prematurity" (Murray & Lopez, 1997). ARI and diarrhoea were the leading causes of infant deaths from infectious diseases, as in most studies summarized in **table 2.2**. Malaria was responsible for only 2% of the infant deaths, reflecting the low transmission in this area. Tetanus was rare and severe measles absent in this well-vaccinated cohort.

Premature birth was the strongest risk factor for neonatal death. LBW and maternal infection within the week preceding delivery were the others main risk factors that could be identified. The latter remained an important risk factor for deaths in the first 3 months of life. There were no risk factors identified for infant deaths that occurred after three months of age, but numbers were small.

Thus malaria itself (i.e. except symptomatic attack close to term) during pregnancy was not directly associated with neonatal deaths. This was similar to previous studies in which malaria during pregnancy did not increase the risk for neonatal deaths (Bruce-Chwatt, 1952; Cannon, 1958; Spitz, 1959; Jilly, 1969; McGregor *et al.*, 1983; Taha & Gray, 1993b; McDermott *et al.*, 1996). Nevertheless, there was obviously an indirect effect of malaria during pregnancy on neonatal mortality, all mediated through LBW. Malaria during pregnancy was responsible for a quarter of LBW newborn, who were more likely to die during the neonatal period. But malaria was mainly associated with LBW due to IUGR, and this group had less risk to die than pre-term infants. Thus the effects of malaria during pregnancy on neonatal mortality are likely to be smaller than expected. This aspect should be integrated into

the estimation of the impact of prevention against malaria during pregnancy on neonatal survival (i.e. sample sizes of further studies should be increased if the outcome is the survival of neonates).

Maternal infections close to term had an additional effect on neonatal deaths, as well as inducing premature birth. This was possibly the effect of a septic environment at birth. Premature delivery is a well-known consequence of symptomatic and/or severe malaria during pregnancy in areas low malaria endemicity or during epidemics (Wickramasuriya, 1937; Le Van Hung, 1951; Endeshaw, 1991; Nair & Nair, 1993; Luxemburger *et al.*, 1997). The risk of premature delivery and/or neonatal deaths was similar for ARI or UTI, reflecting an effect of fever rather than malaria *per se*. Nevertheless, as malaria is a major cause of morbidity in this area, the infant deaths occurring after symptomatic maternal malaria could be prevented by a reduction of this disease during pregnancy.

When adjusted for covariates maternal anaemia was not independently associated with neonatal nor post-neonatal deaths. This result was in contradiction with other studies. Maternal anaemia in malarious areas has been shown to be related with perinatal deaths in several studies (Tasker, 1958; Llewellyn-Jones, 1965; Macgregor, 1963; Brabin *et al.*, 1990a). But women in these studies were severely anaemic whereas most pregnant women in this cohort were moderately anaemic (only 3% had an HCT<25% at delivery; median: 22%; range: 19 to 24.5%) and all anaemic women were receiving iron and folic acid treatments. The association between maternal anaemia and infant death that was also reported in the previous studies conducted in this area (Dolan *et al.*, 1993; Nosten *et al.*, 1994a) and the current work did not confirm this findings. Various hypotheses can be proposed. The number of post-neonatal deaths was very small in the current cohort and thus the study may have lacked of power to detect any impact of maternal morbidity on late infant mortality. The reduction in deaths between the 2 previous studies and the current one was mainly contained in the post-neonatal period and the proportion of deaths from thiamin deficiency decreased considerably. Thus in the previous studies, maternal anaemia may have been a marker of a broader nutritional deficiency. If anaemic mothers were also more often thiamin deficient, their offspring would have had more risk to die later from infantile Beri-Beri. But data on the relationships between thiamin deficiency and anaemia in pregnant women and in infants

are lacking to confirm this hypothesis. Another possibility was that SES factors were confounders in the previous studies, but this type of data was not always collected. Some women were interviewed after the completion of the impregnated bed nets study and there was no association between SES factors and infant deaths. In conclusion, this study did not confirm that maternal anaemia was a risk for infant deaths, but IMR had been halved since the previous studies. Moreover, this association was recently found in an area of intense malaria transmission in Malawi (Verhoeff & Brabin, personal communication).

