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Clinical epidemiology of malaria under differing levels of transmission

By

Tabitha Wanja Mwangi ,
BVM, MSc

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fulfilment of the requirements for the degree of
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Dedication

To my mother

Maryanne Mwangi

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CHAPTER ONE

INTRODUCTION

Malaria is a major public health problem in Africa. A key issue in studies of epidemiology, treatment, immunity and community-based interventions is the definition of clinical disease due to malaria. However this remains problematic due to both the phenomenon of asymptomatic background parasitisation in endemic areas, and differences in the pattern and amount of clinical disease with differing levels of transmission. This thesis describes attempts to define and quantify malarial disease in two communities in coastal Kenya with similar ethnic, geographical and socio-economic backgrounds but which differ in the level of malaria transmission.

A sample of 1,500 people of all age groups from two areas within Kilifi District, with differing transmission were observed for two years. The main objective of this study was to define and quantify non-severe malaria within the two areas of differing malaria transmissions. The thesis is divided into nine chapters, the first three being an introduction to malaria and study design and the other seven chapters being analysis of data and discussion of various results.

Chapter two is a literature review of the epidemiology of severe and non-severe clinical malaria in Africa. It discusses the pathophysiology and clinical presentation of both non-severe and severe malaria and the difficulties involved in its malaria diagnosis. Effects of age and transmission on severe malaria and mortality are reviewed. Finally, other factors that affect malaria morbidity are discussed.

Chapter three is a discussion of the overall design and methodology. The study area is discussed in terms of location, population, topography, climate and entomological factors related to malaria transmission. A description is made of the study population, their

occupation, socio-economic status, health seeking practices and main causes of paediatric admissions at the Kilifi District hospital. The study design is described in detail including the selection of the study population, aging of the study participants and weekly follow-up.

Chapter four is a discussion of basic descriptive parameters of the study population. Age patterns of the general population in the two study areas are described. The study participants that attended the study clinic are then described in terms of their age, sex, symptoms and clinical signs at presentation and the overall diagnosis made at the clinic. A total of six cross-sectional surveys were conducted in the course of the study and the parasite prevalence by age in the two areas were compared. The effect of regular treatments on parasite prevalence rates are discussed. Other physiological parameters such as body temperature, haemoglobin and C-reactive protein levels are described and the differences among people of different age groups in the study areas discussed.

Chapter five details the approach to defining non-severe malaria for epidemiological studies in Kilifi. Logistic regression methods were used to derive malaria attributable fractions of fevers and to derive various parasite density cut-offs as malaria case definitions in the two study areas in Kilifi. These definitions were then used to quantify malaria from data of two years of longitudinal follow-up and the rates of malaria and fever by age in the two study areas. Finally, there is a comparison of the rates of malaria treatments provided at the study clinic and that provided at a rural health facility in Ngerenya. The main finding in this chapter was the age differences in non-severe clinical malaria disease presentation with a higher incidence of malaria in the area with lower malaria transmission.

Chapter six is an attempt to derive algorithms for clinical malaria diagnosis from data of patients that presented to the clinic with a history of fever. Using clinical symptoms and

signs that were found to be associated with malaria, four algorithms were derived for three age groups. The four algorithms in each age group were compared with those derived from other African studies and the 'best' algorithms compared in terms of their ability to predict malaria for treatment using hypothetical situations. The difficulties of using clinical algorithms to define malaria for treatment in endemic areas are discussed.

Chapter seven discusses the data on children admitted into the Kilifi District hospital from the two study areas with differing transmission. Two sets of data are described; malaria admissions among those recruited into the longitudinal study and from the wider study area. The admissions were discussed in terms of age, haemoglobin, parasitaemia and associations with clinical criteria that have been used in several African countries to define severe malaria. Despite there being more admissions from Ngerenya (the lower transmission area) than Chonyi (the higher transmission area), those from Chonyi were associated with clinical criteria carrying an increased risk of death compared with those from Ngerenya.

Appendix XI is a systematic review of several studies conducted in areas of differing malaria transmission in Africa that attempted to quantify malaria. Difficulties of comparing data from studies with different methodologies are discussed and comparisons of malaria rates in areas of differing endemicity described. In conclusion, it appears that the incidence of clinical malaria rises with increasing malaria endemicity up to high-moderate transmission, after which there is no further rise with increasing endemicity.

Appendix XII is a paper soon to be published in the 'Transactions of the Royal Society of Tropical Medicine and Hygiene' that describes the effects of untreated bednets on malaria infection and incidence. Only data from Ngerenya was used and the conclusion was that

untreated bednets in good condition were capable of reducing malaria infection and disease compared to use of no nets at all or worn untreated nets.

CHAPTER 2: LITERATURE REVIEW

2.1 Definition and burden of malaria

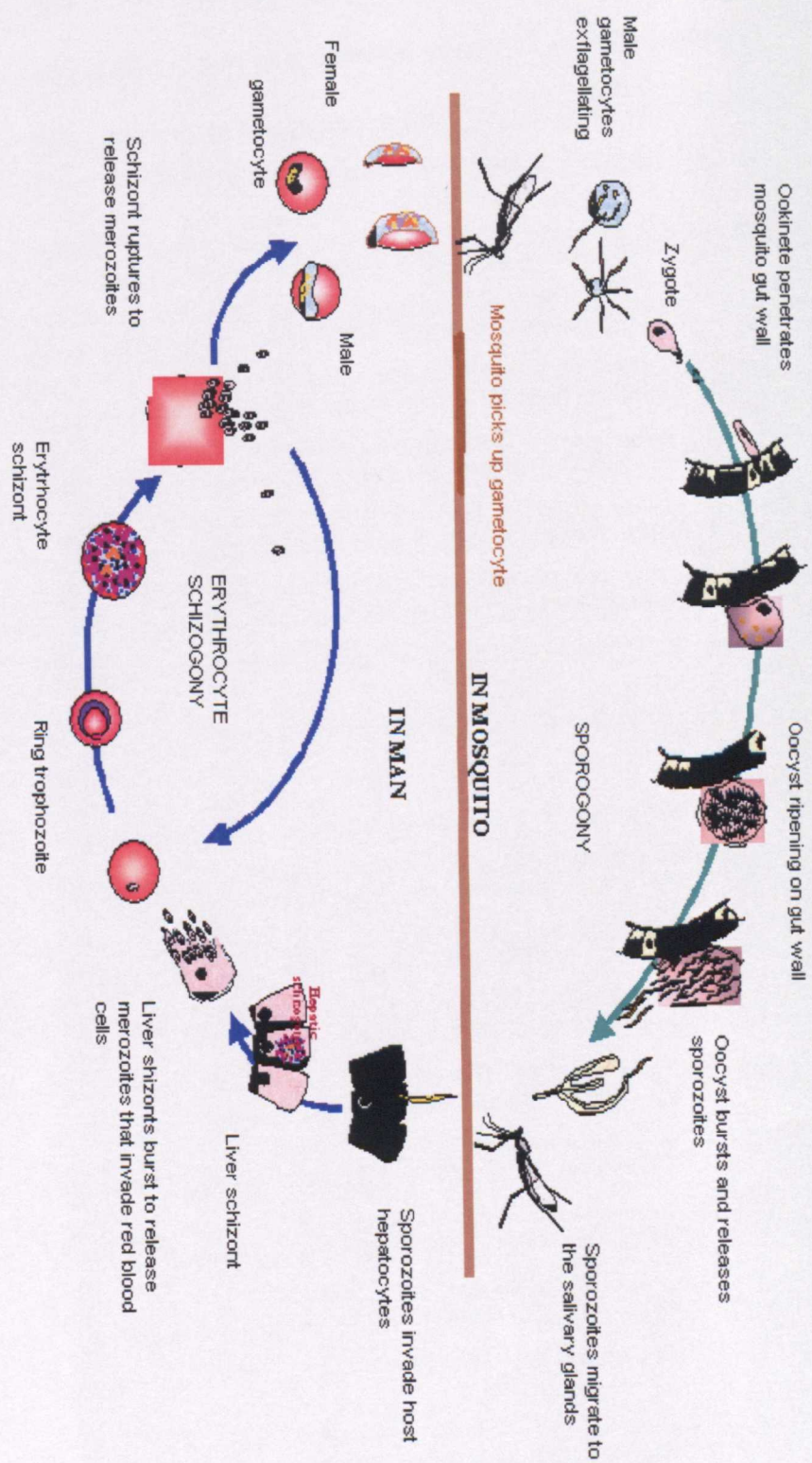
2.1.1. Life cycle of the malaria parasite

Malaria is caused by protozoan parasites of the genus *Plasmodium*. There are about 120 species of *Plasmodium* that infect a wide range of hosts. Twenty-two species are found in primate hosts, 19 in rodents, bats and other mammals whereas there are 70 species described in birds and reptiles. However, there are only four species of malaria parasites that are known to commonly infect humans: *P. falciparum*, *P. malariae*, *P. vivax* and *P. ovale*.

Mosquitoes of the genus *Anopheles* were first identified as the vector of malaria in 1897 by Sir Ronald Ross (Wernsdorfer & McGregor, 1988). There are about 422 species of *Anopheles* mosquitoes throughout the world but of these, only 40 are of major importance in disease transmission. Different species of mosquitoes transmit malaria in various parts of the world. *Anopheles gambiae* complex is the dominant vector in Africa and comprises six sibling species: *An. gambiae sensu stricto*, *An. arabiensis* and *An. quadriannulatus* are fresh water breeders, *An. melas* and *An. merus* are salt water breeders while *An. bwambae* breeds in mineral rich waters (White, 1974). *An. funestus* is the second most abundant vector after *An. gambiae s.s.*

Of the four species of *Plasmodium* that infect man, *P. falciparum* is the most prevalent parasite in sub-Saharan Africa (SSA). The malaria parasite develops in two stages: the sexual cycle in the mosquito host and the asexual cycle in the human as shown in Figure 2.1.

Figure 2.1: The life cycle of the *Plasmodium* parasites in the human and mosquito host (Modified from WHO/TDR, 2002)



The female Anophelene needs a blood meal in order to support egg production. Following development and oviposition, the female vector seeks another blood meal in order to support another brood of eggs.

Within the mosquito, the parasite goes through the sporogonic phase of the cycle. After ingesting a blood meal, the asexual stages are digested in the stomach while the sexual phases develop further. The male and female gametes fuse in the mosquito stomach and the zygote produced at fertilization develops into an ookinete. This ookinete settles on the outer wall of the stomach and there develops into an oöcyte. Sporozoites develop within the oöcyte, which bursts to release them into the mosquito's body. These sprozoites make their way to the salivary glands of the mosquito and await the mosquito's feeding on a vertebrate host. The process of sporogony is affected by ambient temperatures and can take between 7 days, if the environmental temperatures are about 31°C, to 20 days if the temperatures are about 20°C.

After the mosquito bites a human host, the sporozoites are in the peripheral circulation briefly, they then invade parenchymal liver cells and develop and multiply there in a process known as pre-erythrocytic schizogony. The sporozoites develop into schizonts that release merozoites into the circulation. The time from infection to release of merozoites is about 6-16 days depending on the parasite species. About 30,000 merozoites will be released by a single *P. falciparum* schizont in the liver. The early stages of the parasite within the erythrocytes are called trophozoites and these multiply asexually to form schizonts, which when mature have fully developed merozoites. Schizonts burst and release about 10-30 merozoites into circulation to invade fresh erythrocytes. On invading erythrocytes some of these merozoites develop into gametocytes that are taken up by mosquitoes to continue the cycle.

2.1.2 Measuring transmission

During the 1950's when vector control was viewed as a possible method of malaria eradication, mathematical models of vector dynamics were developed with the aim of using them to evaluate the effects of anti-vectorial measures. Some of these mathematical models are described in this section.

2.1.2.1. The basic reproduction rate.

The most important measure of infectious diseases is the basic reproductive rate R_0 defined as the average number of new cases of a disease that will arise from the introduction of an infective host into a wholly susceptible population (Anderson and May, 1991). The R_0 for malaria infections can be estimated using the equation below:

$$\text{Equation 2.1 } R_0 = \frac{a^2 mcbe^{-\mu T}}{\mu r}$$

where 'a' is the vector biting rate, 'm' is the ratio of vectors to host, 'c' is the transmission coefficient from vertebrate to vector (i.e. the proportion of bites by vectors on infected hosts that eventually give rise to mature infections in the vectors), 'b' is the transmission coefficient from vector to vertebrate, ' μ ' is the vector mortality rate, 'T' is the incubation period of the parasite within the vector (sometimes referred to as the extrinsic incubation period) and 'r' is the rate of recovery of the vertebrate from infection. For an infection to persist in an area, the R_0 must be greater than one. The greater the R_0 , the more difficult it is to control a disease. For malaria, estimates of R_0 as high as 25 (95% CI 9-28) have been quoted (Dye *et al.*, 1996). This may explain why malaria has been such a difficult disease to control.

2.1.2.2 Vectorial capacity

Vectorial capacity (C) is the transmission probability index that reflects the mean number of probable inoculations transmitted from one case of malaria in a unit of time.

$$\text{Equation 2.2 } C = \frac{m a^2 p^n}{-\log_e p}$$

where 'm' is the relative density of female anophelines, 'a' the probability that the mosquito will take a human blood meal during a particular day and 'pⁿ', the proportion of vectors surviving the parasites incubation period. In this case 'p' is the probability of vector survival and 'n', the number of days the vector lives. The probability of daily survival is key in determining endemicity levels. For *An. gambiae* and *An. funestus* an average daily survival rate of >60% has been shown to be associated with stable endemicity. Vectorial capacity is a better marker of stability of transmission than R₀.

2.1.2.3 Entomological inoculation rate

Another common term in malaria epidemiology is the entomological inoculation rate (EIR), which is the number of infective mosquito bites received per person per unit of time

$$\text{Equation 2.3 } \text{EIR} = mas$$

Where 'm' is the anopheline density in relation to man, 'a' is the average number of persons bitten by a mosquito in a day and 's' is the proportion of mosquitoes with sporozoites in their salivary glands. The human biting rate 'ma', is usually measured by catching the mosquitoes trying to feed on exposed individuals. In some instances, the human biting rate is calculated by using mosquitoes captured using house spray or mosquito traps. The sporozoite index 's' requires the quantification of the mosquitoes with sporozoites in their salivary glands by dissecting the mosquitoes and directly observing the sporozoites or by the use of Enzyme Linked Immuno-Absorbance Assay (ELISA) techniques.

2.1.2.4 Force of infection

The force of infection is defined as the likelihood of a susceptible individual being infected over a small interval of time. Since the prevalence of malarial infection saturates quickly in

a population, the best way to determine the force of infection is to measure the infant parasite conversion rates. The force of infection (h) among infants can be estimated by using a simple constant risk catalytic conversion model as described by McDonald, (1950).

$$\text{Equation 2.4 } h = [\log (1-x)]/a$$

$X(a)$ denotes the proportion of individuals of age 'a' that have been exposed (infected at least once) to the parasite. This would require following children up and either taking slides or serological tests regularly (Snow *et al.*, 1996). Serological data has some advantage since unlike parasitisation, it is less affected by transmission season or treatment. For this model to be of use in estimating the force of infection, one has to assume that there are no conversions from sero-positive to sero-negative. Using this method, Snow *et al.* (1996) were able to demonstrate a reduction in the force of infection among children sleeping under insecticide treated bed nets (ITBN's) compared to those without nets during a randomised controlled trial of ITBN's in Kenya.

2.1.2.5 Determinants of transmission

Climate, ecology and active control measures determine the ability of the parasite and the vector to co-exist so that transmission can occur. Optimum temperature conditions for sporogony are 25-30°C. Sporogony ceases at <16°C and thermal death occurs at temperatures > 40-42°C. High temperatures are associated with rapid vector development with the duration of the cycle from egg to adult being from 7 days at 31°C to 20 days at 20°C. Altitude and frost have a direct influence on temperature. An altitude of $\geq 1,700$ -1,800 metres has been suggested as a limiting factor for malaria transmission due to frequent low temperatures.

Relative humidity and rainfall are also associated with mosquito abundance. Rainfall is a determining factor especially in arid areas where temperature is ideal but rainfall is rare,

some examples in Africa being Somali and North-eastern province of Kenya (El Hassan *et al.*, 1995; Brown *et al.*, 1998). A relative humidity of >60% is ideal for mosquito survival.

Man-made changes brought on by agriculture such as the building of dams and irrigation schemes greatly alter transmission in an area. An example is the introduction of micro-dams in the Tigray highlands of Ethiopia, which, resulted in a seven-fold increase in the risk of malaria in the villages closest to the dams (Ghebreyesus *et al.*, 1999).

Population movement and urbanisation affect the incidence of disease. People moving from endemic areas to non-endemic areas bring both the vectors and the parasite and this leads to disease importation (Mouchet *et al.*, 1998). Personal protection such as the use of bednets, chemoprophylaxis and house spraying reduce the risk of malaria. Sleeping in a house with closed eaves also reduces risk (Lindsay and Snow, 1988). Levels of education and economic status of these households determine the use of personal protection and good house structures, hence affecting disease transmission (Lindsay *et al.*, 1990; Gamage-Mendis *et al.*, 1991; Carne *et al.*, 1994; Wolff *et al.*, 2001).

2.1.3 Using transmission to describe malaria

Malaria transmission may be broadly categorised as stable or unstable and this depends on the amount of variation present in malaria transmission over time. Under stable transmission, there is year-to-year transmission though there is variation from year-to-year and within the year. The population in stable endemic areas shows high levels of immunity whatever the seasonal variations. In unstable malaria transmission, there is great variability in transmission over space and time and there are low levels of immunity in the populations involved. Areas with stable malaria are said to be endemic and those with unstable malaria as non-endemic areas. Non-endemic areas are prone to epidemics.

2.1.3.1. Describing endemic malaria

A more detailed scheme for the classification of malaria transmission was presented in a WHO conference in Kampala in 1950 and detailed in a paper by Metselaar & Van Thiel in 1959. These definitions have not changed over the years and are presented in Table 2.1. Spleen and parasite prevalence rates derived from cross-sectional studies are used to determine endemicity. The course of the spleen rate age curve is determined by the frequency of infections and the degree of immunity in that population in response to these infections. If the frequency of infections is high as in holo-endemic areas, then the spleen rates are high in the young age groups but as immunity develops with age, the spleen rate decreases and is low in adults. Where the level of exposure is low (hypo-endemic), then the spleen rates are low in children and the decrease with age is not marked in these areas. The areas of low endemicity have higher adult spleen rates compared to areas with a high endemicity.

Table 2.1: Classification of malaria transmission as described by Metselaar & Van Thiel, (1959).

Type	Spleen rates	Parasite rates	Description
Hypoendemicity	Not exceeding 10% in children aged 2-9 years	Not exceeding 10% in children ages 2-9 years but may be higher for part of the year	Areas where there is little transmission and the effects, during the average year, upon the general population are unimportant
Mesoendemicity	Between 11-50% in children aged 2-9 years	Between 11-50% in children aged 2-9 years but may be higher for part of the year	Typically found among rural communities in sub-tropical zones when wide geographical variations in transmission risk exist
Hyperendemicity	Constantly over 50% in children aged 2-9 years. Also high in adults (>25%)	Constantly over 50% among children aged 2-9 years	Areas where transmission is intense but seasonal where immunity is insufficient in all age groups
Holoendemicity	Constantly over 75% in children 2-9 years but low in adults	Constantly over 75% among infants aged 0-11 months	Perennial, intense transmission resulting in a considerable degree of immunity outside early childhood

The basic factor that determines the degree of malaria endemicity is the infection risk and since this is difficult to assess, its most direct reflection in the population is the parasite rate. Therefore, parasite rates are the most commonly used measure when attempting to

compare malaria endemicity on an international scale. However, this measure has a number of limitations, in particular where there is widespread treatment or control measures in place.

2.1.3.2 Epidemic malaria

A malaria epidemic can be described as a sharp rise in the incidence of malaria out of proportion to the normal incidence that a community is subject to (Mac Donald, 1957). Epidemics therefore occur not only where malaria is unknown but in areas of unstable endemicity where a modification in any of the transmission factors may completely upset the equilibrium and where herd immunity is not restraining or is absent. Malaria epidemics follow the typical epidemic curve with a pre-epidemic rise in disease incidence due to an increase in transmission occasioned by high gametocyte rates and greater density and infectivity of the Anopheline populations. The epidemic wave is accompanied by increased mortality due to malaria. In the post-epidemic phase, there is a reduction in the incidence down to the 'normal' population level. Malaria epidemics are characterised by a lack of age-dependent protection with all age groups being at equal risk of clinical disease and mortality (Elhassan *et al.*, 1995; Warsame *et al.*, 1995).

In Africa, There have been recent spates of malaria epidemics described as 'highland malaria' (Marimbu *et al.*, 1993; Mouchet *et al.*, 1998; Lindblade *et al.*, 1999). These epidemics have occurred at altitudes thought to be 'safe' from malaria. They are however not a new phenomenon as 'highland' malaria epidemics were observed in the 1940-1950's in Kenya and Ethiopia (Garnham, 1945; Heisch & Harper, 1949; Fontaine, 1961). Vehicles and immigrants were blamed for those early epidemics. Epidemics associated with the El-niño rains occurred in various areas for most of 1997 (Brown *et al.*, 1998; Lindblade *et al.*, 1999). Although one of the main factors implicated in many of the malaria epidemics is long-term climatic change, Hay *et al.* (2002) have challenged that generalisation by

showing that despite the recent upsurge of malaria epidemics in the East African highlands, there have been no major climatic changes in the last decades. Other factors more likely to be associated with the rise in epidemics include: rise in resistance to anti-malarial drugs, land use changes, population migration, the breakdown of health service provision and malaria control efforts. The effects of drug resistance on these epidemics is discussed in section 2.4.7.1.

2.1.4 Burden of malaria in sub-saharan Africa

About 90% of the global burden of malaria is concentrated on sub-saharan Africa and is caused by *P. falciparum* (Breman, 2001). Malaria has been estimated to result in between 0.5-2 million deaths per year in Africa and about 100-190 million clinical cases of malaria a year (Sturchler, 1989; Murray & Lopez, 1997). Though these figures have been quoted for many years, the evidence on which these estimates were based was not clear. Recently, Snow *et al.* (1999a) have reviewed all the data available in Africa in order to come up with more evidence-based estimates. From this analysis, it was estimated that approximately one million people die as a direct consequence of *P. falciparum* malaria infection in sub-Saharan Africa each year and 75% of these deaths are in pre-school children. Morbidity was estimated to be about 200 million clinical attacks of malaria in people resident in endemic areas in Africa.

The burden of malaria is not just related to direct morbidity and mortality due to the parasitic infections. It has been observed in intervention trials that the drop in mortality was higher than that expected from a drop in malaria deaths only though this is not observed in all studies (Bradley, 1991; Snow *et al.*, 1997). This has often been thought to be due to the difficulty of attributing a death to any particular cause due to the lack of particular signs that are characteristic of malaria and presence of mixed diagnosis (Smith *et al.*, 2001). However, it is also possible that malaria infection leads to an immuno-depressed

state that makes children especially at risk of other life-threatening infections. This may result in secondary or indirect mortality and morbidity attributable to malaria which is however difficult to quantify.

Between 5-20% of survivors of cerebral malaria may have gross neurological sequelae (Holding and Snow, 2001). About 80% of the children with cerebral malaria have status epilepticus, which is associated with diminished cognitive function in about 30% of the cases. Children that have severe malaria are 4.5 times more likely to have cognitive impairment than children from the same community that have not experienced severe malaria (Holding and Snow, 2001).

Maternal, placental and foetal malaria infections during pregnancy adversely affect development and survival of the foetus and newborns through premature births, low birth weight, maternal anaemia and possibly abortion and still-births (Murphy and Breman, 2001). In Africa there may be up to one million malaria associated low birth weight children born each year and approximately 400,000 of these children die before the age of 5 years. In a review of several studies conducted in malaria endemic areas of sub-Saharan Africa, Guyatt and Snow (2001), reported a median malaria anaemia prevalence of 8.2% among all-parity pregnant women. This suggests that about 400,000 pregnant women may have developed severe anaemia as a result of malaria in the year 1995.

Gallup and Sachs (2001) have described the effect of malaria on the economic welfare of affected countries. They showed that countries that had eliminated malaria experienced increased economic growth. A 10% decrease in malaria was associated with a 0.3% increase in the Gross Domestic Product (GDP) per capita annually while countries with intensive malaria had 1.3% lower growth in GDP per capita.

2.2 Pathophysiology of clinical malaria

2.2.1 The biology of malaria fevers

Fever is the commonest clinical feature of malaria disease in the human host. Fever also plays a central role in the epidemiological definition of clinical malaria which will be the central subject in chapter five. This section describes the way in which the body regulates body temperature and the pathogenesis of fever in the human host infected with malaria parasites.

2.2.1.1 Temperature regulation

Human beings generally maintain their body temperature at about 37°C though this varies both within and between individuals and with the time of day. Each individual has a circadian temperature rhythm, the body temperature being lower in the morning and at its maximum in the evening (4-6pm). The body temperature is maintained within the individual's normal range, despite variations in the environmental temperature, via the thermoregulatory centre located in the hypothalamus. The thermoregulatory centre comprises a cluster of neurons in the anterior and posterior hypothalamus that receives signals either from the body periphery (warm and cold receptors) or from the blood going through the hypothalamic region. Moderate changes in the environmental temperature will lead to vasodilatation and sweating to reduce body heat or vasoconstriction and shivering to produce more heat. If there are more severe changes in the environmental temperatures, signals are sent from the hypothalamus to the cerebral cortex to trigger behavioural changes such as seeking cooler or warmer places or a change of clothing.

2.2.1.2 Fever

Fever is an elevation above the normal diurnal variation resulting from infectious or non-infectious agents (e.g. neoplastic and immunologically mediated conditions). During fever, the hypothalamic 'setting' for what is the normal body temperature is raised and so the

body responds (in the normal manner) to raise the body temperature to the required set point. Hence there is shivering and behavioural changes (e.g. covering oneself with clothing to keep warm) that result in more heat production. When the new set temperature is achieved, heat loss mechanisms are activated including sweating and behavioural changes (e.g. removal of warm coverings).

It is important at this stage to make a clear distinction between fever and hyperthermia. During fever, all the body mechanisms involved in heat regulation are in good working condition but respond to a 'wrong' (modified) signal from the hypothalamus whereas in hyperthermia, the signal from the hypothalamus is 'right' but the body heat regulation mechanisms either cannot function or are restrained from functioning by external forces. Hyperthermia occurs under conditions where the heat loss mechanisms are not working either due to too much insulation or environmental factors like high humidity that make heat loss difficult. Hyperthermia cannot be corrected by the use of anti-pyretics.

2.2.1.3 Pathogenesis of fever

Most fevers are induced by substances called pyrogens that are either produced from the body (endogenous pyrogens) or from microbes or their products (exogenous pyrogens). The host itself produces endogenous pyrogens in response to infection, injury, inflammatory response or antigenic challenge. Phagocytic cells such as neutrophils, monocytes and Kupffer cells produce most endogenous pyrogens. Endogenous pyrogens include Interleukins (e.g. IL-1 α , IL-1 β , IL-6, IL-11), Leucocyte inhibitory factor (LIF), Tumor necrosis factor (TNF) and Interferons (ILF- α , ILF- β & IL- γ). It is not clear how these endogenous pyrogens cause fever but the most convincing theory is that they interact with the endothelium of capillaries in the hypothalamic tissues which then produce prostaglandins (PG) mainly PGE₂. The elevation of the PG levels in the hypothalamus induces changes in the thermostat 'settings'. Most anti-pyretic drugs are known to be

prostaglandin inhibitors thus giving more weight to this theory but it is still not clear what other agents may be at work in this process or how the prostaglandins cause the re-setting of the hypothalamic thermostat (Kluger, 1980; Hull, 1989).

The fever causation process also leads to an elevation of plasma proteins known as 'acute phase proteins'. These proteins are present in normal serum or plasma but there is increased synthesis of these proteins in the liver during infections, they include haptoglobulins and caerulo-plasmin, C-reactive protein (CRP), Serum amyloid-associated protein (SAA) and fibrinogen. CRP is produced in large quantities in response to infection and has been shown to facilitate phagocytosis of the invading organisms. The function of SAA and fibrinogen is however unclear. At the same time that there is increased production of some serum proteins, there is also a reduction in the synthesis of other proteins especially albumin.

2.2.1.4 Pathogenesis of malarial fever

A challenge in malaria pathogenesis research is to explain how malaria parasites cause so much pathology despite being located within host cells most of the time. It was initially thought that malaria illness was caused by the parasites competing for resources in the host or release of toxins by the parasites. It has however become clear that some aspects of the host illness such as fever are due to substances produced by the host in response to the presence of the parasites (Clark *et al.*, 1989). The observation that non-specific shock-syndrome in rats after the injection of LipoPolySaccharide (LPS) was similar to the clinical picture of malaria led Clark *et al.* (1978) to suggest that LPS-like parasite products initiated the pathology due to malaria. It was suggested that the malaria toxin thought to induce this pyrogen-like response may not have been a single entity and that this response would be induced by a range of substances (Bate *et al.*, 1989; Taverne *et al.*, 1990). The most clearly identified TNF-inducing factor is Glycosyl-Phosphatidyl-Inositol (GPI).

Monoclonal antibodies to this factor were demonstrated to neutralise toxic effects of the parasite (Bate *et al.*, 1992; 1993, 1994; Schofield *et al.*, 1993, 1994). GPI was suggested to be the main cytokine inducer but was shown not to directly cause the host response. GPI is capable of inducing the production of TNF and IL-1 from monocytes and macrophages. These cytokines are a normal part of the host immune response but when produced in excess, cause pathology. Using a *P. vinckei* model, Clark *et al.* (1981, 1987) demonstrated the TNF injected into infected mice caused malaria like symptoms. Asymptomatic mildly infected mice exposed to the endotoxin at doses that would not cause disease, developed symptoms. This was consistent with an interaction between endotoxin and parasites, which had also been demonstrated in humans (Clark *et al.*, 1989).

Increases in TNF levels during malaria infection have been demonstrated in several studies (Clark *et al.*, 1987; Kwiatkowski *et al.*, 1989; Grau *et al.*, 1989; Kern *et al.*, 1989; McGuire *et al.*, 1998). Through *in vitro* experiments, Kwiatkowski *et al.* (1989) demonstrated that peak TNF production occurred after schizont rupture and may therefore mediate malaria fever. Several studies have shown an association between high circulating TNF concentrations and poor outcome (Grau *et al.*, 1989; Kern *et al.*, 1989; Kwiatkowski *et al.*, 1989). Patients with higher mean values of TNF either had a more complicated clinical course or greater risk of death compared to those that had lower levels of TNF. Other studies have shown that the level of TNF production depended on parasite strain (Allan *et al.*, 1995; Kwiatkoswki *et al.*, 1993). Kwiatkowski's group (1993) showed that parasites with a higher rosetting frequency tended to produce larger amounts of TNF while Allan *et al.*(1995) demonstrated that parasites that caused cerebral malaria had a tendency to generate larger amounts of TNF than those that caused mild malaria.

As both human and mouse models have demonstrated that very high levels of TNF are a predictor of poor clinical outcome, attempts were made to use anti-TNF therapy in the *P.*

berghei mouse model (Clark *et al.*, 1987). This resulted in the prevention of most of the pathology due to malaria and led to a trial of murine monoclonal anti-TNF antibodies in Gambian children as treatment for malaria (Kwiatkowski *et al.*, 1993; van Hensbroek *et al.*, 1996). This therapy was found to suppress the fever response, however there was no improvement in survival from cerebral malaria and there was an increase in the rate of neurological sequelae and the idea of using anti-TNF monoclonals for therapy was not pursued further.

However, a setback was encountered in the understanding of malaria fever pathogenesis with the discovery that many of the *P. falciparum* cultures that had been used across the world for this work were contaminated with *Mycoplasma* (Turrini *et al.*, 1997; Rowe *et al.*, 1998). *Mycoplasma* is known to produce proteins that induce the production of TNF, IL-1 and IL-6 by macrophages and monocytes. It therefore became necessary to re-access the cytokine response in malaria with uncontaminated parasites. Using *Mycoplasma* free cultures, it was reported that monocytes were not recruited into the early cytokine production in malaria, which was different from what happened with endotoxins. The production of cytokines in *Mycoplasma* free malaria cultures was therefore one that involved lymphocytes and these cytokines were released within a day of exposure unlike in the contaminated cultures where the release occurred in 5 or 6 days (Scragg *et al.*, 1999). Scragg also showed that this TNF response required both CD3 (+) and CD14(+) T cell populations whereas the endotoxin like response did not require these cells. Therefore the malaria response is dependant on lymphocyte populations that have no role to play in the bacterial endotoxin response. Hensman *et al.* (2001) demonstrated that TNF and IFN- γ production via T-cells occurred within 18 hrs of exposure and that schizont rupture was not a pre-requisite, the earliest production of cytokines being from intact parasitized erythrocytes. Since the RBC's were not ruptured it was suggested that either there was direct contact between the cells or that schizont rupture itself without cell lysis was enough

to trigger the T-cells. This initial exposure of T-cells to these cytokines might prime them for the massive outpouring that occurs during schizont rupture.

One of the functions of pro-inflammatory cytokines like TNF is to stimulate host cells to produce factors that are protective to the host and at the same time destroy the parasites. One of the most studied of these factors is Nitric Oxide (NO). Host cells are stimulated to produce NO through an enzyme, inducible nitric oxide synthase (Burgner *et al.*, 1999). Inducible Nitric Oxide Synthase (iNOS) is absent in resting cells but the gene is rapidly expressed in response to stimuli such as pro-inflammatory cytokines. Once present, iNOS induces the production of larger amounts of NO than all the other NO enzymes (endothelial NOS & neuronal NOS). Since NO is a short-lived molecule and therefore difficult to measure, most studies measure the presence of its metabolites, mainly nitrates and nitrites known collectively as Reactive Nitrogen Intermediaries (RNI). These RNI's are influenced by diet (intake of foods high in RNI) and renal insufficiency (inability to secrete RNI's), which can both lead to elevated levels. These confounders may have contributed to some of the conflicting results reported in studies on nitric oxide and malaria (Kremsner *et al.*, 1996; Taylor *et al.*, 1998, Al-Yaman *et al.*, 1996). NO has been reported in high concentrations among asymptomatic parasitised subjects and this was interpreted as evidence for a protective role (Anstey *et al.*, 1996) but on the other hand, NO has been reported to be elevated in cases of severe malaria and is associated with pathology (Al-Yaman *et al.*, 1998).

2.2.2 Clinical presentation of malaria fever

A lot was learned about the natural history of malaria disease in the 1920's when induced malaria started to be used to treat neurosyphilis (James, 1931; Shute, 1951; Chernin, 1984). The syphilis organisms (*Treponemes*) invade almost any organ in the body but at later stage focus on the central nervous system leading to widespread progressive neurological

deterioration. Before the 1920's, 60-80% of the patients with syphilis in England and Wales died of syphilitic complications but after the onset of malaria therapy, the case fatality rate did not exceed 5-10% (Chernin, 1984). Malaria fever was thought to kill the syphilis organisms but it was not entirely clear whether the fever was the main mode of action in this therapy. Initially any species of malaria was used for malaria therapy but it soon became apparent that *P. falciparum* caused a much more severe disease hence requiring more patient care. *P. ovale* was shown to produce a mild illness in man resulting in spontaneous cures after 7-8 untreated fevers and it usually resulted in the development of a solid immunity to any future *P. ovale* infections (Shute, 1951). *P. vivax* produced the required fever response, which would be allowed to go on for a week without deleterious effects to the patient and despite numerous passages, it did not lose virulence or become attenuated. *P. vivax* (especially the Madagascar strain) was therefore used in most of the malaria therapy that took place.

2.2.2.1 Fever paroxysm

The classical pattern of the fever paroxysm for vivax malaria has been summarised by Wernsdorfer and McGregor (1988) as having three stages. This classical fever paroxysm pattern is typical of *vivax* malaria but not of *falciparum* malaria whose fever course will be described later in section 2.2.2.2. The first stage is the cold stage in which the patient feels extremely cold and may shiver, leading them to cover themselves to keep warm. Although the temperature is rising, there is intense peripheral vaso-constriction; the skin is cold, dry, pale, cyanosed and goose-pimpled. This stage lasts 15 minutes to an hour and the temperature gradually rises and the shivering ceases. The second stage is the hot stage when the patient discards extra clothing, the skin is hot, dry and burning and the face is flushed. The pulse is rapid and pounding, respiration rate is high and the blood pressure tends to fall. There may be headache, parched throat, extreme thirst, vomiting, restlessness and excitability that may progress to confusion and delirium. Temperature reaches its peak

of 40-41°C. Children under five years of age may at this time develop febrile fits. The hot stage lasts two hours or more. The last stage is the sweating stage where the patient bursts into profuse sweating. Sweat first appearing at the temples and then becoming generalised. The temperature falls and may become sub-normal. There is great relief at this time and the patient may feel tired and fall asleep. The entire paroxysm, (the cold to sweating stage) lasts 6-10 hours and coincides with the rupture of erythrocyte schizonts. These paroxysms are more frequent in the late afternoons or evening than in the mornings. There is usually an interval between paroxysms. In *P. vivax* and *P. ovale* these paroxysms become regular and occur every 48 hrs and are called tertian paroxysms. In *P. malariae*, these occur every 72 hrs and are called quartan paroxysms. The main factor differentiating *P. falciparum* fevers from fevers caused by the other species of *Plasmodia* is that the fever has an irregular pattern and does not follow the classical paroxysmal pattern.

2.2.2.2 Fever and other clinical symptoms due to *P. falciparum*

The following account of the clinical presentation of *P. falciparum* malaria is derived from work on induced malaria in England by James (1931) and a review of malaria therapy work by Kitchen (1949). Both presented observations on neuro-syphilis patients treated with malaria. Inoculation with *P. falciparum* was observed to lead to certain prodromal symptoms, which occur days or hours before the first febrile attack and are more severe in people with low malaria immunity. These symptoms may include one or a combination of: malaise, headache, dizziness, chilliness, shivering, sweating, pains in the back, legs, or elsewhere in the body or gastro-intestinal disturbances (anorexia, nausea, diarrhoea or vomiting) and a transient fever usually not exceeding $\geq 37.7^{\circ}\text{C}$. The induced *P. falciparum* fevers did not follow the classical paroxysm pattern followed by other *Plasmodium* infections but tended to present with irregular fevers with a long paroxysm. The fevers were classified roughly as continuous, remittent or intermittent. Intermittent fevers that usually followed a tertian cycle were rare in *falciparum* malaria but did sometimes occur in

the primary attack or in people that had a higher immunity to malaria. Intermittent fevers with long intervals were associated with more pernicious signs than fevers with short intervals if not treated early. *P. falciparum* infections rarely fully synchronise as there were usually several broods of parasites sporulating at different times hence causing fever at different times.

A range of symptoms accompanied fever in the studies of induced malaria. They ranged from mild symptoms like low level fevers and headache to more severe symptoms like prostration. Greater severity was not necessarily associated with a higher parasite load but depended more on the person's immune status. Asynchronous infections lead to a more severe symptomatology than synchronised infections.

The common symptoms were: fever with temperatures reaching between 39.4°C-40.6°C accompanied with discomfort, prostration and terminating with perspiration. Other symptoms included headache, backache, abdominal pain, pain and muscular stiffness in the back of neck and shoulders, vomiting and rigors. Vomiting occurred in about a third of the patients while rigor occurred rarely in *falciparum* malaria and was found in less than 3% of the patients. Some of the symptoms like fever, nausea, vomiting, headache, generalised or localised aches and pains, abdominal discomfort and prostration may be limited to or accentuated during the paroxysm or at the start of the attack.

2.2.2.3 Clinical presentation of malaria fevers in Africa

The above descriptions are mainly based on induced infections in controlled circumstances among individuals with little initial immunity to malaria. However, several observational studies in Africa have described symptoms associated with *falciparum* malaria under a range of endemic conditions. Schmitz and Gelfand (1976) in Zimbabwe found that headache (73%), vomiting (39%), abdominal pains (37.8%), cough (26%) and chest pains

(20.7%) were the commonest signs. Mkawagile and Kihamia (1986) looking only at adult patients from Tanzania found that the main symptoms were: fever (90%), headache (95%), myo-arthralgia (90%) nausea and vomiting (15%). In a study conducted in Zimbabwe, Bassett *et al.* (1991), found the most commonly presenting symptoms to be feeling hot (73%), headache (85.7%) and bodily weakness (79%). Freeman (1986), compared clinical features among people living in a meso-endemic area to those living in a holo-endemic and found that disease presentation differed between the two areas. Those from the meso-endemic areas presented mainly with musculo-skeletal symptoms (i.e. general malaise, muscle and joint pains) while those from the holo-endemic presenting with gastro-intestinal symptoms (i.e. diarrhoea, vomiting, anorexia, abdominal pain and splenic enlargement).

Malaria disease has a very similar clinical presentation to other infections making it hard to differentiate from conditions such as typhoid, pneumonia and bacteraemia (Richens *et al.*, 1992; Akpede *et al.*, 1992; O'Dempsey *et al.*, 1993). As early as 1940, Williams described bronchitis as a presentation of malaria. It has been repeatedly shown that pneumonia and malaria are hard to differentiate (Redd *et al.*, 1992, O'Dempsey *et al.*, 1993). Redd *et al.* (1992) suggested that when clinical signs corresponding to pneumonia and malaria parasites are found in a patient, the person ought to be treated for both conditions. Bacteraemia has also been shown to be associated with signs similar to those of malaria (Akpede *et al.*, 1992; Berkeley *et al.*, 1999)

2.2.3 Clinical presentation of severe, life-threatening *P. falciparum* malaria in Africa

The majority of children who experience clinical malaria are treated with drugs as outpatients or recover without intervention. However, a small number go on to develop life-threatening features and these are discussed in the following section.

2.2.3.1 Introduction

Severe *falciparum* malaria is defined by the presence of potentially fatal manifestations or complications of malaria in patients with asexual forms of *P. falciparum* for whom other diagnoses have been excluded (WHO, 1986). A functional approach is to define as severe malaria those needing in-patient care. Some approaches are based on the presence of certain signs and laboratory findings that results in an increased risk of death (Marsh *et al.*, 1995). There are three main criteria used in defining severe malaria: Impaired consciousness, severe anaemia and respiratory distress. Children without any of these three features have been found to have a relatively low mortality (Marsh *et al.*, 1995). The following sections discuss each of these syndromes individually and the last section discusses all other syndromes that are also manifested in those with severe malaria.

2.2.3.2 Impaired consciousness

Any degree of impaired consciousness is associated with increased mortality in malaria (Marsh *et al.*, 1995). The term cerebral malaria is reserved for those patients in deep coma, defined by the inability to localise a painful stimulus in a patient with a *P. falciparum* parasitaemia in whom other causes of encephalopathy have been excluded (WHO, 1986). In around 70% of cases the onset of coma coincides with a seizure. Patients are however not considered as cerebral malaria cases if they improve within one hour of a convulsion or when restored to a normoglycaemic state (Marsh *et al.*, 1995).

Other neurological signs associated with cerebral malaria include: abnormal posturing including decerebrate rigidity, opisthotonus, abnormal tone, or transient or persistent hemiplegia (Molyneux *et al.*, 1989; Waller *et al.*, 1995; Newton *et al.*, 1997a). Other clinical signs include retinal haemorrhages or oedema and abnormal respiratory patterns (Lewallen *et al.*, 1993).

The clinical syndrome of cerebral malaria has differing underlying pathologies and there are four distinct syndromes that have been recognised among children with cerebral malaria (Marsh *et al.*, 1996). First, children that experience convulsions may remain in a coma for hours after the cessation of the coma but regain consciousness and have a good prognosis (Crawley *et al.*, 1996). It is not clear why convulsions associated with malaria should have such a prolonged post-ictal period. Although fever in young children may precipitate febrile fits, convulsions in severe malaria are frequently not associated with fever (Waruiru *et al.*, 1996). Second, are the group of children with persistent seizures with no obvious clinical signs, this condition known as covert status epilepticus is associated with a high mortality (Crawley *et al.*, 1996). Third, are the children defined as having cerebral malaria that are severely acidotic who are resuscitated with aggressive fluid resuscitation and regain consciousness within a few hours (English *et al.*, 1996b). The three syndromes listed above differ from the last syndrome of 'true cerebral malaria' that is thought to be associated with parasite sequestration in the brain.

The majority of children admitted to hospitals recover fully from coma within 48 hours. However about 10 -16% of the children that survive a cerebral malaria attack have persistent neurological abnormalities (Molyneux, 1989; Brewster *et al.*, 1990; Walker *et al.*, 1992; Crawley *et al.*, 1996). The most common neurological sequelae on discharge include: ataxia (43%), hemiplegia (39%), speech disorders (39%) and 30% with blindness (Molyneux *et al.*, 1989; Brewster *et al.*, 1990, Bondi *et al.*, 1992; Peshu (personal communication). The case fatality rate for cerebral malaria ranges from 14-27 % (Molyneux, 1989; Brewster *et al.*, 1990; Krishna *et al.*, 1994; Waller *et al.*, 1995; Marsh *et al.*, 1995; Modiano *et al.*, 1995; Jaffar *et al.*, 1997; Biemba *et al.*, 2000).

2.2.3.3 Severe malaria anaemia

In many African studies, severe anaemia is defined as a haemoglobin count < 5g/dl or a haematocrit < 15%. Severe malarial anaemia presents with a range of features from marked pallor in a febrile child through weakness to respiratory distress (Newton *et al.*, 1998).

There are two main mechanisms by which malaria anaemia is thought to occur. First, haemolytic anaemia occurs when erythrocytes rupture or are removed by the spleen or liver. Red cell destruction is usually in excess of that which can be accounted for by rupture or removal of infected cells. Uninfected cells may become sensitised and may rupture or be cleared by a number of mechanisms including modification of membrane proteins or deposition of complement or antibody on their surface which may facilitate their removal. Second, severe malaria anaemia may result from reduced erythropoiesis due to bone marrow depression (McGuire *et al.*, 1999).

It is however rarely possible to attribute anaemia purely to malaria infestation and it is worth bearing in mind that in malaria endemic areas, there are likely to be multiple causes of anaemia which include helminth infestation and nutritional deficiency (Newton *et al.*, 1997b).

The case fatality rate due to severe malaria anaemia is less than 10% in most studies: 4.7% in Kenya (Marsh *et al.*, 1995), 7.8% in The Gambia (Brewster *et al.*, 1990), and 7.3% in Zambia (Biemba *et al.*, 2000). However, in one study conducted in Burkina Faso, it was estimated to be 24% (Modiano *et al.*, 1995).

2.2.3.4 Respiratory distress

Respiratory distress is defined simply as difficulty in breathing and the main clinical sign is increased depth of breathing (English *et al.*, 1996a). There are various potential causes

for respiratory distress in children with malaria, these include: cardiac failure, co-existent pneumonia, direct sequestration of malaria parasites in the lungs or metabolic acidosis (Marsh *et al.*, 1996). Studies carried out in Kilifi, Kenya have shown one of the main causes of respiratory distress in malaria is severe metabolic acidosis defined as a base excess $\leq -12\text{mmol/L}$ (English *et al.*, 1996a). Processes that lead to high levels of lactate ($>5\text{mmol/L}$) and other acids that precipitate metabolic acidosis in malaria include: anaerobic metabolism due to poor tissue perfusion as a result of anaemia and probably parasite sequestration, production by the parasite, lack of clearance due to renal failure and anaerobic glycolysis (Krishna *et al.*, 1994; Marsh *et al.*, 1995; English *et al.*, 1996a, 1997a & 1997b).