9.4. Infant morbidity

9.4.1. Overall impact of morbidity during pregnancy on morbidity in infancy.

ARI and diarrhoea were the leading causes of morbidity during the first year of life, whereas malaria was not frequent. Several morbid events during pregnancy (ARI, UTI, paresthesia, malaria in early pregnancy) were associated with an increased infant morbidity. Malaria during pregnancy had also an indirect effect mediated through LBW. Term-LBW infants were at higher risk of morbidity from all causes than NBW children, mainly through an increased number of diarrhoeal episodes. This findings was expected from the results of previous studies (Victora *et al.*, 1990; Bukenya *et al.*, 1991; Ittiravivongs *et al.*, 1991; Barros *et al.*, 1992; Lira *et al.*, 1996). Surprisingly, preterm-LBW infants seemed to have lower morbidity than NBW children, except for dirrhoeal episodes. One explanation could be that only healthier premature infants survived.

Several SES factors were also associated with infant morbidity. A recent arrival in Thailand, a low father's education, a low economic level, and a large family (3 children or more) were risk factors for infant morbidity. It was slightly surprising that father's education appeared as a more important factor that mother's education (except for ARI) to prevent morbidity throughout pregnancy and infancy. Mother's education is usually recognized as an important element in public health (Ware, 1984). In this study, a low mother's education was often a risk factor for morbidity in univariate analysis, but did not remain in model including father's education. This was observed by other authors who pointed out that the association between mother's education and infant

health might be confounded by other aspects of SES (Desai & Alva, 1998). Male infants had a higher morbidity during infancy than female. In this community, there are no obvious gender-related behaviours towards infant health. The birth of a girl is welcomed, and female children are encouraged to attend school. Adult women have important roles and may become community leaders. It is therefore unlikely that male infants would have been taken more easily to health facilities. Moreover, morbid events were actively reported by home-visitors.

The mechanisms through which maternal ARI or UTI could be a risk factor for infant ARI or diarrhoea remained unexplained. They could be related to some environmental factors of the household (tobacco, hygiene) that the current work was unable to detect. Malaria during infancy was influenced by the stage at which the first malaria episode occurred during pregnancy and this finding will be discussed below.

9.4.2. Malaria during infancy and its relationships with maternal malaria during pregnancy.

Morbidity and mortality from malaria during infancy were low, as expected in this hypo-endemic malaria area. As a result, malaria was only marginally associated with anaemia and did not influence growth. This is a very different picture from that of areas with high malaria transmission, where malaria is the single most important risk for infant anaemia (Renaudin & Lombart, 1994; Achidi *et al.*, 1996b; Kitua *et al.*, 1997) and seems to influence growth (Shiff *et al.*, 1996; Snow *et al.*, 1997b). This could be explained by the high proportion of *P. vivax* malaria which is only rarely associated with anaemia, but the more likely explanation remains that malaria incidence is too low to have a prolonged effect on HCT levels. Vivax malaria has been associated with malnutrition (Williams *et al.*, 1997), but this was not confirmed in this study. The risk to develop severe falciparum malaria was higher than in areas of high transmission and when severe malaria occurred, the CFR was of similar magnitude than that reported in areas of high transmission (Duren, 1951; Greenberg *et al.*, 1989). The definition of severe malaria in this area differs from that of high malaria endemic regions. Most of the severe cases presented with hyperparasitaemia without vital organ dysfunction. But, this clinical feature has been shown to be associated with mortality in this population

(Luxemburger *et al.*, 1997). This previous work also gave more reliable CFR than the estimation obtained in this cohort, in which numbers were small. The present work confirmed that falciparum malaria is rare in infants from this population, but when it does occur, the risk of developing severe disease and death is high.

As noticed previously in the study area (Luxemburger *et al.*, 1996) and in all places where *P.vivax* co-exists with *P.falciparum* (Khan & Talibi, 1972; Genton *et al.*, 1995; Maitland *et al.*, 1996), vivax malaria developed earlier in infancy than falciparum malaria. The mechanisms of this phenomena remain unexplained. Infection with *P.vivax* did not influence the course of subsequent *P.falciparum*, in term of severity, but numbers were very small.

The children were protected against malaria in early infancy (<3 months) and were 5 times more likely to have a clinical malaria attack later in life (3-12 months). Protection of young infants against clinical malaria is widely recognized (Akanmori *et al.*, 1989; Velema *et al.*, 1991; Petersen *et al.*, 1994; Binka *et al.*, 1994; Kuate Defo, 1995; Achidi *et al.*, 1996a; McGuiness *et al.*, 1998; Snow *et al.*, 1998; Wagner *et al.*, 1998), but the current work is the first report in a low malaria endemic area, and regarding *P.vivax* as well as *P.falciparum*. Protection against malaria in the first 3 months of life was independent from malaria history during pregnancy. Moreover, maternally derived antibodies were likely to vary widely, as it has been described previously in areas of low malaria transmission (Collins *et al.*, 1987). Thus this work supported the hypothesis that other factors may act with maternal transmitted antibodies to protect against malaria in early infancy.