Ingestion of exogenous acids, especially salicylates that are common anti-pyretics in Africa may complicate severe malaria (English *et al.*, 1996b). Dehydration is a contributing factor to acidosis and may occur in children with a history of vomiting, diarrhoea and sweating (English *et al.*, 1996c). It is however common for children in malaria endemic areas to have both malaria and lower respiratory tract infection at the same time, in which case the respiratory tract infection may be confused for respiratory distress due to malaria (O'Dempsey *et al.*, 1993). Respiratory distress carries a high mortality of about 20% that rises to 30% in the presence of impaired consciousness (Marsh *et al.*, 1995).

2.2.3.5 Other clinical features of life threatening malaria

The majority of malaria deaths occur in individuals with one of more of the major clinical features described earlier. However, there are other clinical features that may occur singly or together as part of the clinical picture of severe malaria and these are described in the following section. These include: hypoglycaemia, renal dysfunction, pulmonary oedema, gastro-intestinal conditions, jaundice, multiple complicated seizures, hyperparastitaemia, and circulatory collapse.

Hypoglycaemia is defined as a blood glucose level of less than 2.2mmol/L. The possible causes of hypoglycaemia in severe malaria are: high parasite biomass which exerts a large glucose demand, hepatic impairment in which case the gluconeogenic pathway is also disturbed or hyperinsulinaemia which is caused by quinine treatment especially so in vulnerable groups e.g. pregnant women (Taylor *et al.*, 1988). The main presentation for children with hypoglycaemia is impaired consciousness. Hypoglycaemia is associated with increased mortality (Molyneux *et al.*, 1989; Walker *et al.*, 1992; Marsh *et al.*, 1995).

Renal dysfunction is defined as creatinine levels above normal (>3.0mg/dl). There are several possible mechanisms for renal dysfunction in severe malaria which include parasite sequestration in the renal microvasculature compounded by hypovolaemia, endotoxaemia, associated hepatic impairment and intravascular haemolysis (Newton *et al.*, 1998). This condition is rare in African children but common and frequently fatal in adults living in non-endemic areas (Blumberg *et al.*, 1996).

Pulmonary oedema is a rare condition that is characterised by an increased respiratory rate. Dyspnoea increases rapidly and death can occur in a few hours. It can result from over-hydration of malaria patients during treatment or toxin release by parasites in the lungs (WHO, 1986). It is rare in children but common in adults where it is associated with the development of adult respiratory distress syndrome (ARDS) and has a high case fatality (Waller *et al.*, 1995; Soni and Gouws, 1996; Newton *et al.*, 1998).

Gastro-intestinal manifestations like vomiting and diarrhoea are common in children with severe malaria with prevalences of 62 and 47% respectively (Waller *et al.*, 1995). In a study by Schellenberg *et al.* (1999) conducted among paediatric in-patients at a district Hospital in Ifakara Tanzania, there was evidence that vomiting and diarrhoea increased the

risk of dying in younger children (1-7 months old) as opposed to older children (8 months-5 years).

Jaundice in malaria patients is diagnosed by raised bilirubin to $>50\mu\text{mol/l}$ (Soni & Gouws, 1996). Normally, the prevalence is low about 1 to 4.7% but rarely associated with mortality (Marsh *et al.*, 1995; Schellenberg *et al.*, 1999).

Multiple complicated seizures occur in about 18-32% of the children with severe malaria (Marsh *et al.*, 1995; Jaffar *et al.*, 1997). About 54% of children admitted to the paediatric ward in Kilifi District Hospital with non-cerebral malaria seizures had rectal temperatures below 38°C (Waruiru *et al.*, 1996), which shows that these were not febrile seizures. About 86% of these seizures were complex i.e. those that are focal in nature or repetitive.

Hyperparasitaemia is defined as a parasitaemia of $>5\%$ or $>500,000$ parasites/ μl of blood with prevalences of 18-30% reported (Marsh *et al.*, 1995; Soni & Gouws, 1996; Jaffar *et al.*, 1997). However, the risk associated with any level of hyperparasitaemia depends on the patient's immune status and ought to be defined locally. In general, in areas of stable malaria transmission, a parasitaemia $>20\%$ should be considered a risk factor as whereas in areas of unstable endemicity, a parasitaemia of 4% should indicate potentially severe malaria (WHO, 1986).

Circulatory collapse or clinical shock is an infrequent presenting feature of severe malaria. This occurs when the blood pressure cannot be maintained within normal limits and is characterised by systolic blood pressure $<50\text{mmHg}$ and cold, clammy cyanotic skin with constricted peripheral veins (WHO, 1986). The patient may be prostrated and may have impaired consciousness ranging from confusion to deep coma. Shock is rarely recorded in

clinical series because it is a late development in children and is quickly followed by death (Marsh *et al.*, 1995).

2.3. Defining & measuring disease during clinical and epidemiological studies

Having described in detail the pathogenesis and clinical presentation of malaria, the next section describes the problems with routine malaria diagnosis in clinical settings across Africa. The use of clinical algorithms to improve the diagnosis of malaria will then be discussed followed by a section on the improvement of malaria diagnosis for epidemiological studies.

2.3.1 Defining clinical malaria in clinical settings

2.3.1.1 The problems of malaria diagnosis in Africa

A history of fever or a raised body temperature are common symptoms and signs used in the diagnosis of malaria (Delfini *et al.*, 1973; Schmitz and Gelfand, 1976; Mkawagile and Kihamia, 1986; Stein and Gelfand, 1985; Bassett *et al.*, 1991; Redd *et al.*, 1996 among others). The WHO recommendations state that in an endemic area, children presenting with a fever or a history of fever with no other obvious signs ought be treated with an anti-malarial (WHO, 1986). This inevitably results in considerable over-diagnosis of malaria as shown in Table 2.2.

The table summarises published data from various clinical settings across Africa. Two of the studies reported data on adults only (Mkawagile and Kihamia, 1986; Jonkman *et al.*, 1995), three combined data on adults and children (Rees *et al.*, 1971; Stein and Gelfand, 1985; Freeman, 1986) and the rest of the studies reported on different child age groups. Some of the studies were conducted only during the high transmission period (Mkawagile and Kihamia, 1986; Redd *et al.*, 1996) when more malaria cases were expected and some of the data was reported according to season (Olivar *et al.*, 1991; Muhe *et al.*, 1999). The

study period was between 2 weeks to several months ranging in time from 1969 to 2000 involving from between 62 to 1,686 study participants. Some of the data was derived from large government managed hospitals run by well-trained physicians while some of the rural health centres were manned by medical aides.

Table 2.2: The level of misdiagnosis of malaria under health centre conditions across Africa.

Reference	Study settings	Age group	Treated for malaria	Slide Positive
Hendrickse <i>et al.</i> (1971)	Nigeria, admissions to hospital	6 months -5 yrs	500	184 (36.8%)
Rees <i>et al.</i> (1971)	Kenya, 2 weeks data from a filter clinic in referral hospital, 1969	All ages	85	1 (1.2%)
Okeahialam <i>et al.</i> (1972)	Tanzania, dispensary attendants; March-May 1971	≤ 6 yrs	422	86 (20.2%)
	Tanzania, admission data; Jan-Dec 1971	≤ 6 yrs	218	35 (16%)
Stein and Gelfand, (1985)	Zimbabwe, hospital admissions, 1982-1983	All ages	261	72 (26%)
Mkawagile and Kihamia, (1986)	Tanzania, dispensary attendances, June 1981 end of heavy rains	Adults	126	60 (47.6%)
Freeman, (1986)	Kenya, health centre cases, 1985-1986; Meso-endemic area	All ages	182	95 (52.2%)
		6 months-14yrs	117	74 (63.2%)
	Kenya, health centre cases, 1985-1986; Holo-endemic area	All ages	127	42 (33%)
		6 months-14yrs	62	39 (62.9%)
Basset <i>et al.</i> (1991)	Zimbabwe, health clinic, Jan-Feb 1989	All ages	287	80 (27.9%)
Olivar <i>et al.</i> (1991)	Niger, Health clinics – rainy season	1-5 yrs	297	^a 160 (53.8%)
	Niger, Health clinics - dry season	1-5 yrs	220	^a 10 (4.5%)
	Niger, Health clinic – both seasons	≥15 yrs	490	252 (51%) ^a [45 (9%)]
Jonkman <i>et al.</i> (1995)	Malawi, adult outpatient clinic	Adults	1174	344 (29%)
Redd <i>et al.</i> (1996)	Malawi, hospital out-patient, high transmission season from April-May 1993	< 5 yrs	983	672 (68.4%)
Weber <i>et al.</i> (1997)	The Gambia, MRC clinic	2 months-5 yrs	404	31 (7.7%)
Olaleye <i>et al.</i> (1998)	The Gambia, health centre, Sept 1993-March 1994	6 months –9 yrs	^b 407	^c 159 (39.1%)
Muhe <i>et al.</i> (1999)	Ethiopia, Outpatient clinic, low transmission season	2 months-5yrs	^d 804	^a 48 (6%)
	Ethiopia, Outpatient clinic, high transmission season	2 months-5yrs	^d 1,686	^a 511 (30.3%)
Tarimo <i>et al.</i> (2001)	Tanzania, Health clinic April-July 2000.	2 months-5 yrs	^c 395	277 (70%)

Key:

^a - In brackets is malaria defined as parasitaemia $\geq 10,000$ parasites/ μ l of blood

^b -Those treated for malaria had an axillary temperature $\geq 37.5^{\circ}\text{C}$ or a history of fever of at least 3 days.

^c -Malaria defined as an axillary temperature $\geq 38^{\circ}\text{C}$ + $\geq 5,000$ parasites/ μ l of blood

^d -Those treated for malaria had a rectal temperature $\geq 38^{\circ}\text{C}$ or a history of fever of at least 3 days.

^e -Those treated for malaria following the IMCI guidelines of axillary temperature $\geq 37.5^{\circ}\text{C}$ or a history of fever of less than 4 days or palm pallor

The rate of non-severe malaria begins to fall at different age point (older) than that of severe malaria. The rates of severe malaria drops very rapidly and there are very few cases of severe malaria in older children and adults. Immunity is characterised by a reduction in occurrence of disease even in the presence of high parasitaemia.

It has been thought for many years that immunity against malaria results from continuous uninterrupted exposure to infections and will not develop where malaria is epidemic or hypo endemic, is lost if there is a temporary loss of exposure and is not sterilizing (Day and Marsh, 1992). However since exposure is a function of age in endemic areas, it is hard to tease out the effects of age from those of exposure. One way to study this is to look at disease in people that are suddenly exposed to high infection pressure. Baird *et al.* (1991) recorded observations of Javanese transmigrants to Irian Jaya. In Java, the parasite rates were < 1% whereas, they ranged from 33-45% in Irian Jaya and these transmigrants were exposed to about 0.037-0.016 infective bites/person/night. Brief exposure established age-dependent protection independent of cumulative exposure to malaria (Baird *et al.*, 1991). When these observations were extended to five years and over five villages recruited, it was established that for most of the villagers, this age-dependent immunity developed within two years and it was only in one village that it developed within a year (Baird *et al.*, 1993).

Results from these studies of transmigrants to Irian Jaya from Java demonstrated that age is an independent predictor of the ability to develop anti-parasite immunity (Baird, 1995). Age-related intrinsic factors such as the involution of the thymus, degeneration of lymphoid tissues and subtle changes in the number of circulating immune cells were hypothesised to be involved in the development of immunity.

The patients in these studies were treated according to the judgement of the health worker or clinician without any microscopy except the last three studies (Olaleye *et al.*, 1998; Muhe *et al.*, 1999; Tarimo *et al.*, 2001). The criteria for most of the malaria treatments were health worker or physician specific. In these three studies, a measured fever or a history of fever of less than four days were used as criteria for treatment.

In the adult studies (Mkawagile and Kihamia, 1986; Jonkman *et al.*, 1995), 30 to 48% of the patients treated for malaria had a positive smear. In the studies involving only children, a median of 37% of the children treated for malaria had a positive slide or malaria defined as parasitaemia $\geq 10,000$ parasites/ μ l blood. Using a malaria definition of rectal temperature $\geq 38^{\circ}\text{C}$ accompanied by a parasitaemia $\geq 5,000$ parasites/ μ l of blood, Olaleye *et al.* (1998) found that only 39% of the patients that had been treated for malaria should have received treatment. Despite the effort in the last three studies to standardise malaria definition for treatment, there was still a large amount of misdiagnosis as only between 6 and 70% of those treated had malaria parasitaemia above the cut-off..

Studies that looked at clinical episodes of malaria across different transmission seasons reported showed a 5-10 fold increase in the rate of misdiagnosis in the low malaria transmission period compared to the high transmission season (Olivar *et al.*, 1991; Muhe *et al.*, 1999). This was thought to be as a result of the observation that clinical diagnosis did not change with season and whereas most fevers will be due to malaria during the high transmission season, this will not be so in the period of low transmission.

There was no marked difference in the rate of misdiagnosis when comparing large government hospitals to rural health facilities, suggesting that physicians and health workers misdiagnosed malaria at almost the same rate. Rooth and Bjorkman (1992) in a study in Tanzania showed that the sensitivity of identifying a malaria case was similar

between physicians and health workers but the physician diagnosis had a slightly higher specificity than the health workers diagnosis

Although over-diagnosis of malaria was not necessarily a major problem in the era of chloroquine (which was a safe and cheap drug), over time, there has been an increase in drug resistance leading to practitioners offering more expensive and dangerous drugs as a first line of treatment (Weber *et al.*, 1997). Over-prescription of these anti-malarials may lead to quick acquisition of drug resistance as it did in the case of chloroquine resistance hence the need to find a more effective way of diagnosing malaria. On the other hand delayed malaria treatment caused by mis-diagnosis in children that have other fever related conditions may lead to increased morbidity and mortality (Bojang *et al.*, 2000).

It has been suggested that one of the ways that malaria diagnosis can be improved is the introduction of microscopes in health facilities across Africa. A study conducted in Malawi by Jonkman *et al.* (1995) demonstrated a reduction in anti-malarial prescription with the introduction of microscopes in a rural health centre from 21.1 to 6.6%. In non-study conditions however, Barat *et al.* (1999) demonstrated that the introduction of microscopy did not change the clinical diagnosis of malaria or the use of anti-malarials as 20-54% of those that were slide negative still received anti-malarials. This may have been as a result of clinicians set criteria for treating malaria.

However, even if people were treated correctly on the basis of slide results, many people in endemic areas have asymptomatic infections (Greenwood *et al.*, 1987). Therefore any disease presenting with a fever may have a parasitaemia and it is difficult to tell apart the fevers in which parasites are the cause and those in which parasitaemia is coincidental. Introduction of microscopy may therefore not necessarily make a major impact on malaria diagnosis in endemic areas, especially in cases where the background parasite rates are

very high. Another potential way to improve malaria diagnosis is the use of combinations of clinical symptoms and signs associated with clinical malaria (Redd *et al.*, 1996). The next reviews this approach to malaria diagnosis.

2.3.1.2 The application and value of clinical algorithms for the diagnosis of malaria

Some studies have provided evidence that taking a good history may improve the diagnosis of malaria (Redd *et al.*, 1996; Olaleye *et al.*, 1998) whereas other studies have shown that there is no improvement in the diagnosis of malaria by improving history taking (Bassett *et al.*, 1991; Luxemburger *et al.*, 1998). Table 2.3 gives results of a comprehensive review of studies that have looked at clinical symptoms as diagnostic of malaria in Africa (Chandramohan *et al.*, 2002).

Table 2.3: Clinical algorithms derived for the diagnosis of non-severe malaria in children under 10 years of age in studies conducted in Africa (Chandramohan *et al.*, 2002)

Setting	Parasite Prevalence	Age group	Criteria with best predictive values	Sen	Spec	PPV	NPV
Rural Tanzania ¹	59%	<9 years	Intermittent fever 2-3 days	73	98	99	71
Rural Malawi ²	60%	< 5 years	Rectal temperature $\geq 37.7^{\circ}\text{C}$ or nailbed pallor or splenomegally	85	41	68	64
Peri-urban Gambia ³	7%	< 5years	Chills, sweating and shaking	94	19	5	99
Rural Ethiopia ⁴	30%	2 months-5 years	History of fever +(history of previous malaria attack or absence of cough or pallor)	83	51	42	87
Peri-urban Gambia ⁵	39%	6 months-9 years	*Weighted malaria score ≥ 8	88	64	65	87
			*Weighted malaria score ≥ 9	61	82	72	73
Peri-urban Gambia ⁶	35%	5 months-9 years	*Weighted malaria score ≥ 7	89	63	56	90
			*Weighted malaria score ≥ 8	70	78	63	82

Parasite prevalence = slide positive rate ≥ 1 parasite of mostly *P.falciparum*

Sen- Sensitivity (%) Spec- Specificity (%)

PPV- Positive predictive value (%) NPV- negative predictive value (%).

*Weighted malaria score was calculated from the following nine predictors: sleepy, reduced feeling, absence of cough, shivering, feels hot, cough not heard, pallor of palm, absence of rash, increased respiratory rate. Each predictor was given a score of '1' if present and '0' if absent except feeling hot that was scored '3' if present and '0' if absent

¹Rooth and Bjorkman. (1992); ²Redd *et al.* (1996); ³Weber *et al.* (1997); ⁴Muhe *et al.* (1999); ⁵Olaleye *et al.* (1998); ⁶Bojang *et al.* (2000).

All the studies were conducted in children less than 10 years old in malaria endemic areas with parasite prevalences ranging from 7 to 60%. A child was classified as a malaria case if they had parasitaemia and a fever or a history of fever. The studies by Weber *et al.* (1997), Olaleye *et al.* (1998) and Bojang *et al.* (2000) used a fever and a cut-off of $\geq 5,000$ parasites/ μ l of blood as diagnostic of malaria.

The strength of each predictor (measured either as relative risk or odds ratios) for clinical malaria was determined. However, none of these clinical symptoms or signs were good predictors of malaria on their own but when all strong predictors were put together in an algorithm, the sensitivities and specificities were higher. Clinical signs associated with clinical malaria (as defined above) include feeling hot on palpation, raised measured temperature, pallor of conjunctiva or palm, increased respiratory rate, palpable liver or spleen, normal chest examination and being abnormally sleepy. Symptoms associated with clinical malaria included intermittent fever, vomiting, headache, and absence of cough, shivering, chills, reduced feeding and increased sleepiness. Shivering, chills (Odds ratio of 1.7) and reduced feeding (Odds ratio about 2) were identified as malaria predictors in the Gambian studies (Olaleye *et al.*, 1998; Bojang *et al.*, 2000) but not so in Ethiopia (Muhe *et al.*, 1997). The odds of malaria for those children with palpable liver and increased respiratory rate ranged from 1.6 to 3.7 and 1.3 to 2.7 respectively. Palm pallor was a predictor of malaria in both The Gambian (OR=1.8) and Ethiopian studies (OR=2.8).

One disadvantage of the use of algorithms is that they differed depending on the time of the year (which determines the intensity of transmission) and the age of the study participants. This may explain the fact that even though both Bojang *et al.* (2000) and Olaleye's group (1998) worked in different parts of The Gambia with similar endemicity, they found the algorithm to be slightly different for the two areas. The sensitivity and specificity of some of the signs and symptoms have been shown to decrease with age and

increased with transmission intensity with the malaria algorithm having a higher specificity during the high malaria transmission season than during the dry low transmission season (Gomes *et al.*, 1994; Weber *et al.*, 1997; Muhe *et al.*, 1999; Bojang *et al.*, 2000). Algorithms also tend to be better at predicting malaria in areas with low rather than high malaria endemicity (Chandramohan *et al.*, 2002). In addition not all algorithms would be easy to implement, for example some require the detection of an enlarged spleen, which can be difficult for staff in health clinics in rural areas. Others require taking history and scoring the signs and symptoms, which gives rise to a lot of errors. In one of the scoring studies 7% of the cases were misdiagnosed due to wrong counting of scores (Bojang *et al.*, 2000). As it is not possible to get a good history of disease symptoms from most sick children, one has to rely on the observation of the carer and the way that the carer understands the symptom description. Lack of appropriate local words for certain symptoms may make it difficult to assess the presence of these symptoms (Weber *et al.*, 1997). Even if the symptom history is correctly taken, the sensitivities of the clinical algorithms range from 61% to 93%, suggesting that some of the algorithms will not be able to pick almost 40% of children under 10 years of age with malaria. Their specificity range of 19% to 98% suggests that a lot of children without malaria would continue to be wrongly treated. As malaria is a potentially fatal disease, the inability of the diagnostic test to pick such a large number of cases may be dangerous.

2.3.2 Defining malaria morbidity for epidemiological studies

The requirements of a diagnostic tool for malaria are different for clinical management and for epidemiological studies. Since malaria is a potentially fatal disease, there is need to treat every possible case, therefore a high sensitivity of the diagnostic test is required, while in the case of epidemiological studies, precision is more important. The aim of many epidemiological studies is to assess the impact of various interventions, such as insecticide treated bed nets, introduction of prophylaxis, household spraying or currently, malaria

vaccine trials. Table 2.2 indicates that malaria diagnosis is fraught with difficulty. Intervention studies require a form of case detection in order to know which of the two groups (intervention and control) have more clinical disease. Case detection either by passive detection at a clinic or by active case detection through regular follow-ups in the homesteads requires a standard malaria definition in order not to over or under-estimate the efficacies of various interventions.

2.3.2.1. Background to morbidity surveillance in Africa

Malaria is basically defined using fever and parasitaemia. The following sections describe how the temperature and parasitaemia data is collected and interpreted for epidemiological studies in Africa.

2.3.2.1.1: Measuring parasitaemia

Parasitaemia is an approximation of the density of circulating parasites within a host and is recorded as number of asexual parasites/ μl of blood.

2.3.2.1.1.1 Microscopy

Parasitaemia is determined by reading thin or thick smears, which are made from blood taken from finger pricks. Thin film smears are rarely used as a screening test in surveys but are useful for confirming the species of *Plasmodium* causing infection (Trape, 1985). The threshold of microscopy detection of malaria infections is about 40-50 parasites/ μl of blood (Payne, 1988).

Three methods of measuring parasitaemia are in regular use, firstly the use of the parasites/leukocyte ratio. If the white blood cell count (WBC) is taken at the same time as the slides, then an accurate extrapolation of ratio to density can be made. Otherwise, a standard mean leukocyte count of 8,000/ μl , is used in most studies. Secondly, the mean

number of parasites per oil immersion field of a thick blood film is also used but for good precision, the slides have to be evenly spread (Killian *et al.*, 2000). Proportion of positive fields requires that 200 fields be examined and the number of fields with at least one parasite noted. Thirdly, the proportion of slides that have all fields positive is used as an indicator of fluctuations in the morbidity levels in populations (Coosemans *et al.*, 1994). This third measure is only helpful in areas of low or moderate transmission. It is worth noting however that there could be variations in parasite density readings when slides are read by different persons (Delley *et al.*, 2000). It is therefore important to consider quality control in slide readings.

2.3.2.1.1.2 Rapid diagnostic tests (RDT's)

These tests rely on the detection of antigens derived from malaria parasites in lysed blood. Most employ a dipstick or test strip bearing mono-clonal antibodies directed against the target parasite antigen. The main antigen used in these kits is the Histidine-Rich Protein II (HRP II). Two main versions of this test are available: ParaSight™/® - F kit which is a rapid dip stick antigen capture assay while ICT Malaria Pf™ is an ImmunoChromatographic Test (Shiff *et al.*, 1993; Kodisinghe *et al.*, 1997; Singh *et al.*, 1997). An alternative kit, OptiMal®, detects dLDH (d-lactate dehydrogenase) which is produced by *Plasmodium* parasites (Tarimo *et al.*, 2001). These rapid tests have a sensitivity of >90% of detecting parasitaemia as long as the parasitaemia is above 1000 parasites/μl of blood and specificity >95% (Perri *et al.*, 1997; Pieroni *et al.*, 1998).

The advantages of RDT's over microscopy is that they are faster, requiring less training and experience and have higher sensitivity as they detect circulating antigens therefore whether the parasites are sequestered or not, their presence can be detected. The disadvantage of the tests is that they are more expensive than microscopy and not quantitative, hence of limited use in epidemiological studies.

2.3.2.1.2. Defining fevers during active malaria surveillance

Axillary temperatures are often taken in preference to rectal temperatures in field trials as they are non-invasive facilitating compliance. Several studies have been carried out comparing axillary and rectal temperatures in children (Morley *et al.*, 1992; Haddock *et al.*, 1996; Shann & Mackenzie, 1996; Craig *et al.*, 2000). Morley *et al.* (1992) demonstrated that axillary temperatures were only able to pick 73% of the fevers detected by rectal temperatures. Since rectal temperatures are considered to be a better indicator of body temperature than axillary temperatures, then the difference between these two readings in an individual demonstrates the level of accuracy of axillary readings.

Craig *et al.* (2000) conducted a meta-analysis of 20 studies that compared rectal and axillary temperatures and found that there was a wide range in these differences depending on the thermometer used and how long it was placed in the axilla. There was little difference between the rectal and axillary temperatures in neonates but differences were larger in older children and adults.

The studies above were conducted in controlled hospital environments but other factors are likely to affect fever detection in a field setting. A study was conducted in The Gambia where axillary temperatures were taken five times a day (every three hours) in 93 children aged between 1-5 years (Armstrong-Schellenberg *et al.*, 1994). There was a mean difference of about 1.2°C in normal body temperatures in the course of the day, this depended on the time of the day and the ambient temperature. The highest temperatures were in the early afternoon and the lowest in the early morning. Nigerian children living in a rural area were found to have a higher body temperature than those living in an urban setting (Fasan, 1974). These variations make it difficult to define 'normal' ranges for children's body temperatures.

Since malaria fevers are periodic, some studies have investigated the detection of abnormally raised acute phase proteins as a proxy or additional measure of fever. An example of a commonly used acute phase protein is the C-reactive protein (Ree, 1971, Naik and Voller, 1984; Hurt *et al.*, 1994; McGuire *et al.*, 1996). C-reactive protein (CRP) remains in the circulation for at least seven days from the onset of the fever though raised levels of these acute phase proteins is common with many illnesses and is non-specific (Deodhar *et al.*, 1989). In the case of cross-sectional surveys or longitudinal studies where patients are visited weekly or monthly, the presence of CRP could validate reported histories of fever. CRP on its own would have a low specificity but so would raised body temperature. Therefore the presence of both could improve on fever detection. Some studies that have used additional CRP data did not show an improvement in malaria diagnosis (Ree, 1971, Naik and Voller, 1984) whereas other studies have shown it to be useful (Hurt *et al.*, 1994; McGuire *et al.*, 1996).

2.3.2.2. Attributing fever to malaria infection

Since malaria is a febrile illness, case detection studies use fever as a screening tool. In a study done in Western Nigeria, Delfini (1973) observed a steep rise in the prevalence of parasitaemia from 25% to 50% over the temperature range of 37.2°C to 37.7°C but at higher temperatures, the level of parasitaemia remained relatively constant. In another study of hospital staff in Western Nigeria, Cobban (1960) found parasitaemia in 66% of the patients presenting with a febrile illness and a temperature $\geq 37.8^\circ\text{C}$ but in only 18% of those with a temperature below this level. In a study by Greenwood *et al.* (1987), a steep rise in the level of parasitaemia from about 30% to 60% was observed at temperatures of 37.5°C. Most studies conducted across Africa that use axillary temperatures have adopted the cut-off axillary temperature of $\geq 37.5^\circ\text{C}$ as a measure of fever (Appendix XI).

Concurrent fever and peripheral malaria parasitaemia form the basis of most definitions of malaria. However, in endemic areas, many individuals will have asymptomatic parasitaemia and so not all episodes of fever with accompanied parasitaemia indicate a causal relationship. This section shall discuss various methods of calculating malaria attributable fevers and to define malaria cases.

2.3.2.2.1 Fever and parasitaemia

A study conducted by Delfini (1973), demonstrated that an increase in parasite density was associated with an increase in the risk of fever in endemic areas. Therefore although the number of parasitaemic individuals in the febrile and afebrile group are often quite similar in endemic areas, there are higher parasite densities among those febrile than among those afebrile (Bruce-Chwatt, 1963). Low parasite densities were associated with low grade fevers (<38°C) or no fevers at all. An increase in parasite density resulted in an increase in body temperature but only up to 39.5°C after which no matter how large an increase in parasitaemia, there is no matching rise in body temperature (Barber & Olinger, 1931; Bruce-Chwatt, 1963). Despite the association between increasing parasite density and the risk of fever, there will always be people in endemic areas that will have a moderate parasitaemia without fever as a result of acquired immunity (Ross & Thomson, 1910). Nevertheless, studies conducted by Colbourne *et al.* (1955 a, b) showed that the probability of finding parasites in a febrile person were 2 to 4 times higher than that of finding parasites in an afebrile person.

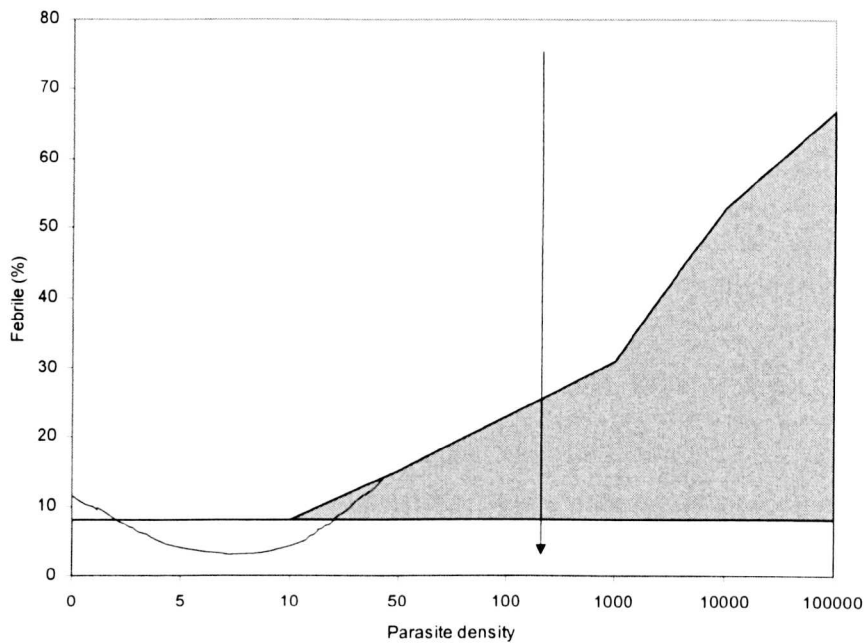
To what extent is it possible to define cut-off parasite densities above which the risk of fever starts to rise? Ross & Thomson (1910), set the pyrogenic threshold (parasite density at which the risk of fever starts to rise) at 600-1,500 parasites/ μ l of blood. Miller (1958), derived a threshold of 1,644 parasites/ μ l of blood for adults and 11,000 parasites/ μ l of

blood in children in Liberia. In this study Miller (1958) found that parasite counts as low as 30 parasites/ μl of blood would induce symptoms in adults and counts over 4,550 parasites/ μl of blood would undoubtedly cause clinical malaria, whereas children had a much higher threshold. People with parasitaemias above their age threshold would be considered malaria cases.

In a later study by Trape *et al.* (1985), a cut-off of a parasite/leukocyte rate ≥ 2 (approximately $\geq 16,000$ parasites/ μl of blood) was chosen as the pyrogenic threshold for children under the age of 5 years and there was little variation in the threshold within that age range. Velema *et al.* (1991) used the proportion of people that were febrile using various parasite cut-offs and concluded that with about 100-1,000 parasites/ μl of blood only 24% of the people were febrile while when using a cut-off of $\geq 1,000$ parasites/ μl over 84% of people were febrile. The cut-off with the highest number febrile was therefore taken to be the pyrogenic threshold for this area. Figure 2.2 demonstrates the difficulty of cut-offs chosen arbitrarily.

The rising curve represents the risk of a child having a fever with increasing parasite density. The shaded area under this line represents the fevers due to malaria and the unshaded area represents the fevers not attributable to malaria. The vertical line shows a typical case definition of parasites equal to and above a certain density. Any definition will miss some cases, (those under the shaded area to the left) and some fevers due to other causes will be classified as malaria (unshaded area to the right). One of the reasons why a cut-off chosen arbitrarily leads to over or under-estimation of malaria is because the relationship between parasitaemia and illness is affected by immune status of the host. The extent of the bias generated by using cut-offs depends on the age group that is being studied and the malaria transmission in that area.

Figure 2.2: The relationship between fever and parasite density in areas of high malaria transmission (Armstrong-Schellenberg *et al.*, 1994).



The small curve to the left of the chart shows negative risk of fever with low parasite densities and this is thought to be due to the fact that in the young age groups, fevers due to other conditions tend to lower parasitaemia (Rooth and Bjorkman, 1992). Measles and influenza are good examples of this, the importance of which will be demonstrated in the discussion of attributable fractions (section 2.3.2.2.2). One way to circumvent the problem is to ignore the whole issue of cut-offs and look at the fraction of fevers attributable to parasites or find a way to evaluate the accuracy of these cut-offs by calculating sensitivities and specificities.

2.3.2.2.2 Calculating attributable fractions

Attributable fraction is defined as the fraction of the total disease experience in the population that would not have occurred if the effect associated with the risk factor of interest were absent (Bruzzi *et al.*, 1985). In the case of malaria, this is the fraction of fevers that would be eliminated if malaria were eradicated. This can be calculated by using

the numbers of fevers among those that do not have parasitaemia and the number of fevers among those with parasitaemia. Two formulae have been used to describe the malaria attributable fraction

1- Greenwood *et al.* (1987)

Incidence of fever among those without parasitaemia – I_u

Incidence of fever in the population = I_p

Proportion of fevers in the entire population that are attributable to parasitaemia = AF_p

$$\text{Equation 2.5. } AF_p = \frac{I_p - I_u}{I_p}$$

2- Armstrong-Schellenberg *et al.* (1994) used a slightly different approach.

$$\text{Equation 2.6. } AF_p = p [(R-1)/R]$$

Where ‘p’ = proportion of fevers cases with parasitaemia and ‘R’ is the relative risk. The relative risk is calculated by comparing the risk of fever among those with parasitaemia compared to those without parasitaemia.

These two formulae will only hold if the prevalence of parasitaemia in those with fever is higher than that in those that are afebrile. In highly endemic areas, this does not always hold, which results in a negative attributable fraction estimate (Smith *et al.*, 1994). For example in a study conducted by Smith *et al.* (1994), in a population of children under the age of six years living in a high transmission area in Tanzania, 88.3% of febrile children had parasitaemia compared to 91.4% of the afebrile controls. One way to deal with this problem is to group those with low level parasitaemia with those that had no parasitaemia. Smith *et al.* (1994) demonstrated with data from Tanzania that this may result in varying figures of the attributable fraction depending on the cut-off at which the low parasitaemias are defined, which is normally chosen arbitrarily.

2.3.2.2.3. Use of logistic regression

The logistic regression model does not rely on the number of people without any parasitaemia. This method considers models that constrain the fever risk to a monotonic function of the parasite density. These models then estimate for each individual case of fever, the probability that that fever is attributable to parasitaemia (malaria). The logistic regression model equation is:

$$\text{Equation 2.7 } \text{Log } P_i / (1 - P_i) = \alpha + \beta_1 x_i^{\lambda}$$

P_i is the risk of fever for the observation 'i' with a parasite density 'x'. Maximum likelihood estimates of α and β were computed conditional on the estimate of ' λ ' which is the power parameter used to transform 'x'.

The Relative risk (R_i) of fever for parasite density x_i , relative to a density of zero is therefore

$$R_i = \exp \beta_1 (x_i)^{\lambda}$$

The attributable fraction (λ) is therefore estimated as:

$$\text{Equation 2.8 } \lambda = 1/N \sum_i (R_i - 1) / R_i$$

The summation is over the N cases of fever

The logistic regression method can also be used to derive sensitivities and specificities of various malaria definitions. The method is described in detail in chapter five where it is used to calculate malaria case definitions in data collected in Kilifi.

2.4 The epidemiology of severe malaria

The majority of clinical malaria cases are mild or uncomplicated because progression is limited by either treatment or host response. However, a proportion of episodes progress to become severe and complicated and may lead to death. In this section, I consider the factors affecting the epidemiology of severe malaria in sub-saharan Africa, mainly age and transmission intensity through their effects on the acquisition of immunity.

2.4.1 Description of hospital surveillance studies linked to population studies.

The following section will be a summary of studies that have provided data on the pattern of severe malaria. This data will be used to discuss how differences in malaria transmission alter the pattern of severe malaria. These studies were conducted in a number of hospitals in the following countries: Kenya, The Gambia, Malawi, Burkina Faso, Tanzania and Senegal.

Kenya and The Gambia

Data from five study sites: Bakau and Sukuta in The Gambia, Kilifi north, Kilifi south and Siaya in Kenya have been described by Snow *et al.* (1997). Within these four study sites, severe malarial anaemia was defined as a haemoglobin level ≤ 5 /dL, whereas cerebral malaria was defined using a Blantyre coma score of ≤ 2 . A primary diagnosis of malaria was made only when the child had a peripheral blood parasitaemia with no other cause for admission.

Malawi

A study was conducted in Malawi comparing hospital admissions in the Queen Elizabeth Central Hospital (QECH), which is located in Blantyre city, and the Mangochi District Hospital (MDH) in the townships (Slutsker *et al.*, 1994). The populations served by MDH were from an area with sustained transmission whereas QECH admissions were from an urban area where there was fluctuating malaria risk. This study discusses only severe malarial anaemia, which was defined as a haematocrit $\leq 15\%$ in the presence of *P. falciparum* parasitaemia.

Burkina Faso

The Burkina-Faso study on severe malaria compared two populations under differing transmission (Modiano *et al.*, 1998). One was an urban population in Ouagadougou

exposed to approximately 5-10 infective bites/person/year compared to a rural population around Ouagadougou that was exposed to 300-500 infective bites/person/year. The rural population was involved in an ITBN trial (Habluetzel *et al.*, 1999). Severe anaemia was defined as the presence of *P. falciparum* parasitaemia and a haemoglobin level ≤ 5 /dL. Coma (cerebral malaria) was defined as a score between 0 and 2 on the Blantyre coma score in the presence of *P. falciparum* parasitaemia.

Tanzania

In Tanzania, data from hospital surveillance at the St. Francis Designated District Hospital (SFDDH) in Ifakara, Kilombero District was used (Snow *et al.*, 1994; Schellenberg *et al.*, 1999). The people in this area were exposed to about 300 infective bites/person/year. Severe anaemia was defined as the presence of *P. falciparum* parasitaemia and a PCV $< 15\%$ whereas impaired consciousness was assessed subjectively i.e., the inability to localise pain and the inability to sit unsupported for those ≥ 8 months old.

Senegal

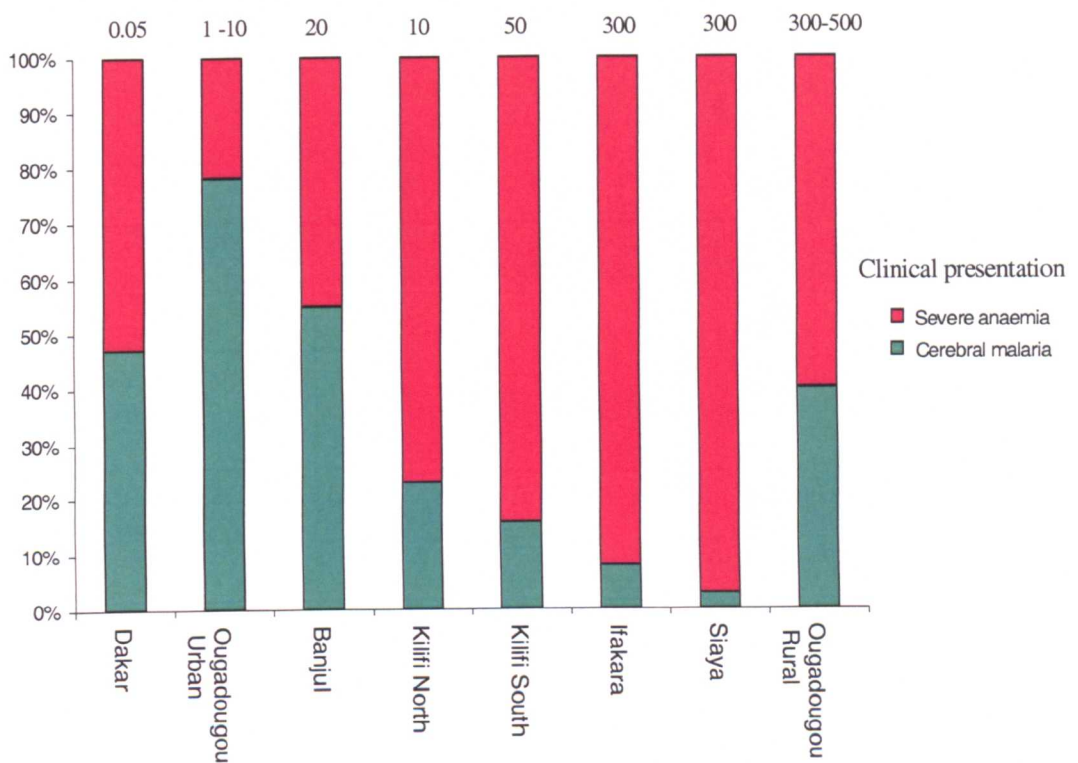
Data from 1990–1996 was analysed from a paediatric hospital in Dakar where the drainage population was exposed to about 0.05 infective bites/person/year (Imbert *et al.*, 1997). Only 161 (34%) of the patients admitted in those six years were classified as severe malaria though there was careful evaluation of each patient through laboratory and clinical investigations to eliminate the possibility of malaria. Severe malaria anaemia was defined as the presence of *P. falciparum* parasitaemia and haemoglobin levels ≤ 5 /dL or a haematocrit of $< 15\%$ whereas cerebral malaria was defined as parasitaemia and unarousable coma.

The following sections will discuss clinical presentation and age patterns of severe malaria under varying levels of transmission as observed from these studies.

2.4.2 Clinical patterns of severe malaria by transmission and age

Studies on severe malaria conducted in the various African settings have demonstrated that there are differences in presentation relative to transmission intensity. Figure 2.3 illustrates these findings. The range of EIR that are represented in this figure are from the left 0.05 infective bites/person/year in Dakar to 300 infective bites/person/year in Siaya.

Figure 2.3: Presentation of severe malaria anaemia and cerebral malaria in areas with differing levels of malaria transmission in Africa. The intensity of transmission defined as the number of infective bites/person/year (EIR) is indicated at the top of each column (Marsh and Snow, 1999).

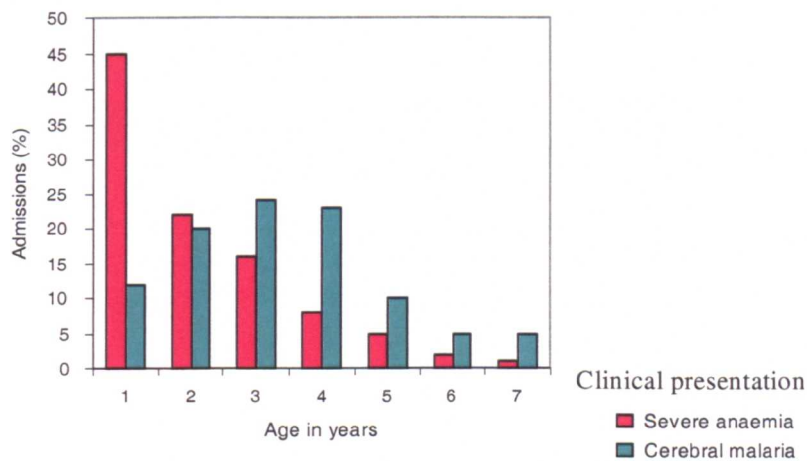


This summary of data from East and West Africa suggests that severe malarial anaemia is more common in high transmission areas whereas cerebral malaria has its highest incidence in the low transmission areas (Marsh and Snow, 1999). In three of the four areas with an EIR >50, over 80% of the severe malaria cases were classified as severe anaemia

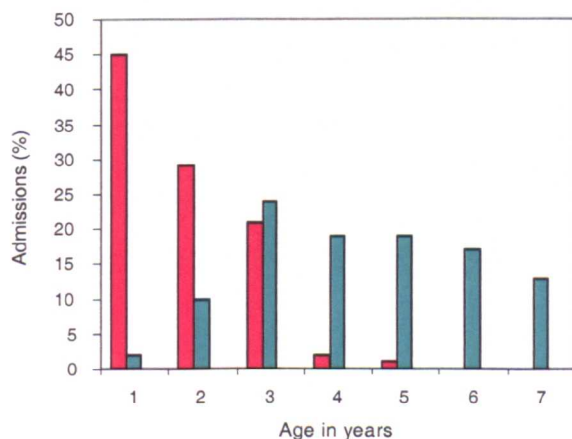
whereas in three of the four areas with an EIR<20, less than 50% of the severe malaria cases presented as severe malarial anaemia. There seems to be a higher rate of cerebral malaria in West Africa compared to East Africa irrespective of transmission (Marsh and Snow, 1999). However even within West Africa, there was more severe anaemia in the high transmission areas and more cerebral malaria in the low transmission areas. This difference in the clinical presentation of malaria with transmission is strongly related to differences in the clinical presentation of severe malaria with age. These differences are illustrated in Figure 2.4 that compares data from The Gambia and Kenya.

Figure 2.4: Clinical presentation of severe malaria anaemia and cerebral malaria in children 1-7 years of age in two study areas in Africa with differing transmission.

Kenya (Marsh and Snow, 1997)



The Gambia (Marsh, 1992)



The malaria admission rate due to severe malarial anaemia is highest in those in the under two year age groups. About 45% of malaria admissions in those less than or equal to one year of age were severe anaemia admissions, this dropped to about 25% in the two year olds and about 15% in the three year olds. By the age of five, less than 3% of the malaria admissions were due to severe anaemia. Cerebral malaria however showed a different age presentation, with a maximum in the 3-4 year olds of about 20% of all malaria admissions. By the age of five years, the rate of admissions due to cerebral malaria had dropped to less than 10% whereas in The Gambia, the rate of cerebral malaria admissions remains at about 10% beyond the age of seven reflecting the pattern of more cerebral malaria in West Africa than East Africa. The mean age for severe anaemia in The Gambia was 27 months and 45 months for cerebral malaria while in Kenya, the mean age for severe anaemia was 22 months and 40 months for cerebral malaria (Marsh, 1992).

Similar results were obtained by Modiano *et al.* (1998), in Ougadougou when they compared two areas with high (EIR=300) and low (EIR=10) transmission. The highest rate of severe malarial anaemia was in children ≤ 2 years old irrespective of transmission and there was a rapid decline in the rate of severe anaemia in both areas with age. Like the data from The Gambia and Kilifi, by the age of three years, the prevalence of severe malaria anaemia was very low in Burkina Faso.

The reasons for this pattern of clinical presentation of malaria with age are not clear. A possible explanation as to why severe anaemia is more common in very young children maybe related to the small absolute volume of blood and total numbers of erythrocytes. The destruction of erythrocytes as occurs during a malaria attack therefore leads to the rapid depletion of total erythrocyte numbers leading to severe anaemia.

A number of hypotheses have been put forward to explain why cerebral malaria is more common in older children than their younger counter-parts (Snow and Marsh, 1998a;b). One hypothesis is based on physiological maturation with age that might be related to the ability to express key endothelial receptors for parasite sequestration in internal organs. There is no direct evidence of this but it is consistent with the observations that when non-immune adults and children are suddenly exposed to high malaria transmission, adults are at higher risk of developing cerebral malaria than children (Baird *et al.*, 1991). Secondly, cerebral malaria may have an immunopathogenic element and be exacerbated by previous exposure to malaria but against this is the observation that non-immunes do develop cerebral malaria on first exposure (Gupta *et al.*, 1999). The third hypothesis postulates that cerebral malaria is caused by a distinct group of parasites that are rare and encountered by chance by older children but against this is the observation that severe malaria including cerebral malaria is caused by relatively common malaria parasites (Bull *et al.*, 2000).