The stage at which malaria occurred in pregnancy influenced malaria in infancy. Children born to mothers who had their first falciparum episode in early pregnancy were more likely to have malaria during infancy than those born to mothers who had malaria later in pregnancy or no malaria. This remained true when analysis was restricted to women without *P.vivax* infection, in order to avoid potential interactions between *P.falciparum* and *P.vivax*. Half of the episodes of falciparum malaria that occurred during the first trimester of pregnancy were detected prior to the registration of the women in ANC (i.e. they were detected passively), whereas the majority of episodes occurring later in pregnancy were detected during ANC follow-up (i.e. by a

combination of weekly active detection and passive detection if the women attended the clinic with fever in between two visits). This may have lead to a higher proportion of symptomatic episodes during the first trimester and thus higher parasite densities that could induce a different materno-foetal immuno-reaction. But, among the first detected falciparum malaria infections, the proportions of symptomatic and/or severe malaria episodes were comparable in each trimester of pregnancy.

These results could be the first clinical support to the hypothesis that early *in utero* exposure to malaria antigens leads to tolerance towards malaria during infancy (Rasheed, 1994). Soluble *P.falciparum* antigens are transferred to the foetus (Druilhe *et al.*, 1976; Jakobsen *et al.*, 1998), whereas this has not been reported, and indeed never studied, for *P.vivax*. If only *P.falciparum* antigens reached the foetus, this could explain that the timing of vivax malaria during pregnancy did not influence malaria in infancy. Surprisingly, falciparum malaria in early pregnancy was associated with a higher risk of malaria by either species during infancy. If the hypothesis of tolerance is retained, this findings imply that early *in-utero* exposure to falciparum malaria antigens induces a non-specific cross-species immuno-tolerance. Further studies are urgently needed in order to support or refute this hypothesis, because it could modify the policy of interventions against malaria during pregnancy. In most areas, women are reached only late in pregnancy, whereas if the hypothesis of tolerance against malaria when the exposure occurs in early pregnancy is true, prevention should start much earlier.

Another hypothesis to explain this finding is linked to behaviour and environmental factors. Pregnant women who had falciparum malaria in early pregnancy were also more likely to have a past history of malaria. They were therefore either more mobile, or living closer to a vector breeding site, than other pregnant women. Although it could not be demonstrated, it is possible that children born to these women were also at higher risk of malaria than their counterparts, because at this age they stay with their mothers (including going to the forest) and therefore share their behaviour and environment. Similarly, the excess of malaria in Karen children compared to those from other ethnic group was more likely to be related to the life of these infants than to genetic differences. Karen women regularly go to the forest, which is a risk factor for infection with malaria (Luxemburger *et al.*, 1996), and they take their infants with them,

whereas Black Karen women and their babies are more likely to stay in the camp where they have shops.

9.4.3. Infant anaemia

Anaemia increased with age and reached 20% in 12 month old infants. Studying the HCT profiles revealed that infants from the cohort had an HCT level only slightly lower than that of a reference population (Saarinen *et al.*, 1978) until 2 months of age, but they did not recover from physiological anaemia. From 3 months onwards, HCT levels regularly decreased. This phenomenon was even more marked in LBW infants. Anaemia in LBW infants is a well-reported fact, but is usually related to prematurity (Stockman & Oski, 1978) and not well-described for full-term LBW infants. In this study, LBW was associated with anaemia throughout infancy. Infant anaemia was thus an indirect consequence of malaria during pregnancy. Nevertheless, as age increased, SES (time spent in Thailand, father's education) and other factors (ethnic group, gender) became more important determinants of infant anaemia than LBW.

Contrary to the findings of Redd *et al.* (1994) and Cornet *et al.* (1998) there was no direct relationship between malaria during pregnancy and anaemia in early infancy. But the authors of these studies reported an association between placental malaria and infant anaemia. They suggested that physical alterations of the infected placentas may decrease nutritional exchanges or that immunologic mechanisms were responsible for haemolysis. Although placental malaria is sometimes detected by histological examination in our study site, the parasite burden does not reach the level seen in areas of intense malaria transmission. This may explain why malaria during pregnancy was not associated with infant anaemia in this study. Nevertheless, malaria in early pregnancy possibly had an effect on infant anaemia through an increase in infant malaria.