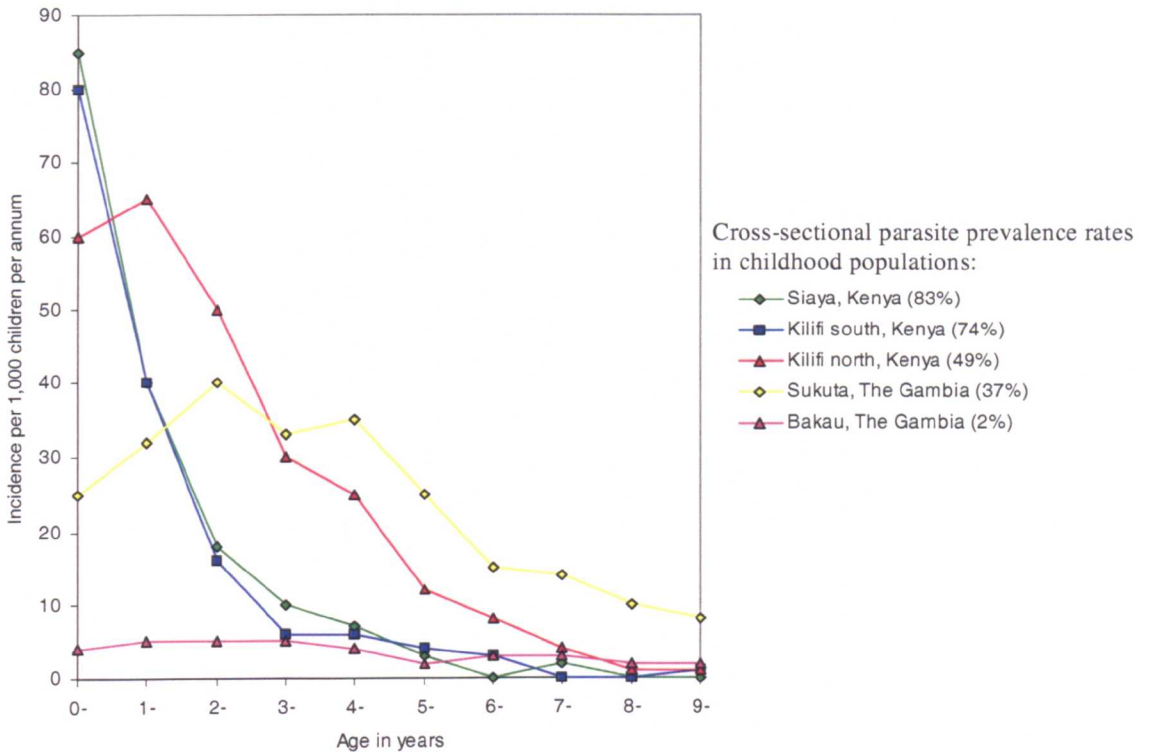
2.4.3 Pattern of severe malaria by age and transmission intensity

This section will discuss overall severe malaria patterns (combining all syndromes considered to be severe malaria) in relation to variations in the level of transmission. It has been noted earlier that in areas of high transmission, severe malaria is concentrated in the very young (Snow and Marsh, 1998a;b). Figure 2.5, compares age data on severe malaria admissions in five study sites in The Gambia and Kenya with differing transmission. The figure describes the incidence of severe malaria per 1,000 children per annum in children under 10 years of age from areas with differing transmission.

Transmission ranged from highest in Siaya and Kilifi south with parasite prevalence among children being 74% and 83% to moderate transmission with a parasite prevalence of 37% and 49% in Kilifi north and Sukuta and low transmission in Bakau with a parasite prevalence of about 2% in the childhood population. In the high transmission areas of

Siaya and Kilifi south, the incidence of malaria dropped from over 80 episodes/1,000 children/annum to about 40 episodes/1,000 children/annum in the one year olds and to less than 20 episodes/1,000 children/annum in the two year olds. Severe malaria was therefore concentrated mainly in the first two years of life.

Figure. 2.5: Age specific patterns of severe malaria admissions at five sites in Kenya and The Gambia (Snow *et al.*, 1997).



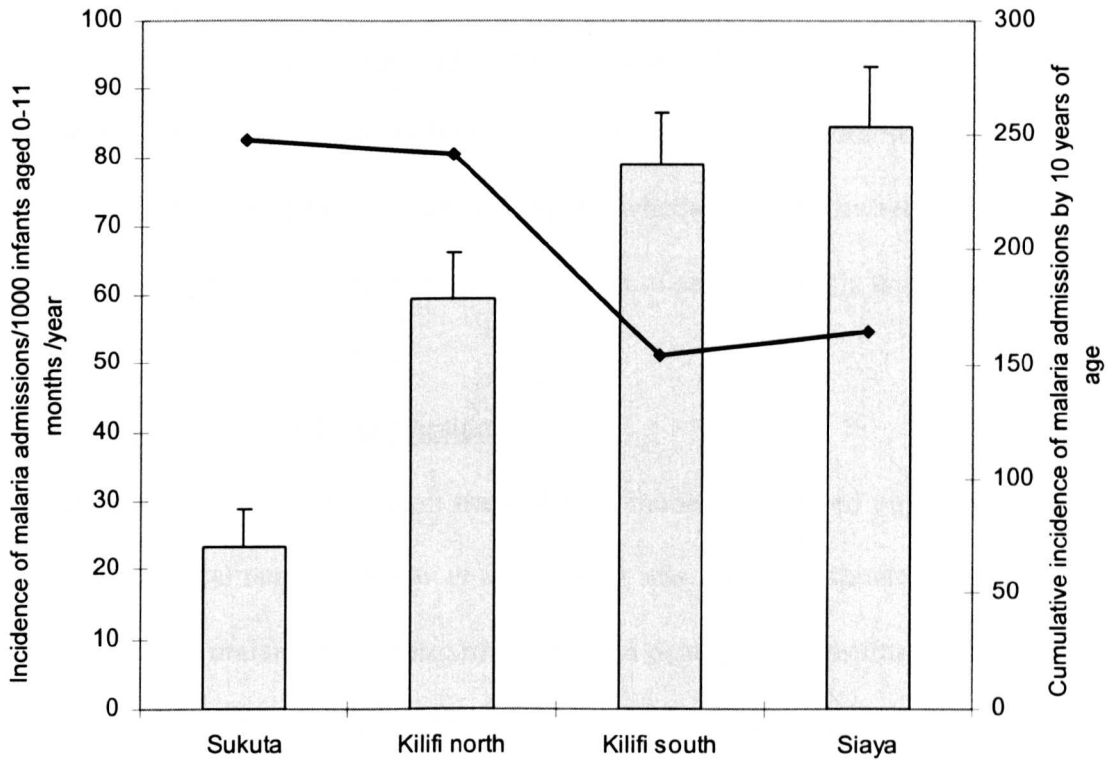
In Kilifi north and Sukuta, areas of moderate transmission, the incidence of severe malaria peaks at the age of one year and then declines gradually to a level of about 25-10 episodes/1,000 children/annum in the five year olds. In Sukuta, the incidence of malaria persisted at a low level (<15) throughout childhood whereas it had declined significantly (<5) by the age of 8 years in Kilifi north. However, in areas of low transmission like Bakau, severe malaria is spread out evenly throughout childhood but does not rise above 10-episodes/1,000 children/annum.

It might be expected intuitively that populations living in areas of high transmission would have an overall higher rate of severe malaria than populations living in areas with low malaria transmission. However, two studies conducted in Brazzaville and Kilifi demonstrated that there was no linear increase in the rate of severe malaria disease with increase in EIR (Trape *et al.*, 1987; Mbogo *et al.*, 1995). When comparing admissions due to severe malaria among children residing in area with an EIR ranging from 0 to 69, Mbogo *et al.* (1995) found that there were no differences in the rates of severe malaria. Trape *et al.* (1987) studied areas with EIR ranging from <1 to 100 infective bites/person/year in Brazzaville, Congo and found that there were no differences in the rate of severe malaria attacks across these ranges of transmission.

A study that observed the number of episodes of severe malaria in children less than a year old showed that immunity developed relatively fast in areas of high transmission (Snow *et al.*, 1998c). The main result of this study was that the overall rate of malaria with increasing malaria transmission plateaus and probably declines with increasing transmission. Figure 2.6 compares the incidence of malaria admissions in 1,000 infants aged 0-11 months per year to the cumulative incidence of malaria admissions by the age of 10 years in areas with differing transmission.

Data from four of the five sites described by Snow *et al.* (1997) were analysed. The admissions/1,000 infants in the 0-11 month age bracket were highest (84.6) in Siaya, that was the area with the highest transmission and lowest (23.3) in Sukuta, the area with the lowest transmission. The pattern among those under a year old demonstrated a pattern of increase in severe malaria admissions with increasing transmission in these four sites.

Figure 2.6: Changes in severe malaria admissions with increasing transmission in infants (bars) and children under the age of 10 years (line) from four sites in The Gambia and Kenya.



The comparison of overall admissions in the 0-9 year olds in the five study sites described showed that there was an actual decrease in the rate of severe malaria in areas of high transmission compared to areas with less transmission which was the opposite effect to that found in the infants. A more detailed look at the data showed that in Siaya and Kilifi south, peak admissions occurred at the age of 3-5 months, the decline in the rate of admissions starts in the sixth month whereas in Kilifi north and Sukuta, the rate of admissions continues to rise throughout infancy and there is no decline in the rate of admissions right to the 11th month (Snow *et al.*, 1998c).

In all study areas, there was minimal severe malaria in children under the age of three months. This is thought to be due to protective mechanisms that operate at this time that include foetal haemoglobin, maternal antibodies and riboflavin deficiency.

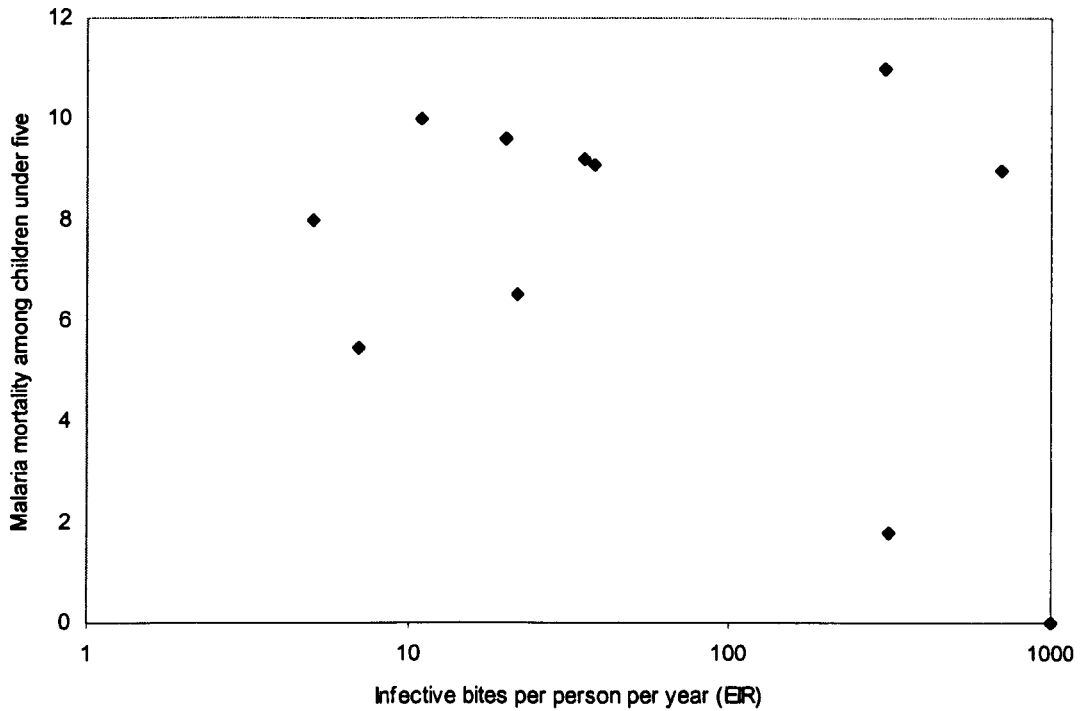
In some situations, severe malaria admissions appear to increase with transmission and in others there appears to be no differences in admission rates with wide differences in transmission. The next section seeks to find out whether or not the relationship between malaria mortality and transmission is similar to that of severe malaria and transmission.

2.4.4 Malaria mortality and transmission

Using integration of high-resolution transmission models, projected population structures and epidemiological surveys, Snow *et al.* (1999a) was able to estimate the population in Africa exposed to malaria and approximate malaria deaths. They estimated that about 360 million people in Africa are exposed to the risk of malaria transmission and about a million deaths occurred due to malaria in 1990. The median estimate of malaria deaths was 8.0 (Inter quartile range: 4.6-10.3) malaria deaths/1000 children under five years of age/year.

It might be expected that the majority of these deaths would occur in areas with the highest malaria transmission. However in a review of 11 studies for which malaria mortality and transmission data was available, Snow and Marsh (1995) showed that though the data was poor, it was consistent with different interpretations including a rise in mortality with increasing transmission, a plateau or a fall in the mortality with increasing transmission. This data is illustrated in Figure 2.7. These observations of malaria mortality and transmission created a lot of debate (Sauerwein & Meuwissen, 1995; Carme, 1996; Lengeler *et al.*, 1995 & 1997; D'Alessandro and Coosemans, 1997; Shiff, 1997; Trape, 1997; Greenwood, 1997; Lines, 1997).

Figure 2.7: Malaria mortality in children under five years of age from areas with varying malaria transmission in Africa (Snow and Marsh, 1995).



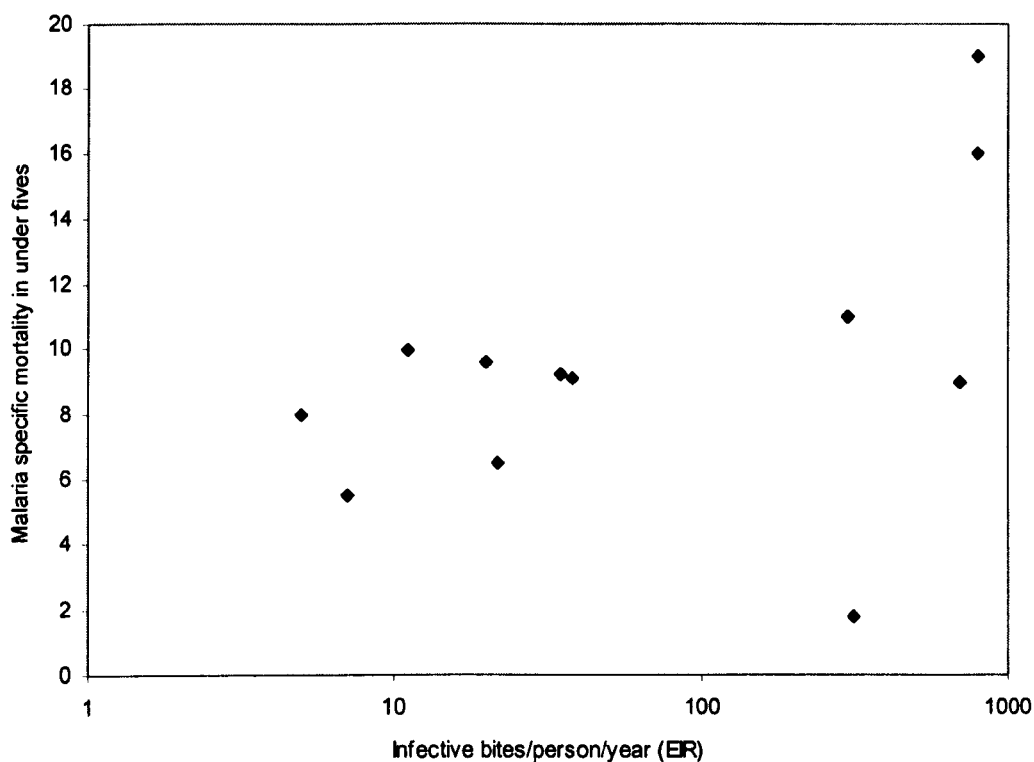
The next section concentrates on the debate on whether reduction in transmission will actually cause a reduction in the rate of severe malaria and death and who would be able to benefit from the reduction of malaria transmission in Africa.

2.4.5 Effect on mortality of reducing malaria transmission

One of the interpretations from Figure 2.7 is that malaria mortality decreases with increasing malaria transmission. This would imply that benefits of transmission reduction would only be transitory and deaths and disease may not be prevented but postponed as efficient vector control will only prevent the development of natural immunity (Snow and Marsh, 1995, 1997). The whole issue creates doubt on the value of such interventions as ITBN's, house spraying, genetically engineered mosquitoes, pre-erythrocytic and transmission blocking vaccines and conventional vector control (Smith *et al.*, 2001).

Lengeler *et al.* (1997) re-analysed the same data presented by Snow and Marsh (1995) after removing the data points from the 1950's, as they were not comparable to current data and also without the data from Brazzaville. Figure 2.8 illustrates this data and an additional two extra data points from areas in Tanzania with high malaria transmission and high mortality.

Figure 2.8: Modified graph of malaria mortality in children under five years of age from areas with varying malaria transmission in Africa (Lengeler *et al.*, 1997).



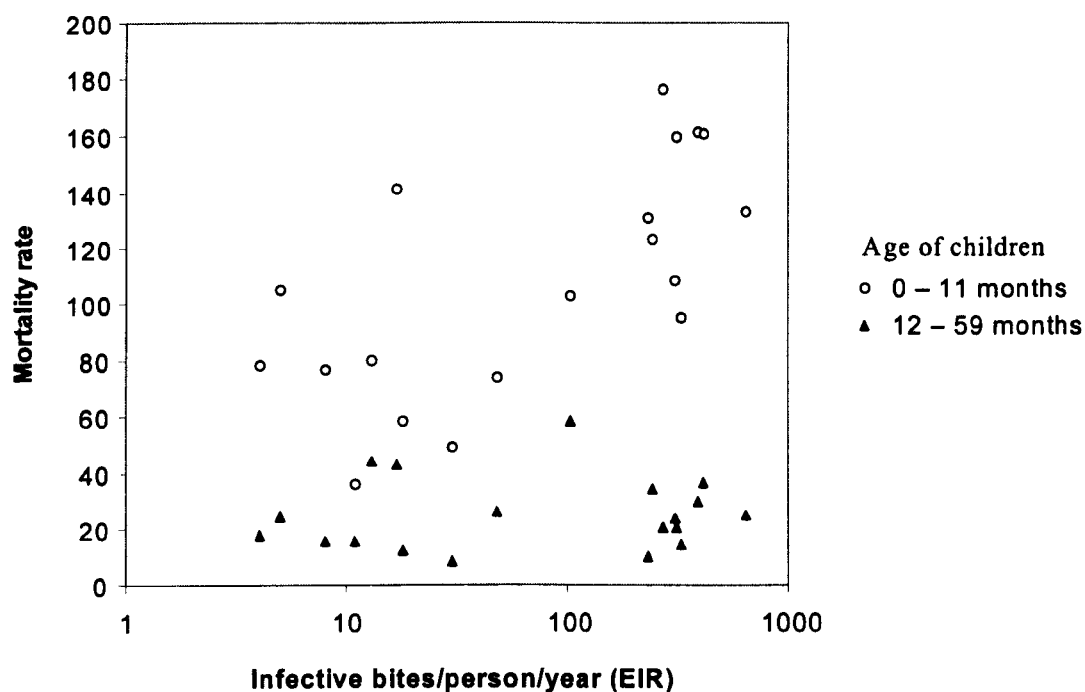
The Brazzaville data was considered biased as the mortality rates from this region are not typical of Africa due to the high usage of anti-malarials and high literacy rates compared to other parts of Africa (Carne, 1996). Secondly, Kinshasa which is across the river Congo from Brazzaville with similar exposure recorded mortality rates as high as those in the rest of Africa (Greenberg *et al.*, 1989) suggesting that another factor other than transmission

had a large role to play in the prevalence of severe malaria and mortality in Brazzaville.

The analysis suggested a linear relationship between malaria transmission and mortality.

Smith *et al.* (2001) considered published and unpublished data from 1980-1999 and looked at all-cause mortality in infants (0-11 months of age) and children 1-5 year olds. Figure 2.9 compares all cause mortality rate in infants and children 1-5 years old with increasing malaria transmission.

Figure. 2.9: Mortality rates in infants (0 to 11 months) and children (12 to 59 months) with increasing EIR (Smith *et al.*, 2001).



Key:

Infant mortality rate- Deaths per 1,000 live births in children 0-11 months old

Child mortality rate – Deaths per 1,000 child-years-at-risk in children 12 – 59 months old

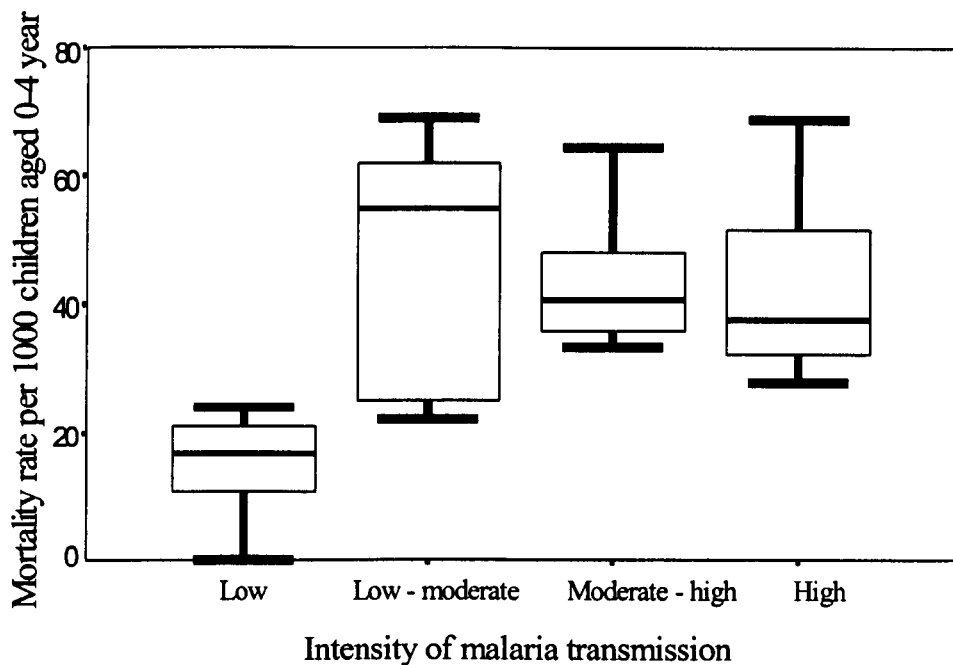
The reasons why they looked at all-cause rather than malaria specific mortality was first, malaria specific mortality is hard to determine from verbal autopsy especially that which is caused by severe anaemia. Second, mortality from malaria may be associated with co-

infections and it is erroneous to ascribe many of these deaths to one cause. Third, malaria may be associated with indirect mortality from other conditions and last, malaria control measures are always associated with a larger reduction in all-cause mortality that cannot be attributed solely to the reduction in malaria deaths alone. The data showed that all-cause mortality in infants increased with transmission intensity. On the other hand, there was no difference in the mortality rate in the children 1-5 years of age with increasing transmission.

The results suggest that a reduction in malaria transmission resulted in a reduction in the infant mortality rate and this is the group that is likely to benefit from any malaria control measures that are aimed at reducing transmission. This is also the age group susceptible to severe anaemia. Although severe malaria anaemia is associated with low mortality where blood transfusions are easy to perform, in the era of HIV, there is benefit in reducing the number of blood transfusions, which would reduce indirect mortality as a result of transfusion transmission on the virus (Snow *et al.*, 1999a). These data also suggest that interventions that may lead to a reduction in malaria transmission may not necessarily lead to an increase in malaria mortality in older children. This idea has been supported by data from a four year follow-up of children involved in a trial of insecticide treated curtains in Burkina Faso (Smith *et al.*, 2001).

The most recent and comprehensive contribution to this debate is a review of studies (26 in total) conducted since 1980 where all cause mortality from 0-5 years were recorded prospectively (Snow and Marsh, 2002). Figure 2.10 demonstrates the mortality rates/1,000 children between 0-4 years in areas of differing endemicities. Instead of EIR, parasite prevalence rates were used and endemicity was categorised as low if parasite infection was <25%, low-to-moderate if 25-50%, moderate-to-high if 51-74% and high if >74%.

Figure 2.10: Box plots showing the median (central lines) 25%, 75% quartile ranges around the median (box width) and upper and lower limits (T) of all-cause childhood mortality per 1,000 children aged 0-4years per annum recorded during the surveillance of communities with different malaria endemicities (Snow and Marsh, 2002).

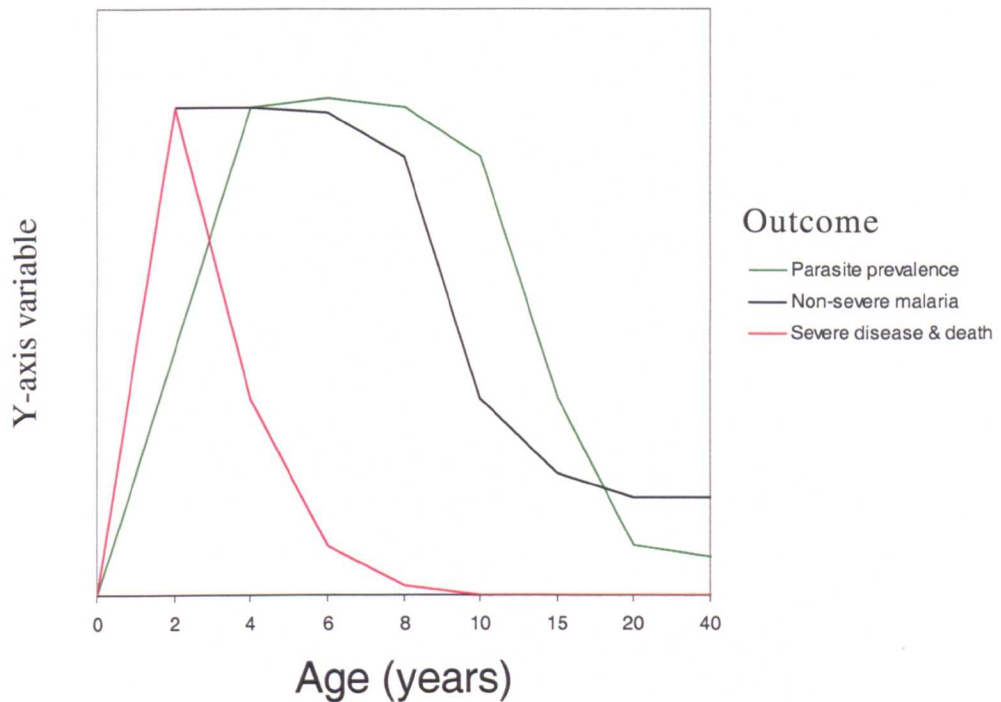


Children living under low endemicity have low mortalities compared to those in moderate to high endemicities. There was little difference in all-cause mortality under the age of five years over a broad range of malaria transmission intensities. Within a small increase in endemicities from to low-moderate, there was a sharp increase in all-cause mortality and soon after saturation at an unknown point.

2.4.6 Teasing apart the effect of age and transmission on severe malaria disease.

Age impacts on parasite prevalence, non-severe and severe malaria disease patterns in endemic areas. Figure 2.11 is a hypothesised illustration of the ‘characteristic’ picture for the three outcomes with age that is typical of many malaria endemic areas.

Figure 2.11: Hypothetical representation of the relationship between parasitaemia, non-severe malaria and severe malaria with age in endemic areas



The curves shift right with increasing intensity of transmission and left with decreasing transmission if all other factors are held constant. Parasite prevalence rises sharply with age and stays high in young children and then drops. Parasite prevalence peaks at an older age group in the moderate areas of transmission and much earlier for those in the areas of high transmission. This drop in the parasite prevalence with age is a reflection of immunity, which is characterised by low parasite prevalence in adults relative to children in endemic areas.

The rate of non-severe malaria begins to fall at different age point (older) than that of severe malaria. The rates of severe malaria drops very rapidly and there are very few cases of severe malaria in older children and adults. Immunity is characterised by a reduction in occurrence of disease even in the presence of high parasitaemia.

It has been thought for many years that immunity against malaria results from continuous uninterrupted exposure to infections and will not develop where malaria is epidemic or hypo endemic, is lost if there is a temporary loss of exposure and is not sterilizing (Day and Marsh, 1992). However since exposure is a function of age in endemic areas, it is hard to tease out the effects of age from those of exposure. One way to study this is to look at disease in people that are suddenly exposed to high infection pressure. Baird *et al.* (1991) recorded observations of Javanese transmigrants to Irian Jaya. In Java, the parasite rates were < 1% whereas, they ranged from 33-45% in Irian Jaya and these transmigrants were exposed to about 0.037-0.016 infective bites/person/night. Brief exposure established age-dependent protection independent of cumulative exposure to malaria (Baird *et al.*, 1991). When these observations were extended to five years and over five villages recruited, it was established that for most of the villagers, this age-dependent immunity developed within two years and it was only in one village that it developed within a year (Baird *et al.*, 1993).

Results from these studies of transmigrants to Irian Jaya from Java demonstrated that age is an independent predictor of the ability to develop anti-parasite immunity (Baird, 1995). Age-related intrinsic factors such as the involution of the thymus, degeneration of lymphoid tissues and subtle changes in the number of circulating immune cells were hypothesised to be involved in the development of immunity.

2.4.7 Other factors that contribute to severe malaria and mortality

2.4.7.1 Drug resistance

Using 38 studies with endemicity and malaria mortality data from 1912-1995 from sub-Saharan Africa, Snow *et al.* (2001) were able to show that although overall childhood mortality has been going down over the decades (Table 2.4), whereas malaria mortality has risen since the 1990's.

Table 2.4: Changes in malaria-specific mortality among children in selected community-based demographic studies in Africa (Snow *et al.*, 2001).

Area	Date of mortality survey	Malaria-specific mortality per 1000 children aged 0-4 years/year	Malaria as a percentage of all-cause mortality in children aged 0-4 years
Tanga, Tanzania	1933 – 1934	15.8	29
	1992 – 1993	15.4	36
Bagamoyo, Tanzania	1983 – 1985	7.8	23
	1992 – 1994	15.4	46
Niakar, Senegal	1984 – 1989	6.4	10
	1990 – 1995	10.2	23
Bandafasi, Senegal	1984 – 1989	3.8	5
	1990 – 1995	6.7	10
Mlomp, Senegal	1985 – 1989	0.6	3
	1990 – 1995	4.7	17
Coast, Kenya	1932 – 1935	6.6	14
	1991 - 1995	6.7	35

There was an increase in malaria-specific mortality across various sites in Africa over the years and in most areas there was a doubling of the rate of malaria. There was also an increase contribution to all-cause mortality by malaria. The most likely explanation of rising malaria-specific mortality was the advent of chloroquine resistance. A study of a stable population in the Brooke Bond tea growing company in Kericho in the Kenyan highlands showed that there has been an increase in the number of malaria admissions since the early 1990's (Shanks *et al.*, 2000). Malaria case fatality has risen from 1.3% in the 1960's to 6% in the early 1990's. There have been no major climatic changes in the decades of follow-up or changes in health delivery services; the major change was a marked increase in drug resistance. Hospital data collected in Kinshasa from 1982-1986

showed a rise in mortality in 1986 that was coincidental with the rapid development of chloroquine resistance to *P. falciparum* malaria (Greenberg *et al.*, 1989).

Data from Mlomp (Senegal) suggests that the increase in the mortality due to malaria started in the early 1990's and there was at the end of the decade almost an 11-fold increase in mortality due to malaria (Trape *et al.*, 2002).

2.4.7.2 Host genetics

Malaria has been such a major cause of mortality in Africa that one would expect any host polymorphisms that confer decreased susceptibility to be under strong selection pressure, the classical example is sickle cell trait. More recently, an increasing number of genetic polymorphisms affecting the red blood cell and the immune system have been reported to affect susceptibility to severe malaria, some of these are discussed in the following sections.

2.4.7.2.1 Genetic disorders of the red cell

Genetic disorders of the red cell are the commonest gene disorders involved in protection against malaria, they include: sickle cell trait, thalassaemias and Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency. Haemoglobins S, C or E have been found to be protective across different malaria endemic areas of the world (Weatherall, 1987). G6PD deficiency follows a similar world distribution to the three haemoglobinopathies but β thalassaemias are not as widespread as α thalassaemias.

In some cases, for example sickle cell trait, protection is offered only among individuals with the heterozygous state. The homozygous state of this trait is often fatal. The trade-off is therefore death from the disease condition for which protection is enhanced or death

from the homozygous state. The gene frequency in a population is therefore maintained by the enhanced survival of the heterozygous state.

Studies in The Gambia and in Kenya have shown that the sickle cell trait provides 90% protection from both cerebral malaria and severe malarial anaemia (Hill *et al.*, 1991, Marsh, 1992). Studies in the same areas also reported that G6PD deficiency was associated with a 46-58% reduction in the risk of severe malaria in both male hemizygotes and female heterozygotes (Ruwende *et al.*, 1995). A large case-control study conducted in Burkina Faso demonstrated a 29% reduction in risk of clinical malaria in haemoglobin C homozygotes and 93% reduction in risk among heterozygotes (Modiano *et al.*, 2001).

2.4.7.2.2 Other genetic polymorphisms

Hill *et al.* (1991) reported that 40% protection against severe malarial anaemia and cerebral malaria in those with HLA class I antigen HLA-Bw53. They also found some protection against severe malarial anaemia in those with HLA class II antigen HLA-DRB1*1302. The main role of these HLA molecules is to present foreign antigens to T-lymphocytes. HLA class I molecules are associated with the presenting of antigen peptides to cytotoxic T-lymphocytes while the parasites are in the liver while the HLA class II molecules assist in antibody responses as demonstrated by HLA-DRB1*1302 which is known to generate an effective immune response.

There are several host molecules that bind to parasite molecules expressed on the surface on infected erythrocytes and these include CD36, thrombospondin, intracellular adhesion factor-1 (ICAM-1), E-selectin and chondroitin-4-sulfate (Newbold *et al.*, 1997). Pain and colleagues (2001) were able to show that the heterozygosity of one CD36 mutation was associated with protection from severe malaria.

A number of genetic polymorphisms affect the acute response to infections and may be expected to have an effect on malaria outcome. An example of this is the protection from malaria associated with TNF α promoter polymorphisms. It has been demonstrated that children who are homozygous for the TNF α promoter 2 allele (TNF2) suffer more severe illness than children that are heterozygous for TNF2 or homozygous for TNF α promoter 1 allele (McGuire *et al.*, 1994). This has been proposed to be because TNF2 is thought to increase the expression of TNF α , which is associated with severe disease when it is produced in excess whereas when it is produced at low levels, it is associated with protection. This may explain why the homozygous state is not protective while the heterozygous state is protective. However, in a study conducted among infants in Tanzania, TNF α promoter polymorphisms appeared not to have a protective effect against malaria (Stirnadel *et al.*, 1999).

2.4.7.3 Socio-economic factors

Socio-economic factors are likely to influence the occurrence and outcome of malaria illness in several ways. The length of time to seek medical attention is associated with distance to hospital but also to social-economic factors. Whether the child is taken to a hospital is determined by the mother's level of education as many illiterate families consider severe neurological signs and symptoms (coma and seizures) as treatable by traditional healers and not the formal health centres (Molyneux *et al.*, 1999). If a family does not have enough money for basic needs, there is unlikely to be any spending on malaria protection such as the use of mosquitoes coils, bed nets, aerosol sprays or chemo prophylaxis (Lindsay *et al.*, 1990). Household wealth also determines house structure with affluent homes having ceilings and no eaves, which let fewer mosquitoes in, hence lowering malaria transmission.

The few studies that have been conducted in Africa looking at the relationship between socio-economics status and the occurrence of severe malaria have produced conflicting results. Koram *et al*; (1995) in a study conducted in The Gambia found no relationship between socio-economics and the occurrence of severe malaria, the same was found by Luckner *et al*; (1998) in a study conducted in Gabon. Carne *et al*, (1994) on the other hand found that there was an association between the occurrence of cerebral malaria and socio-economic status in Congo. The probable difference in these results is the study design. Whereas Carne in the Congo study did not take the size of the household into consideration when choosing the control sample, the other 2 studies did. In the Congo study, controls were sampled from the general population whereas in the other 2 studies, the controls were mild malaria cases from health centres. The other issue that may lead to the observed difference was that in The Gambia, (where Koram conducted his study), most deaths do not occur in the hospital (Greenwood *et al.*, 1987).

2.4.8 Shortcomings of these comparisons

The findings in section 2.4 were as a result of comparisons made of data from several sites across Africa to describe disease patterns and presentation with transmission. However, comparisons of such data are fraught with difficulties that could lead to bias in the interpretation of the results. Some of the shortcomings of comparing these data sets are discussed in this section.

2.4.8.1 Use of hospital data

Comparing hospital data from various places across Africa has its limitations as there might have been mobility between exposure settings and even if the populations were stable, there must have been year-to-year variation in disease rates that was not accounted for (Snow *et al.*, 1997). The other source of difficulty is the assumption that the populations in the various areas used the health facilities at the same rate. Comparing the

age distribution of other common conditions like acute respiratory infections in these sites is useful in showing whether there are any age differences in the use of these health facilities (Snow *et al.*, 1997). However, if a population perceives that certain symptoms occur due to 'spiritual' problems (e.g. fits), they are more likely not to take such cases to the hospital and refer them to traditional healers leading to biases in the estimation of the population burden of such conditions (Molyneux *et al.*, 1999).

2.4.8.2 Difficulties in measuring transmission

There is also the difficulty of measuring the force of infection or the entomological inoculation rates. In some cases like Ifakara, passive case detection and force of infection estimates were calculated from different populations. Sometimes year-to-year variation can result in large differences. In a span of three years in Dielmo, Senegal, EIR estimates ranged from 89 to 238 whereas in Ndiop it ranged from 7 to 63 in a span of four years (Hay *et al.*, 2000). There are also biases in the approximation of transmission with preferential data collection being undertaken in areas that are known to have malaria, this data is then extrapolated to large areas and may not be representative (Hay *et al.*, 2000). This problem can be resolved by the use of parasite prevalence rates in children from the community, which are less prone to error (Beier *et al.*, 1999).

2.4.8.3 Difficulties in measuring mortality

One of the shortcomings cited for these comparisons of malaria transmission and malaria mortality is that a number of these studies rely on mortality data that has been derived from verbal autopsy. Verbal autopsies are used to ascertain the cause of deaths that occur outside the hospital setting. They are based on three assumptions, firstly that it is possible to differentiate the causes of death from clinical and historical events, secondly that bereaved parents can accurately recognize and describe their events and thirdly that there is retention of clinical and historical information over time (Snow and Marsh, 1992).

Studies conducted in The Gambia and Kenya comparing the cause of death as recorded in the hospital and by use of verbal autopsy found that verbal autopsy had a sensitivity of 46-50% and a specificity of 80-85% for identifying a malaria death as diagnosed in the hospital (Snow *et al.*, 1992; Todd *et al.*, 1994; Quigley *et al.*, 1996). A particular shortcoming of the use of verbal autopsies is their inability to pick out a severe malarial anaemia death from the other causes of death especially acute respiratory tract infections since these have a very similar presentation. Since severe malarial anaemia occurs mainly in those under two years of age and this is the age when the majority of disease and deaths occur in areas of high transmission, there is a likelihood that looking at malaria mortality in high transmission areas will result in an under-estimate of these deaths (Smith *et al.*, 2001).

2.4.8.4 Few data points

One of the short-comings that is frequently quoted in the comparisons of malaria mortality and transmission is the lack of sufficient data (Snow and Marsh, 1995). It has been therefore difficult to describe the true pattern of malaria mortality with transmission (Greenwood, 1997; Smith *et al.*, 2001). All the arguments stemming from the lack of available data agree that there is a need to look at more data points in order to know for sure what happens to malaria mortality when transmission is reduced and that can only happen if there is long term follow-up of intervention studies for at least five years in order to see whether there are any changes in mortality patterns with time.

CHAPTER THREE

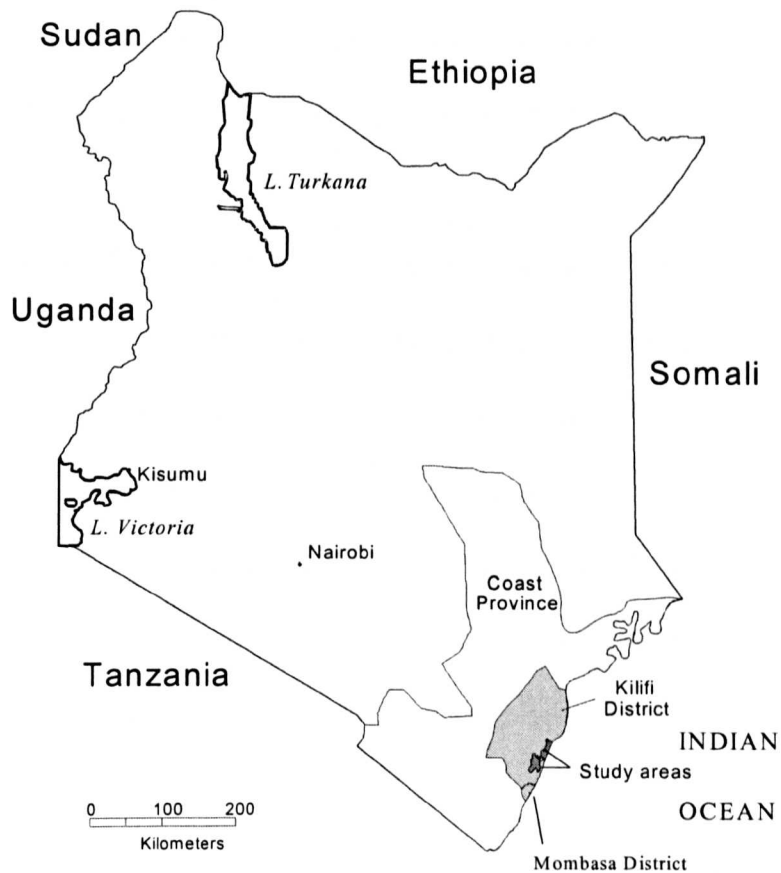
BACKGROUND, MATERIALS AND METHODS FOR COMMUNITY STUDIES IN KILIFI DISTRICT

3.1 Study area

3.1.1 Physical location

Kenya spans the equator on the East Coast of Africa between latitudes 5° North and 5° south. Kenya has a total landmass of 581, 677km² with a population of 28.7million persons in 1999 (Central Bureau of statistics, 2001).

Figure 3.1: The Kilifi study areas in relation to Kilifi District and Kenya.



The selected study area was within the Kilifi District which is located in the Coast Province of Kenya (Figure 3.1). Kilifi District borders the Indian Ocean to the east, Mombasa and Kwale to the south, Taita Taveta District to the north, while to the northeast are Malindi and Tana River Districts. Kilifi District covers an area of 4,779 km² and was occupied by 544,303 people in 1999 (Central Bureau of Statistics, 2001). According to the Kilifi District Development Programme report (March 2001), Kilifi District is one of the least developed districts in the country with 305,000 (55%) of its people described as 'absolutely poor people'. The average household income was Ksh 4,200/month (US\$54) with an average per capita income of Ksh700/month (US\$9). The highest income generating activity was agriculture and more than 80% of the population depend upon it. The population growth rate was high (3%) but the under-five mortality was 111 per 1,000 live births (11.1%). According to the same report, the literacy level in this district was 45%, which was low compared to the national average of 78%.

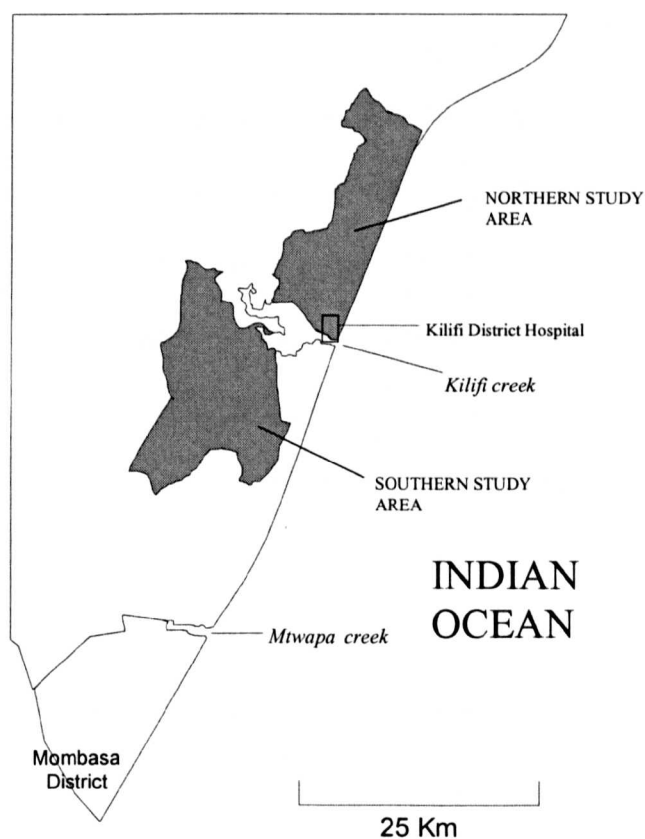
Kilifi District covers four agro-climatic zones that separate out places with differing potential land use (Jaetzold and Schmidt, 1983). There is the coastal plain that is 30m above sea level and extends 10 km inward. Then there is the foot plateau that varies from 60-120m above sea level and is made up of sandstones and impervious clays that is mainly flat surface with a few hills. The third zone is the coastal range that lies between 120-260m above sea level and has good rainfall and fertile soils. The fourth zone occupies the semi-arid and arid areas of the District and is known as the 'Nyika' plateau.

Figure 3.2 shows the position of the two rural study areas that have been identified for long-term demographic surveillance in a section of Kilifi District. The area referred to as the 'northern study area' situated north of the Kilifi creek while the area referred to as the 'southern study area' located south of the creek. The northern study area contains the locations Roka, Tezo, Ngerenya and Sokoke covering an area of 306 km² with a population

of about 57,385 people living in 7,761 households (Central Bureau of Statistics, 2001).

The northern study area was a former settlement scheme in which each homestead was allocated 12 acres of land. The land has been subdivided within families and so currently most of the families own less than the acquired 12 acres. The soils are sandy and not very productive. The northern study area has been under demographic surveillance work since the early nineties (Snow *et al.*, 1994) and was involved in a large randomised control trial of insecticide treated bed nets (ITNB's) from 1993-1995 (Nevill *et al.*, 1996).

Figure 3.2: The larger Kilifi study areas, north and south of the Kilifi creek.



The southern study area contained Chonyi location that included the sub-locations of Mwarakaya, Banda Ra Salama, Chasimba and Ziani covering an area of 202 km² with a population of about 47, 138 people living in 8,115 households (Central Bureau of

Statistics, 2001). Homesteads in the southern study area are clustered in small villages with the farms located some distance from the homesteads. The soils in this area are predominantly red soil that are more fertile and the land more hilly than that of the northern study area.

3.1.2 People

The people of Kilifi District are predominantly of the Mijikenda tribe that consists of nine sub-tribes that include: the Giriama, Jibana, Chonyi, Rabai, Kambe, Kauma, Ribe, Digo and Duruma. These people are said to have originally migrated from the southern tip of Somalia called Shungwaya and settled in and around a network of 'kayas' (forested lands that have remained sacred to date). The Giriama are the largest group comprising about 90% of the total population of Kilifi District. All the nine sub-tribes speak a similar language and share the same traditions. The Mijikenda system is patriarchal and mostly polygamous. The majority of the Mijikenda people are still very traditional and adhere to their traditional religions and cultural practices. In both the northern and southern study areas, 99% of the study participants are Mijikenda. In the northern study area, the majority (83%) are from the Giriama sub-tribe while in the southern study area, the majority (81%) are from the Chonyi sub-tribe.