From 6 months onwards, SES factors and infant malaria history were related to infant anaemia. The time spent in the camp was inversely associated with the risk of developing anaemia. Recent refugees were likely to have a lower income and food diversity, as time is needed to find work and establish a garden, and a different approach

towards health structure. They were usually in poorer health than families already living in the camps for many years. Infant malaria was related to infant anaemia at 6 months but was no longer related at 12 months. Malaria during infancy was not common enough to explain the important drop in HCT levels during late infancy. Hookworm infestation was also rare (4% in a survey conducted in the cohort in 1996). Thus a more likely explanation for these poor HCT levels was nutritional deficiency, mainly iron deficiency, although this could not be confirmed biologically. But, a recent nutritional survey conducted in the camps (BBC, 1997) concluded that the refugee's food ration contained only 50% of the daily requirement in iron. Thus pregnant and lactating women, who need double that amount, did not have adequate iron intake. Most infants were fully breast-fed for the first few months and then received weaning food with poor iron contents (rice, banana). Most infants were probably iron-deficient. Further studies should be conducted in order to assess the effects of iron supplementation in these infants.

Male infants were more likely to be anaemic at 1 year of age than female. This sex-difference was described in previous studies (Serjeant *et al.*, 1980; Hassan *et al.*, 1997; Paracha *et al.*, 1997) without clear explanation. Boys from the cohort had also more morbid episodes than girls, but it was difficult to define which one, between anaemia and morbidity, was the cause or the consequence of the other.

Karen infants were more often anaemic than Black Karen. This seemed independent of their higher risk of developing malaria during infancy. Again, the most likely explanation was nutritional. The Muslims community has a more diverse diet, for cultural and economic reasons. While Karen usually eat large amounts of rice with few vegetables and fish paste, the diet of Black Karen contains various recipes, close to that of South Asia. Moreover, they own the shops and are less poor.

9.5 Infant growth

Growth of this birth cohort was constantly lower than that of the NCHS/WHO reference population. They even did not catch-up temporarily at 3 months of age, like in other population from developing countries (Cohen *et al.*, 1995; Asefa *et al.*, 1996; Kolsteren *et al.*, 1996). By the age of one year, 42% of the infants from the cohort were stunted, a proportion similar to the estimation from WHO for South-East Asia (WHO, 1992). Their attained weight and length at one year of age were in the same ranges as those of a neighbouring population from another ethnic group in rural Thailand (Gershoff *et al.*, 1977).

Mother's morbidity during pregnancy did not influence growth directly, but again was indirectly associated with impaired growth (underweight and/or stunting), for which LBW was the strongest risk factor. The growth curves of LBW Infants never caught up relative to those of their NBW peers. Most of the surviving LBW children in this cohort were growth-retarded rather than premature, and small-for-gestational age infants remained lighter and smaller throughout infancy, compared to children born with appropriate weight-for-age (Adair, 1989; Kedebe & Larson, 1994; Cohen *et al.*, 1995). SES was associated with impaired growth, as it was with anaemia. Children born in 1995 and 1996 were more often stunted than those born in other years. This corresponds to birth in the new camp, in which population movements were restricted and where the number of gardens and poultry was low. Refugees living in this camp could not supplement their food ration, as it was the case in other camps.

Infant's morbidity was also not associated with attained weight and length at one year of age. This result was similar to that of a study conducted in Indonesia, in which morbidity did not affect growth performance (Kolsteren *et al.*, 1997). Nevertheless, male infants were more often stunted at one year of age than female and a possible explanation for this was that they had more morbid episodes, especially from ARI, than the girls. Moreover, the number of morbid events during the first year of age was associated with wasting. The analysis of attained growth by 6 and 12 months was possibly insufficient to demonstrate the relationships between morbidity and growth during infancy.

9.6. Summary and conclusions

This thesis reported the results of the first cohort of mothers and infants conducted from the beginning of pregnancy until the end of the first year of life, in order to assess the effects of malaria and anaemia during pregnancy on the survival, morbidity and growth of the infants. Malaria was an important determinant of LBW due to growth retardation rather than prematurity. Most of the effects of malaria during pregnancy on infant health were mediated through the reduction in birthweight. LBW infants were more likely to die during the neonatal period, and those who survived had a higher incidence of morbid events during infancy, compared to infants weighing more than 2500g at birth. LBW children developed anaemia by three months of age and remained anaemic throughout infancy, although in the second half of infancy SES and environment seemed to become more important risk factors for anaemia than LBW. Similarly, LBW infants never catch-up in term of growth and remained lighter and shorter than NBW infants, but SES factors were important determinants of stunting at one year of age.

Malaria during pregnancy had also an effect on infant survival, when an attack occurred close to term. Fever (due to malaria, ARI or UTI) in the week prior to birth was responsible for 10% of the premature births, and prematurity was the main risk factor for neonatal deaths. Fever prior to birth had an additional direct effect on infant deaths during the first three months of life.

The reduction in birthweight due to malaria during pregnancy has been demonstrated for a long time (Brabin, 1991). It was assumed that malaria had therefore an indirect effect on infant mortality and that prevention of malaria during pregnancy might reduce this effect (Greenwood *et al.*, 1989). This study clearly showed that malaria from both species, were important determinants of LBW, even after adjustment for SES, and had indirect effect on infant survival. Nevertheless, as growth-retarded infants were less likely to die than premature, and that only a small proportion of the latter were due to the fever associated with malaria, the overall impact of malaria on mortality was more limited than expected. Thus very effective large-scale interventions against malaria might be needed in order to reduce infant mortality.

Contrary to previous studies (Brabin *et al.*, 1990a; Dolan *et al.*, 1993; Nosten *et al.*, 1994a) maternal anaemia during pregnancy had no independent effect on infant survival. SES might have be confounders in the previous studies. Other hypotheses were that maternal anaemia was too moderate in the women of this cohort, or that the reduction of infant mortality over time might have led to a lack of power for the study to be able to demonstrate the association.

Infants born to mothers who had falciparum malaria in early pregnancy were more likely to develop malaria from any species during infancy, when compared to children whose mothers had malaria later in pregnancy or who did not had malaria. Although the existence of exposure behaviours common to the mother and her child could not be ruled out, these results were the first epidemiological report supporting the hypothesis of immuno-tolerance in infants exposed to malaria antigens early *in utero*. Similar studies need to be conducted in areas of different endemicity in order to compare the results.

In conclusion, this work demonstrated that the effects of malaria during pregnancy are not limited to the live newborn but extend during the entire infancy. LBW results in higher mortality and morbidity, and impaired growth, and a quarter of LBW is due to maternal malaria during pregnancy. Moreover, malaria during early pregnancy is associated with an increased risk of developing malaria in infancy. Thus, the current work not only confirms that preventing malaria during pregnancy is a major public health goal in malarious areas, but also that interventions against this parasitic infection should be implemented as early as possible during pregnancy.

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CB: |__|__|__|

MOTHER'S MORBIDITY DURING PREGNANCY

CODE: CB |__|__|__| OS CODE: |__|__|__| ANC CODE: |__|__|__|__|

NAME ..... AGE: |__|__|

HOUSE: |__|__|__| SECTION: |__|__|

GRAVIDITY: |__|__| PARITY: |__|__|

DATE OF FIRST CONSULTATION: |____|____|____|

DATE OF DELIVERY: |____|____|____|

NUMBER OF CONSULTATIONS: |__|__|

MALARIA HISTORY

MALARIA BEFORE PREGNANCY: YES / NO

MALARIA ATTACKS BEFORE ANC ATTENDANCE:

NO	DATE	SPECIES	COUNT	SYMTOMS	TREAMENT	STUDY CODE

MALARIA ATTACKS DURING ANC ATTENDANCE:

NO	DATE	SPECIES	COUNT	SYMTOMS	TREAMENT	STUDY CODE

CB: |__|__|__|

HAEMATOCRIT

HCT ON ADMISSION: |__|__|. |__| LOWEST HCT: |__|__|. |__|

HCT AT DELIVERY: |__|__|. |__|

NUMBER OF WEEKS WITH HCT<30%: |__|__|

NUMBER OF WEEKS WITH FFA: |__|__|

TRANSFUSION: YES / NO IF YES, DATE: |____|____|____|

B1 HISTORY

B1 SYMPTOMS: YES / NO IF YES: 1. NUMBNESS
2. CARDIAC SIGNS
3. NEUROLOGICAL SIGNS

NUMBER OF WEEKS WITH B1 TREATMENT: |__|__|

OTHER MORBIDITY

DATE	DIAGNOSIS	TREATMENT

DELIVERY

IN HOSPITAL: YES / NO

NORMAL: YES / NO IF NO, SPECIFY: .....