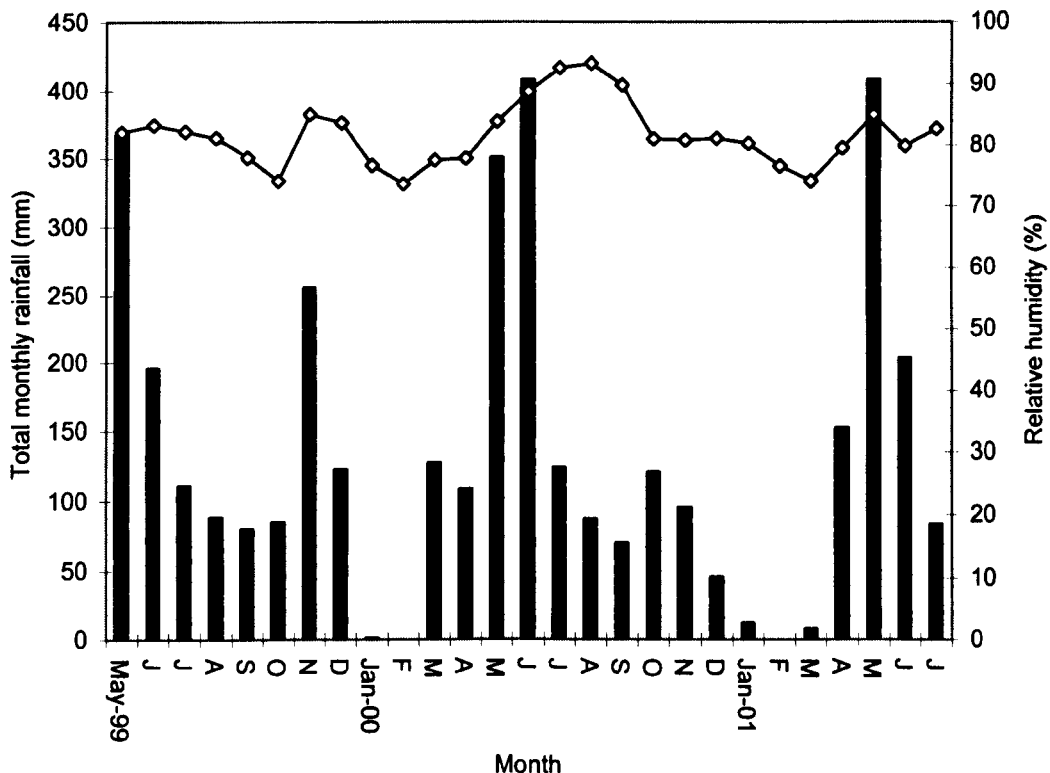
Most of the people living in the rural areas are farmers who grow maize, beans and cowpeas for subsistence while cashew nuts and coconuts are cash crops. Fruits mainly mangoes, tangerines and paw paws also earn money for subsistence in many families. The majority of the men aged 20-50 are not resident within the study area but go to the big towns (Mombassa, Malindi and Kilifi) in search of employment mainly in the tourism industry. In the northern study area, another source of income is the quarrying of coral stone and sand for building houses. There are two main types of housing in the rural study areas, these are the Mijikenda and the Swahili houses. The traditional Mijikenda house is

made of frames of wooden poles and branches that are covered from top to bottom with grass. The Swahili houses on the other hand are made of mud and wattle walls with coral stones occasionally mixed in the mud and a roof of woven dry palm leaves (makuti).

3.1.3 Climate

Daily data on rainfall, relative humidity and maximum and minimum temperatures that had been collected over three years at the Kilifi Agricultural Institute, located less than 2 km away from the Kilifi District Hospital were used. Total rainfall and mean monthly relative humidity for the period of May 1999-June 2001 are demonstrated in figure 3.3.

Figure 3.3: Total monthly rainfall (bars) and mean monthly relative humidity (line) during the study period May 1999 – July 2001.



The total annual rainfall for the two years spanning from 1999-2001 ranged from 1,056 to 1,540 mm/year with a range of 0 to 409 mm of rain a month. Most (37-58%) of the rain fell in the months of May and June, which is the period of the long rains. The highest daily

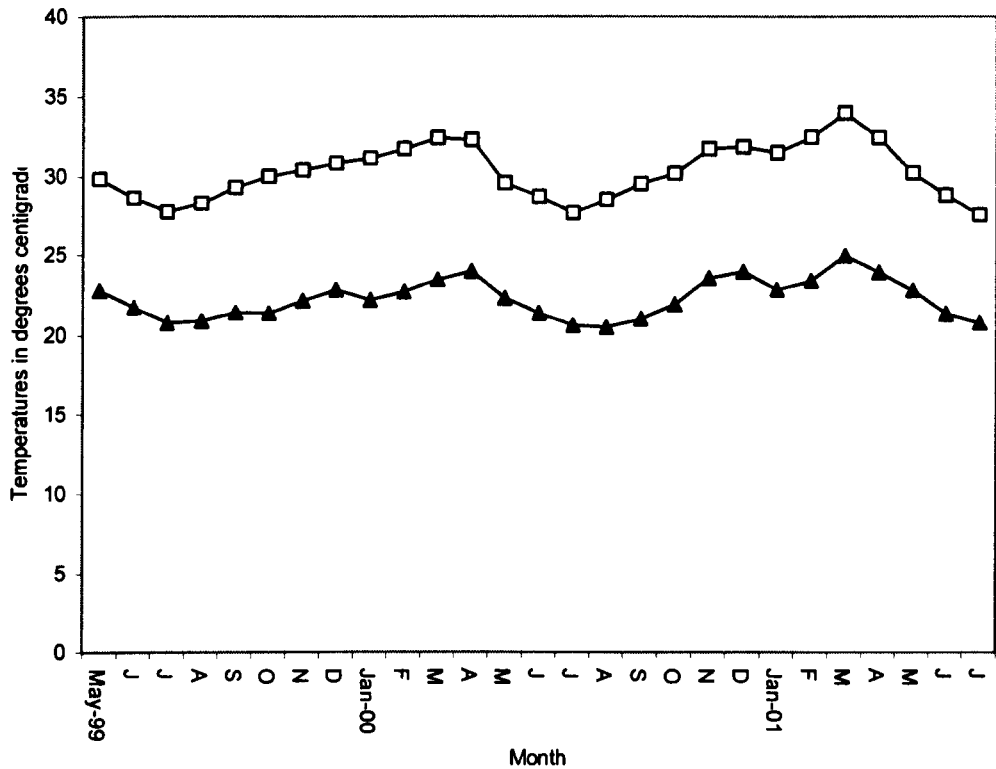
rainfall was 182 mm that fell on the 5th of June 2000. The short rains usually occur in the month of November but this is frequently unreliable and sometimes does not happen. The driest time of the year fell between January and March. There was no rain at all in the month of February either in the year 2000 or 2001 and only very little (3mm) in February 1999.

The average relative humidity during the study period was $81.4 \pm 9.5\%$. The highest humidity (96%) was experienced in the months of July-August 2000, during and just after the long rains. The lowest daily relative humidity (54%) was in the months of February and March when there was usually very little or no rain at all. About 95% of the relative humidity data fell in the range of 64-96%.

Figure 3.4 demonstrates the mean monthly maximum and minimum temperatures from May 1999 to June 2001. Ambient temperatures in this area ranged from a low of 19°C to a high of 36°C. The average monthly maximum temperature was $30.3 \pm 2.1^\circ\text{C}$ with a range of 24°C-36°C while the average monthly minimum temperature was $22.3 \pm 2^\circ\text{C}$ with a range of 19°C-33°C. The hottest month of the year was just before the long rains in April while the coolest month was July right in the middle of the long rains. The mean annual difference between the daily maximum and the minimum temperatures was $8 \pm 1.9^\circ\text{C}$ with February having the highest difference of 9.1°C and July with the least difference (6.9°C). A high ambient temperature leads to an increase in the growth of the vector population and a shortening of the interval from laying of eggs to mosquito emergence.

The optimum temperatures and relative humidity for the development of the malaria vectors are 20-30°C and 70-80% respectively (Wernsdorf and McGregor, 1986).

Figure 3.4: Mean monthly maximum (squares) and minimum (triangles) temperatures in Kilifi District for the period May 1999 – July 2001.



Since temperature and the relative humidity were ideal for malaria vectors during the whole study period, the most likely determinant for transmission was rainfall, which varied widely affecting the proliferation of breeding sites during the course of a year.

3.1.4 Entomological data

Studies conducted in the coast of Kenya have identified the following anopheline vectors: *An. funestus*, *An. coustani*, *An. squamosus*, *An. pharoensis*, *An. nili*, *An. merus*, *An. gambiae sensu stricto* and *An. arabiense* (Mbogo *et al.*, 1993; 1995; Mbogo *et al.*, 2003). *An. gambiae s.l.*, comprised more than 87% of the anophelines caught in the northern study area while in the wider coastal area of Malindi, Kilifi and Kwale, the most common species was, *An. gambiae s. s.* Using Enzyme Linked Immuno-absorbence Assay (ELISA) between 2.2 to 4.1% of *An. gambiae s.l.* were infected with *P. falciparum*. Rural areas had

higher infection rates compared to urban Kilifi township areas (Mbogo *et al.*, 1993; 1995). *An. funestus* mosquitoes were observed to be infected in the wider northern study area with infection rates as high as 10.5% (Mbogo *et al.*, 1995).

Anopheline vectors are present during all the months of the year but there were sharp increases in the vector populations associated with the rains. Transmission was detectable in January and then from June to December during which periods the Entomological Inoculation Rate (EIR) ranged from 0.01-0.09 infective bites/person/night with the highest transmission rates being in the months of June to September. In the wider Kilifi, Kwale and Malindi areas, the EIR ranged from 0.01- 1.07 infective bites/person/night. Due to the low abundance of vectors, it was harder to detect transmission during the months when there were no rains and easiest at the time just after the start of the rains in June-July.

The methods used to collect mosquito samples included night biting collections, daytime resting catches and pyrethrum spray catches. The mosquitoes were dissected and samples assessed visually for parasites and were also tested to detect antibodies against the circumsporozoite protein of *P. falciparum* by ELISA (Mbogo *et al.*, 1995). These methods however had very low sensitivities as there were areas with an EIR of '0' that still managed to generate cases of severe malaria (Mbogo *et al.*, 1995).

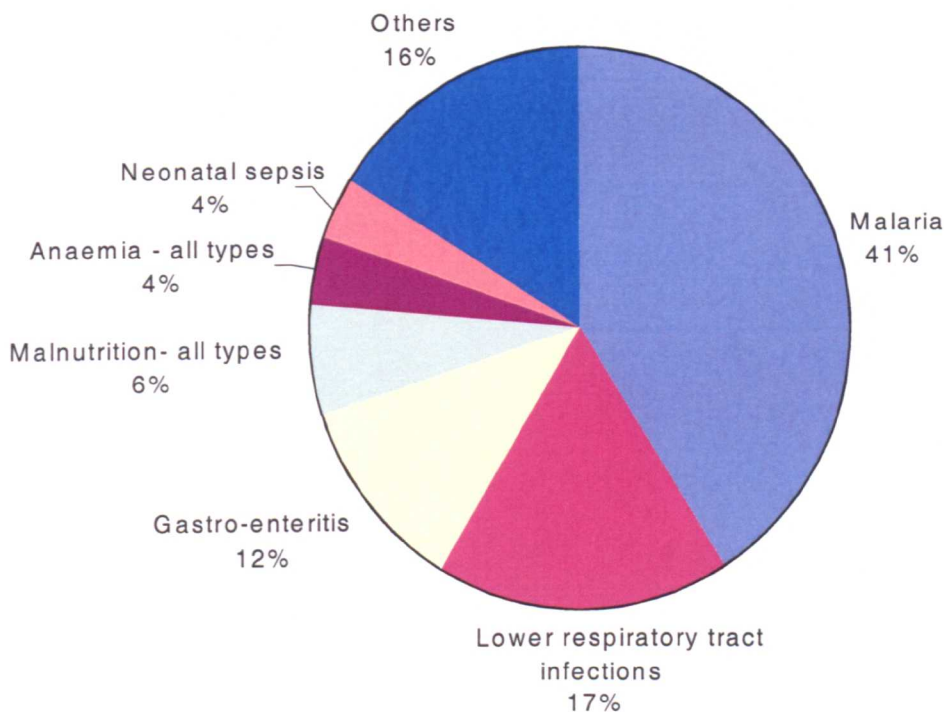
The EIR for the northern study area was estimated as 10 infective bites/person/year (Mbogo *et al.*, 1995). Recent studies in the southern study area have indicated an EIR of 50 infective bites/person/year (Mbogo, personal communication). There was marked spatial heterogeneity within the sites that was thought to be related to the distribution of larval habitats in these areas (Mbogo *et al.*, 2003). Another factor that may contribute to differences in exposure to mosquito bites in the two study areas is bednet use. Only 5% of the children under 10 years of age in Chonyi compared to 69% in Ngerenya used bednets.

Although most of the bednets were not treated, there was evidence of protection offered by the use of untreated bednets in good condition compared to no nets at all (Appendix X11).

3.1.5 Clinical data

The Kilifi District Hospital has a 36-bed paediatric ward and an eight bed high – dependency ward, commonly referred to as the KEMRI ward. In these two wards, intensive paediatric surveillance has been going on since the early 1990's. Figure 3.5 shows the diagnosis at admission for the period May 1999-May 2001.

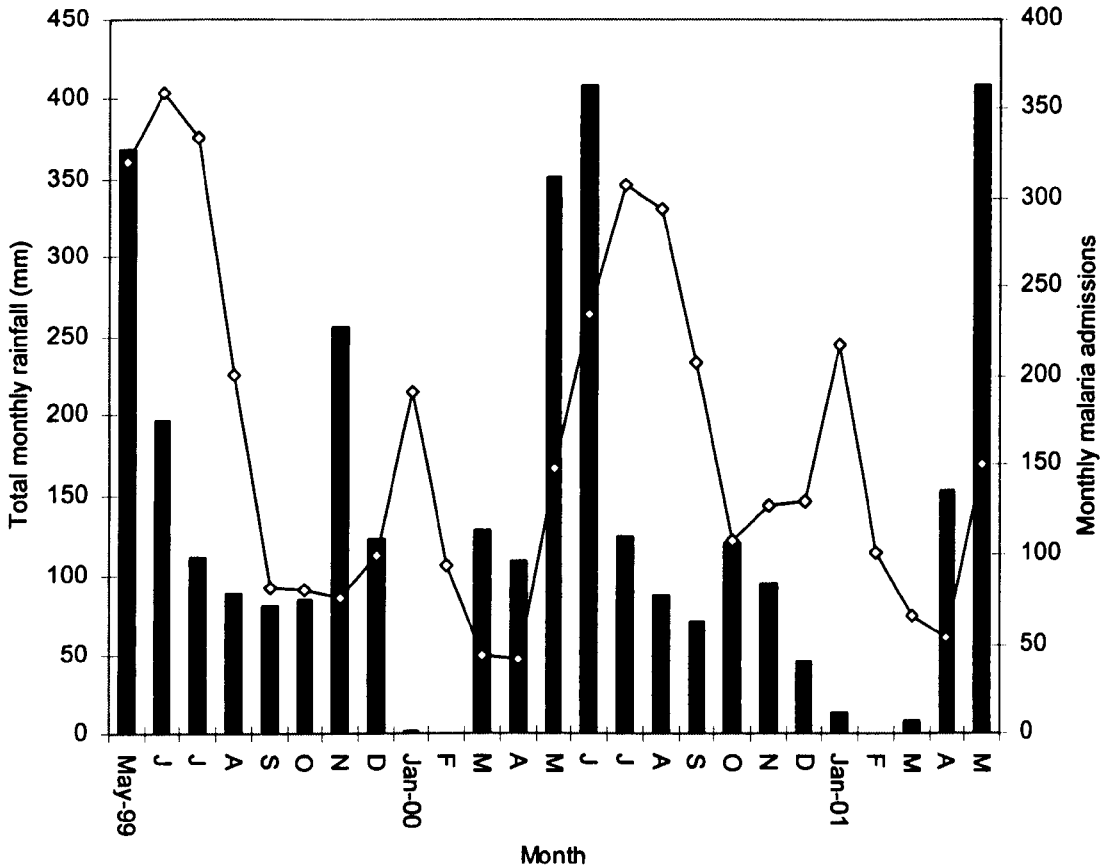
Figure 3.5: Diagnosis at admission in the Kilifi paediatric wards from May 1999 to May 2001.



Most (41.2%) of the admissions were attributable to clinical malaria while 17% of the admissions were due to lower respiratory tract infections. About 12% of the admissions

were due to gastro-enteritis and 6% due to malnutrition. Figure 3.6 shows the distribution of the malaria admissions and the rainfall pattern over the period of the study.

Figure 3.6: Monthly malaria admissions at the paediatric ward of the Kilifi District Hospital (line) and rainfall pattern (bars) from May 1999 to May 2001.



Malarial admissions were very seasonal and followed the rainfall pattern closely. The largest number of admissions being in the months of May to August in association with the start of the long rains and a small peak in the month of January after the short rains. The highest number of admissions were immediately after the month with the highest amount of rainfall.

3.1.6 Health-seeking behaviour

Two studies have been conducted in an attempt to understand the health-seeking patterns of the population living within the study area (Mwenesi, 1993; Molyneux, 1999). Molyneux (1999), studied the treatment seeking patterns of rural mothers in response to uncomplicated fevers after migrating to an urban area. The mothers from the rural area were those from the larger southern and northern study area. Mwenesi (1993), on the other hand, used part of the northern study area and Kilifi town to study mothers understanding of malaria and how treatment was sought for children.

Sources of treatment in this area ranged from shops, community health workers (CHW), private and government clinics, dispensaries, traditional healers and the Kilifi District Hospital (KDH). There are hardly any pharmacies in the rural areas. Most of the mothers opted for shop bought drugs as a first line of treatment (52%) followed by visiting the government hospital (14%), then either a private clinic/dispensary/ hospital (18%) but very few people consulted a traditional healer as a first line option (3.5%) (Mwenesi , 1993).

Molyneux (1999) also found that the first line of action taken by most mothers (69%) with a febrile child was the use of shop-bought drugs. The child was then monitored for 2-3 days before alternative treatment was sought. Only children that were seriously sick with worrisome symptoms were taken to the health centres immediately. Mothers preferred to use shop brought drugs as they were easily accessible with about 87% of the rural population living within 1 km away from a shop (Mwenesi, 1993). Health facilities were further away and many mothers would incur travel costs to get to these facilities. For example, the central point of this population was 10 km away from the KDH.

When biomedical treatment failed to cure the child, then a child was normally taken to a traditional healer so as to determine whether it was a 'normal fever' or one caused by

bewitchment. If the child's fever was accompanied with convulsions, about 36% of the mothers consulted traditional healers first before seeking formal biomedical treatment option. Convulsions within this community were thought to be as a result of evil spirits and therefore, more than ordinary fevers, were more likely to be presented to the traditional healer than the medical facility (Mwenesi, 1993; Molyneux, 1999). These spirits were said to dislike injections and it was believed that children would get worse if brought to the hospital (Mwenesi, 1993). This notwithstanding, the Wellcome-KEMRI ward situated within the KDH had a good reputation for curing malaria related fits quickly. Most people in the area however believed that lumbar punctures (a routine procedure for children with fits) cured malaria quickly by removing the dirty water from the child's backbone (Mwenesi, 1993).

Although the mother was usually the first to notice when her child was unwell, the decision of where the child would be treated was not always hers to make. She had to consult with her husband, her husband's brother or her mother-in-law (Mwenesi, 1993). The direction of treatment would therefore depend on the finances available and the beliefs of those around the mother. In Molyneux's study (1999), 65% of the mothers choice were made through advisory and financial help from these relatives. Mwenesi (1993) found a similar figure (62%). Half of the women that made there own decisions without consulting anyone were married and living together with there husbands. Gender and power relations within the home influence greatly what sort of health care a child receives and how soon.

3.1.7 Selection of the study area

Cross-sectional surveys in the one to nine year olds in Kilifi reported a parasite prevalence of 49% in Kilifi north and 74% in Kilifi south (Snow *et al.*, 1997). Populations living in Kilifi north are exposed to an estimated 10 infective bites/person/year whereas the populations in Kilifi south are exposed to an estimated 50 infective bites/person/year

(Mbogo *et al.*, 1995; Mbogo *et al.*, submitted). The two areas have equal access to KDH although the northern study area has had more contact in terms of past research studies with the research institute. Inhabitants of the two study areas selected were from the same ethnic group with similar customs and beliefs but differed in their malaria exposure. They were therefore ideal for observing the effects of differences on non-severe malaria in people living in a rural area of Africa under differing levels of transmission with most other factors held constant.

3.2 Methodology

The overall study design was divided into two basic processes: weekly follow-up and cross-sectional surveys. The weekly follow-ups involved active detection of fevers in the field and was supplemented by passive case detection at the study clinic, whereas the cross-sectional surveys involved the making of smears from all study participants. The methodology is described in detail in these sections.

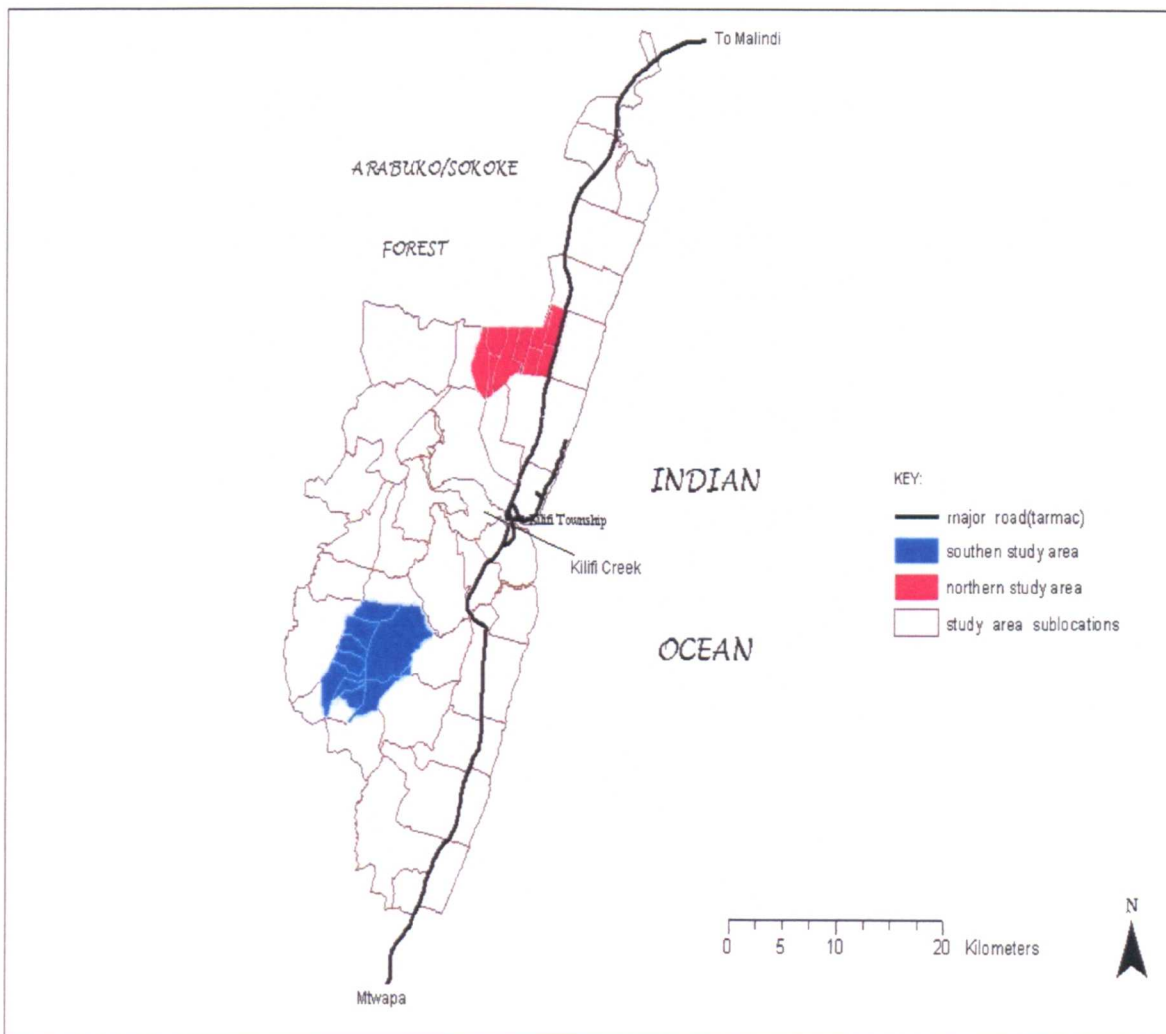
3.2.1 Community sensitisation

The central administration including the Ministry of Health representative (Medical Officer of Health, Kilifi) and the local administration (District Officer, Kilifi) were made aware of the research project first. With their approval, the research protocol was explained to the local authorities in the study area which included the chiefs, elders and divisional officers. Meetings were organised with all the elders in the two areas and the study was explained to them. All the head teachers in and around the study areas were also informed of the study. These community leaders were also given explanatory leaflets elaborating the study in the English and Swahili languages.