POST-PARTUM

NORMAL: YES / NO IF NO, SPECIFY: .....

PREVIOUS CHILD:

TIME SINCE LAST BIRTH (MONTHS): |__|__|

SURVIVED ONE YEAR: YES / NO

CB: |__|__|__|

SOCIO-ECONOMICAL QUESTIONNAIRE

Code: |__|__|__| Name: .....

Age: |__|__| Ethnic group: ..... |__|

House number: |__|__|__| Section: |__|__|

1. When did you arrive in Thailand: |__|__| years.

2. Where did you live before you arrived in Thailand?

   1. village in Karen Side: ..... |__|
   2. township or big city : .....

3. Which activity did you have before you arrived in Thailand?

   1. farmer |__|
   2. home work
   3. student
   4. other (specify): .....

4. How many years did you go to school? |__|__|

5. Standard level: |__|__|

6. What languages do you speak? (one or many answers):

   1. po karen
   2. sgaw karen
   3. burmese
   4. English |__|
   5. other (specify)

7. What languages do you write? (one or many answers):

   1. po karen
   2. sgaw karen
   3. burmese
   4. English |__|
   5. other (specify)

8. Which work did you do during your pregnancy?

   1. house work only |__|
   2. teacher/ nurse/ lab technician
   3. rice field/ forest
   4. other (specify): .....

CB: |__|__|__|

9. Did you get for this work:
   1. some money |__|
   2. some food
   3. other (specify)
   4. no answer

10. Is your husband usually:

   1. living in the camp |__|
   2. working outside
   3. in the front line
   4. other (specify): .....

11. If he is working, does he get:

   1. some money |__|
   2. some food
   3. other (specify)
   4. no answer

12. Which activity did he have before he arrived in Thailand?

   1. farmer |__|
   2. student
   3. other (specify): .....

13. How many years did your husband go to school? |__|__|

14. Standard level: |__|__|

15. What languages does he speak? (one or many answers):

   1. po karen
   2. sgaw karen
   3. burmese
   4. English |__|
   5. other (specify)

16. What languages does he write? (one or many answers):

   1. po karen
   2. sgaw karen
   3. burmese
   4. english |__|
   5. other (specify)

17. What is your religion?

   1 buddhist |__|
   2 christian
   3 muslim
   4 other

CB: |__|__|__|

18. How many of the following do you own?:

1. chicken: |__|__|
2. pig or goat: |__|__|
3. cow: |__|__|
4. radio: |__|__|
5. watch: |__|__|

19. Do you grow your own fruit or vegetables? YES / NO

20. How many people are usually living at your home: |__|__|

21. How many refugee's food ration do you get: |__|__|

Interviewer's impression:

22. General appearance of the visited house:

1. rich |__|
2. average
3. poor
4. very poor

25. Is the house:

1. very tidy |__|
2. tidy
3. dirty

26. Is the house built with:

1. bamboos and leaves only |__|
2. bamboos, leaves and pieces of wood
3. leaves only (roof and walls)

27. What furnitures are there (table, chair, cupboard, bed):

.....

Interviewer's name: .....

Date: .....

CB: |__|__|__|

Home visitor's name: .....

CB code: .....

Child's name: ..... Mother's name: .....

Date	Present (YIN)	Ill (YIN)	Date	Present (YIN)	ill (YIN)

CB: |__|__|__|

INFANT OPD CONSULTATION

Name:.....

Date: .....Consultation number: |__|__|

Symptoms:

* Fever : Y / N / DK .....days

* Convulsions : Y / N / DK .....times

* Sucking well : Y / N / DK .....days
(or eating)

* Vomiting : Y / N / DK .....days
.....times/day

* Diarrhoea : Y / N / DK .....days
..... times/day
type (bloody, watery..): .....

* Abdominal pain: Y / N / DK ..... days

* Runny nose : Y / N / DK ....days

* Coughing : Y / N / DK .....days

sputum: Y / N / DK
type (white, green, blood): .....

* Difficulties to breathe: Y / N / DK .....days

Other symptoms (specify):.....

CB: |__|__|__|

Physical examination:

Temp ..... 0C weight .....Kg

Spleen ..... cm Liver .....cm

Anemia Y / N / DK

RR ..... Pulse .....