3.2.2 Census

Twelve zones located in the previous bednet study area of the northern study area were selected (marked in red on figure 3.7.) The Ngerenya area was well mapped and enumerated in the past during the bed net trial and the last enumeration was conducted in 1997. An enumeration was therefore conducted at the start of this study in April 1998. However, the southern study area was not well mapped and only a single census had been conducted in 1996 and no enumeration had been conducted since.

Figure 3.7: The northern (red) and southern (blue) study areas that were involved in the longitudinal study as part of the wider study area.



It was not possible to conduct a full census for the purpose of this study. However, five clusters of homesteads from the 1996 census were selected and the enumeration conducted among these clusters only. The study area where these clusters came from is marked in blue on figure 3.7.

Trained fieldworkers fluent in the local languages visited all the houses included in the re-census. Using updated household lists (an example of such a form is shown in Appendix I), a reliable respondent was found and information sought regarding the residential status of the household members. This established whether the previous household members were still resident, moved out or had died. Similarly, information was acquired regarding any new members who had moved to the households and also all births since the last census. Any new houses were added to the map and the members enumerated. All new household members were issued with a unique identification number unless they had moved in from one of the enumeration zones within the study area, in which case, they retained their old numbers. During this census, a household was a family group with one head. This would be a nuclear family involving a man with his wives and children. Frequently people lived in homesteads which included a man's family and the families of this man's sons. This elderly man was then considered to be the head of the homestead. During this census, no effort was made to separate a unit household from a homestead and throughout the discussions, heads of homesteads are referred to as heads of households as well.

3.2.2.1 Aging study participants

Since age was of particular importance in this study, it was essential that the dates of birth collected at the census be accurate. However, the literacy levels in Kilifi District were rather low: 30% in women and 70% in men (KDDP, 1996). This made it difficult to get information on dates of birth from the mothers, therefore other methods had to be used to obtain reliable data.

3.2.2.1.1 Age determination of children

None of the study participants under the age of 10 years had birth certificates. The most reliable way to get the birth date of the children was the birth notification which was a form given to mothers who had delivered at a District hospital. These were few since the majority of the children in Kilifi were born at home. The next best option was the use of infant health cards, which are issued by government facilities when mothers brought their infants for vaccination as part of the primary health care programme. Although some of the mothers were late in taking their children for vaccination, they were still just a few months old and so the date of birth on these cards was a reliable estimate. About 71% of the children under five years in Chonyi and 67% in Ngerenya had these health cards. The third option was records kept by literate heads of households of when each child in the homestead was born.

When all these records were unavailable, the next option was to identify an index child with a reliable birth date who physically appears to be of the same age as the child whose age was unknown. The mother was asked how far apart the birth of her child was from that of the index child, and this was used to estimate the birth date of the child. When all else failed, the last option was the use of an event calendar (Appendix III) where the mother was asked to remember any national or local event that was associated with the birth of her child. Age of school attendance was completely unreliable as some of the children start school very late.

3.2.2.1.2 Age determination of adults

Although almost all adults in the study area would have a national identity card, these are not considered to very accurate and were not used for aging adults in this study. The event calendar, which is also used in the national census was used (Appendix III). This calendar had a list of major national and local events that happened in the past within Kenya and

especially Kilifi. Some of the participants may have been told about the event surrounding their births or been named in a way that may remind them of the event at their birth. The year of the event was then directly used to age the person. However, this was not always possible, they therefore described particular events in their life and estimated their age at the time of the event. The event calendar was then used to estimate the time that elapsed since the event and the person aged.

An alternative method was used for aging women in the community to complement the event calendar. A mother was asked to use one of the grown-up girls in the compound whose age was known to approximate how old she was when she got married. The questionnaire proceeded to find out how long she waited before her first birth, and if the child was still alive, then her age was calculated from the age of this child (Appendix IV). If the child was not around, or the child's age was unknown, then more information was sought on the spacing between the births and her approximate age estimated from these data. The data was likely to have its problems since there was a high divorce and remarriage rate in this community and the mother may be unwilling to talk about her previous marriages or children that she gave birth to before joining her current homestead. However if the event calendar was difficult to use, this was the next best option.

3.2.3 Household selection and the consent process

In Ngerenya, random numbers were used to select the household to be recruited into the study. About 40 people were selected in each of the 16 age groups that are: <1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11-14, 15-19, 20-39, 40-59 and >60 years of age. Once the study was underway, all mothers with children under 10 years of age that were involved in the study were recruited as well. Since it was not possible to conduct a full census in Chonyi, five clusters of households were selected and from these clusters, study participants from the

different age groups selected. The same numbers of children were recruited in Chonyi as in Ngerenya.

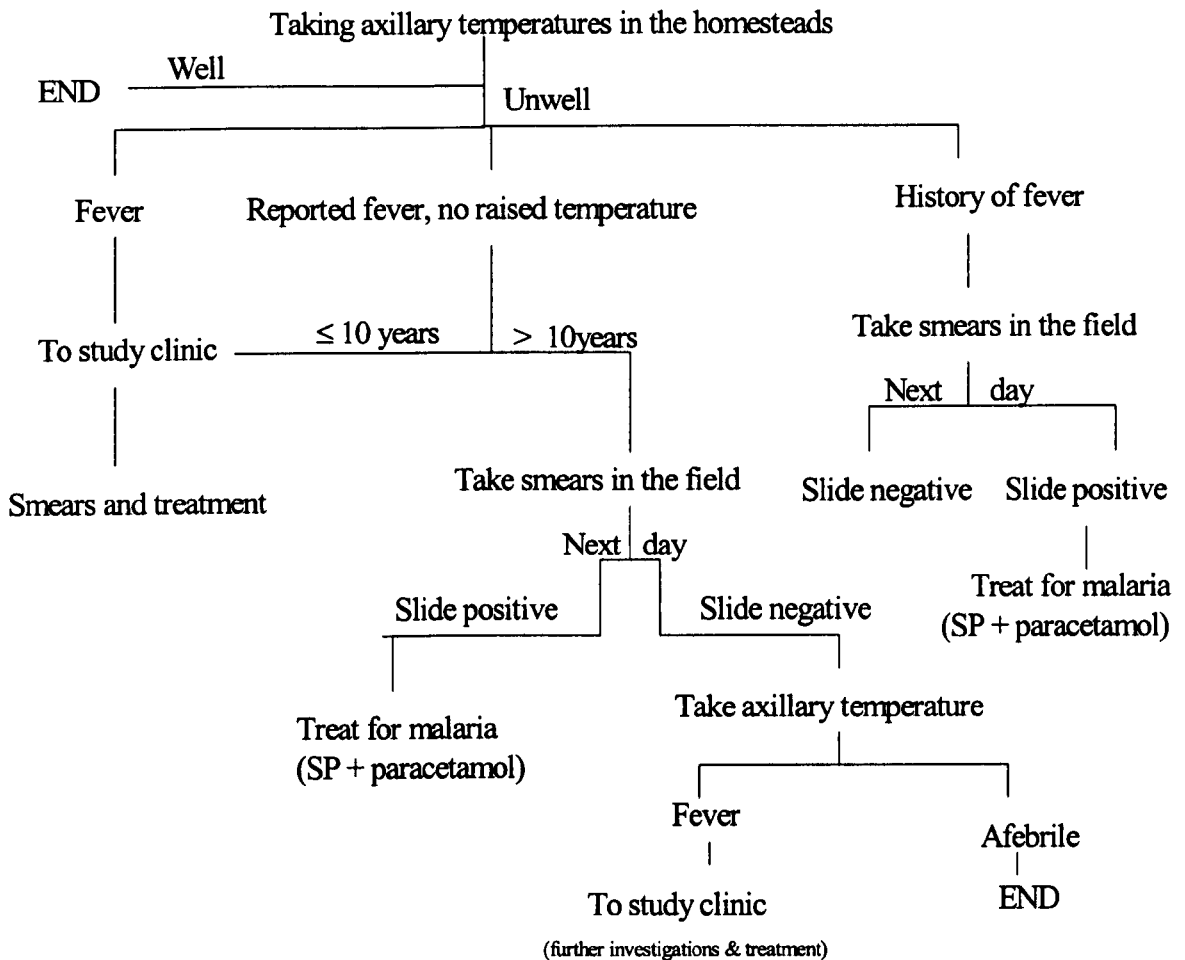
Consent was sought in different stages. During the census process, the local administration, which included the district officers, chiefs, elders and head teachers were informed about the study. Consent for the study was given orally by these groups of community leaders. Within the household, oral consent was first sought from the head of the homestead. The study was explained to the head of the household so that the field workers would be able to obtain written consent for the individual study members and the mothers/guardians of the children who were potentially likely to be included in the study (see below). If the head of the household was not there, no consent was taken from the people in that house. During the consent process, only one head of the house refused to grant oral consent for the family but all others agreed to participate.

The study was explained individually to each of the parents of children under the age of 15 years and to each person over the age of 15 years. The local languages were used in the explanation and the participants given a chance to ask questions. Those who were comfortable with the study and ready to participate were asked to sign or thumbprint on the copy of the consent form (Appendix II). A copy of the consent form was kept in the home so that the participants may read it and ask any questions. Each person recruited into the study got a hospital notebook similar to the ones used in most government facilities which had a sticker with the persons name, serial number and census number on the cover in order to ease identification during clinic visits. New births into the household were recruited throughout the period of the longitudinal study and issued with a similar notebook.

3.2.4 Weekly follow-up

A total of 11 fieldworkers visited each of the 124 households every week. During each visit, the fieldworker took the axillary temperature of each study participant using a digital thermometer. The tip of the thermometer was placed under the armpit of the individual and held in place by pressing the arm to the body. It was removed when it made a 'beeping' sound and the axillary temperature and the time the temperature was taken recorded on a form. The thermometer was swabbed with surgical spirit and dried before being placed under the other armpit. If the axillary temperature was found to be below 36°C, the temperature readings were recorded three times from the same individual and the highest reading recorded on the forms. Figure 3.8 shows the trail of events that followed after taking of the axillary temperatures and these are described here.

The participant (or the parent of the participant for the children under 15) was asked whether the participant was well or not on the day of the visit. If the participant had a fever (axillary temperature of $\geq 37.5^{\circ}\text{C}$), then they were given fare to attend the clinic at the Kilifi District Hospital. If the participant was under 10 and unwell but with an axillary temperature $< 37.5^{\circ}\text{C}$ at the time of the visit (reported fever, no raised temperature), they were also given their fare to attend the clinic. If the participant had a fever or a history of fever and there was no one to accompany them to the hospital (if they were children) or an adult that did not want to go to the hospital, then a smear was made in the field and a form (Appendix VI) filled out.

Figure 3.8: Flow diagram of events at each visit to the home

If the participant was well on the day of the visit, they were asked if they had had any illness in between the visits for which they had not attended the designated clinic at KDH. If they did, then another form (Appendix V) was filled out and a smear taken if a history of fever accompanied the illness. This is represented on the far right of the chart.

Those given fare to the hospital were attended to at a designated clinic by 3 fieldworkers and a clinical officer. Each patient had a form filled out at the clinic (Appendix VII) that had all the signs and symptoms of their illness. Anyone with a fever or a history of fever had a smear made, treated and given fare to go back home. The study clinic remained open from 8am to 5pm on weekdays and from 8am to 12pm on week-ends. There were no charges for treatment. Any person that was severely ill was admitted at the KDH.

3.2.4.1 Overlapping studies

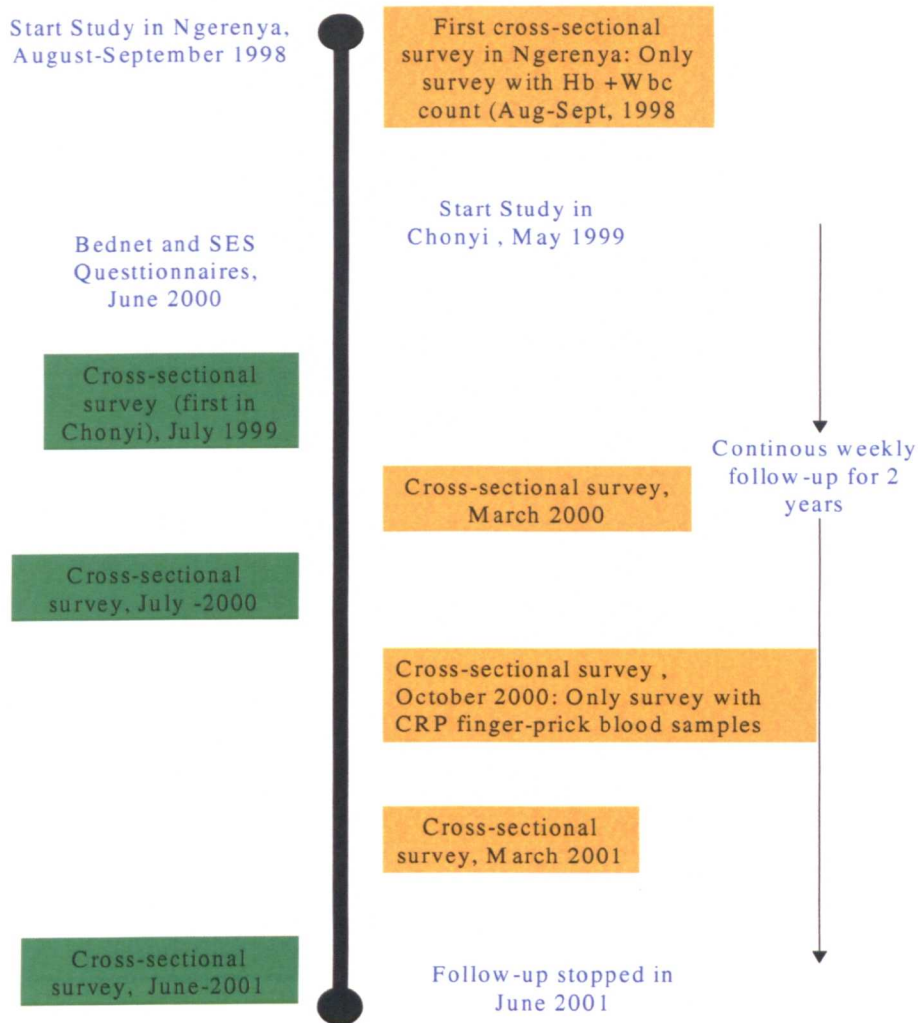
The same study population within the age groups of 6 months to 6 years were also potentially recruited into clinical trials when presenting with clinical malaria at the clinic. This was limited to one episode of recruitment per child during the two years of longitudinal follow-up. The drug studies involved were firstly a study on combination therapy using lapdap and fansidar that lasted the time period February 2000 to September 2000. There was also a study to determine which combinations of artesunate were useful with fansidar that lasted from June 1999 to September 2000. Children recruited in the combination therapy study were followed up for 28 days whereas those in the lapdap study were in the drug study for 2 weeks.

All the children that were recruited into these studies were attended to at the same clinic as the longitudinal study patients. The patients were always first attended to by the longitudinal study clinician and the hospital forms (Appendix VII) filled out, if they fitted the drug trial criteria, they were recruited into the trials. There was therefore no loss of data from recruitment into the drug studies.

3.2.5 Cross-sectional surveys

There were a total of six cross-sectional surveys conducted in the course of this study, three in the low malaria season and the others in the high intensity period of transmission (Figure 3.9). During these cross-sectional surveys, thick and thin smears were made from each study participant. These smears were made in the field, dried and then brought back to the laboratory. They were stained and stored to be read later by the same technicians that read the smears made at the clinic.

Figure 3.9: A plan of the study design (Green colour represents cross-sectional surveys conducted in the rainy season while brown colour represents those conducted during the dry season).



3.2.5.1 Making smears

Thick and thin smears were made simultaneously when slides were made either for treatment purposes or during the cross-sectional surveys. All smears were air-dried then the thin smear was fixed in 100% alcohol but the thick smears were not fixed. The smear was then stained in Giemsa stain (10%) and read after 10 minutes. The parasite count was read per 200 WBC using the thick smear but if the reading went beyond 800 parasites/ 200 WBC then the parasite count was done per 500 RBC using the thin smear.

WBC count was calculated in 10^3 cells/ μ l of blood while the Hb was in g/dL. Parasite density was calculated per microlitre of blood as shown below.

$$\text{Parasite}/\mu\text{l of blood} = \text{count}/200 \text{ wbc} * \text{WBC count}/\mu\text{l of blood} * 5$$

or

$$= \text{count}/500 \text{ rbc} * \ln(\text{Hb count}) * 2000$$

3.2.6 Questionnaire surveys

During all the surveys, the heads of the households had to be made aware of what was happening and verbal consent sought from them before the mothers were approached. The mothers were also informed that the process was voluntary and verbal consent sought in order to administer these questionnaires. Both questionnaires described below were administered in both the southern and northern study areas.

A bednet survey was conducted in the middle of the rainy season – June 2000. A questionnaire was filled out for each study participant (Appendix IX). The aims of the study were to find out mosquito avoidance techniques used by the study participants. The main method of avoidance being the use of mosquito nets. Information was also sought on the use of local repellents, and whether the house structure allowed easy mosquito entrance.

The aim of the mothers' socio-economic questionnaire was mainly to find out the mothers level of education (Appendix VIII). Information was also sought to find out how easy it was for mothers to get money to treat a child with a non-complicated fever.

3.2.7 ELISA for CRP

Samples for C-reactive protein (CRP) were collected from all consenting study participants during a cross-sectional bleed and during a follow-up period of eleven months. The CRP

data was collected to supplement the detection of fevers during both active and passive case detection. This section details the sample collection and the ELISA procedure that was used.

3.2.7.1 Sample collection

Finger prick blood samples of approximately 0.5mls were collected into microtainer tubes from all consenting study participants. The samples collected in the field were kept in a cool box and brought to the laboratory. On the same day the samples were centrifuged at 10,000 revolutions/minute for 5 minutes and stored at -30°C. From July 2000 to May 2001, finger prick serum samples were also collected from a random selection of the study participants that attended the study clinic with a history of fever. On collection, the samples were also put in cool boxes and taken to the laboratory where they were centrifuged at 10,000 revolutions/minute for 5 minutes and stored at -30°C. I performed all the ELISA laboratory tests as described in the protocol that follows.

3.2.7.2 ELISA protocol

Microwells (NUNC[®] Maxisorp flat-bottomed microtitre plates) were coated with 100µl/well of rabbit anti-human CRP (DAKO[®]) diluted 1:88 in coating buffer (Appendix X) and incubated at 4°C overnight. Wells were washed with washing buffer (Appendix X) using an automated washer and 100µl/well of serum samples added. The serum was diluted 1:8,000 in diluting buffer (Appendix X). The assays were conducted in duplicate with blank wells and standard reference positive and negative sera on each plate. After incubation for 2 hours, the wells were washed and peroxidase-conjugated rabbit anti-human CRP (DAKO[®]) diluted at 1:6,000 in diluting buffer was added to the well at 100µl/well and incubated for 1 hour. The plates were then washed and the chromogenic substrate (Appendix X) was added at 100µl/well. After incubation for 15 minutes in the

dark, the reaction was stopped with 100µl/well of 0.5M H₂SO₄. The plates were read at 490nm by an automated ELISA-reader.

3.2.8 Personnel

A total of 14 fieldworkers were involved in this study, all of whom have a minimum school education of eight years. All were conversant with English, Swahili and the local languages (Giriama and Chonyi), the majority of them residing within the study area. A senior fieldworker was selected as a fieldworker supervisor. The author was the study supervisor and was responsible for the overall supervision and training of all fieldworkers. A total of 2 months were spent in training the fieldworkers for the active surveillance which involved a month of piloting the active and passive surveillance. Eleven of the fieldworkers visited the households on a weekly basis. Each fieldworker was assigned 10 households that had a total of 200 persons and were rotated to different parts of the study area every four months.

Three of the fieldworkers assisted the clinicians at the study clinic. There was one clinician who was directly responsible for examining and treating children this study but during bank holidays and week-ends, they were replaced by other clinicians. The study supervisor held weekly meetings with all the field and study clinic personnel in order to iron out any problems in the course of the study.

There were several laboratory personnel involved in staining and reading all the slides that were obtained at the cross-sectional surveys and also routinely at the study clinic. All technicians at the KEMRI-Wellcome Trust labs were involved in reading the slides at some point in the course of the study, no one single technician was assigned to this activity.

There was one data entry clerk. As the data needed double entry, the data entry clerk did one entry and two fieldworkers fed the same data to another computer again. The data entry clerk did the verification of the two data sets and corrected any mistakes made at the point of data entry.

3.3. Quality control

3.3.1 Thermometers

The axillary temperatures were taken using BD[®] thermometer (Becton-Dickson UK Ltd, Oxford). These thermometers gave readings using degree centigrade with two decimal figures. To ensure that the thermometers were not faulty, these were crosschecked using a mercury thermometer in a water bath at the end of every week. Thermometers were considered to be in good condition if the readings were within $\pm 0.02^{\circ}\text{C}$ of the mercury thermometers.

3.3.2 Slide readings

Quality control of slide readings was done every three months in the laboratory as part of the laboratory routine quality control measures. This involved the selection of 10 slides that were rotated between all the 16 technicians involved in slide reading within the research institute. These slides were selected from the regular slides taken in the ward and would include a high, low and negative parasite slide. There were often slides with confusing features and with different species of *Plasmodium* and of different quality. After all the technicians had taken their readings, the mean parasite counts for each slide was calculated along with the standard deviations (SD). The outliers (outside 2 SD's) were removed and the mean re-calculated. Any slide readings outside this range were considered erroneous and the technician asked to repeat the readings. This internal quality control process was repeated every three months to ensure that all the technicians reading the slides within the research unit were at par with each other. There was an external quality

control process where slides were brought in from UK laboratories. The same technicians made their readings and their results were compared to international standards.

Every time fresh Giemsa stain was produced, a known slide was used to test the stain. This ensured that the stain contained no debris and that the staining pattern was the same as the standard pattern. The PH of the staining buffer was also regularly checked and maintained at pH 7.2.

3.4 Data processing and statistical methods

All the records were double entered in Foxpro[®] Version 6.0 (Microsoft Corp., Seattle, WA, USA) and verification done followed by checking the data for inconsistencies that were corrected. All the data was analysed using STATA[®] software, Version 7.0 (Stata Corp., College Station, TX, USA). Details of the analysis conducted are described in the materials and methods section of each chapter. I conducted all the statistical analysis described in this thesis but also consulted Amanda Ross, the unit statistician, for advice and ideas. It is worth noting that there was a lot of analysis conducted within this set of data and some of the statistically significant differences might be a chance occurrence. Associations were considered to be statistically significant if the p-value was <0.05 unless otherwise indicated.

CHAPTER FOUR

GENERAL RESULTS

4.1: Introduction

This section provides basic