Lungs .....

Ears .... Throat:.....

Tonicity: ..... Dehydration: .....

Other signs:

Laboratory results:

malaria smear: .....Hct: ..... %

WBC :.....

Pl:.....

Other:

Diagnosis: Diag1:..... Diag2:.....

In case of malaria: severe Y / N specify:
failure Y / N

Treatment: Tt1.....
Tt2.....
Tt3.....

Date: .....

Signature:

photocopy of IPD chart in case of transfert to MSF hospital

CB: |__|__|__|

INFANT FOLLOW-UP

MOTHER'S NAME: .....

HOUSE: |__|__|__| SECTION: |__|__|

CHILD'S NAME ..... DATE OF BIRTH: |__|__|__|

SEX: M / F DUBOWITZ SCORE: |__|__|. |__|

NUMBER OF IMMUNIZATION CARD:.....

REMARKS (abnormalities, familial disease, reasons for special care)

AGE	BIRTH	1 WEEK	1 M.	2 M.	3 M.	4 M.	5 M.	6 M.
DATE								
WEIGHT								
HEIGHT								
HEAD								
ARM								
TEMP.								
PULSE								
RR								
SPLEEN								
LIVER								
REMARKS								
IMMUN. DATE								
BI MOTHER								
BI CHILD								
HCT								

CB: |__|__|__|

AGE	7 M.	8 M.	9 M.	10 M.	11 M.	12 M.
DATE						
WEIGHT						
HEIGHT						
HEAD						
ARM						
TEMP.						
PULSE						
RR						
SPLEEN						
LIVER						
REMARKS						
IMMUN. DATE						
B1 MOTHER						
BI CHILD						
HCT						

AGE SIT: |__|__| MONTHS AGE CRAWLER : |__|__| MONTHS

AGE WALKER: |__|__| MONTHS

LOST: Y / N IF YES, REASON: .....

DATE: |___|___|___|

DEAD: Y / N IF YES, DIAGNOSIS .....

DATE. |___|___|___|

CB: |__|__|__|

INFANT FOLLOW-UP; 6 MONTHS-OLD BABIES

NAME: ---------- DATE: |____|____|____|

I-GROWTH MONITORING

1. Line aspect:
   1. regular increase within normal range
   2. regular increase under the lower line
   3. growth temporary stopped followed by regular increase
   4. growth stopped for ..... weeks/months
   5. other

2. Percentage weight for height:

II-DEVELOPMENT

Is the baby: 3. sitting with help Y / N
4. babbling Y / N
5. reaching for toys Y / N
6. playing with toes Y / N

III-NUTRITION

7. Is the baby breast-fed Y / N If not: 8. at what age breast feeding was stopped? .....

9. what milk is now given to the child? .....

Does the baby eat:

10. rice	Y / N	11. age beginning:
12. fruits	Y / N	13. age beginning:
14. vegetables	Y / N	15. age beginning:
16. eggs	Y / N	17. age beginning:

IV-CARE AND HEALTH FOLLOW-UP

18. Who is taking care of the child: 1. mother
2. grand-mother
3. sister/ aunty
4. other, specify .....

CB: |__|__|__|

19. Is the child immunized?

1. complete immunization for age
2. incomplete immunisation for age
3. vaccine card lost, BCG scare+ mother history
4. not immunized

When the child is sick, is the gardian:

20. bringing him to the MSF hospital/dispensary Y / N
21. bringing him to the SMRU dispensary Y / N
22. bringing him to the Thai hospital Y / N
23. buying medicines in shops Y / N
24. calling a private medic Y / N
25. using traditional medicines Y / N

26. Is the mother going to the weekly feeding and B1 distribution from MSF

Y / N

27. Number of visits in the CB study: ..... monthly follow-up

28. Number of consultations in the CB study: ..... OPD consultations

V-MORBIDITY

(mother interview confirmed by health card whenever possible; document every pathological episodes on separate forms)

29. How many times did the child get fever: .....

30. How many times did he had a blood smear taken .....

How many times did he get: 31. malaria: .....
32. ARI.....
33. diarrhoea: .....
34. other relevant disease: .....

CB: |__|__|__|

VI- Physical examination

35. general appearance: .....

36. cardiac auscultation: .....

37. pulmonary auscultation: .....

38. spleen: .....

39. liver: .....

40. other: .....

Physician 's signature: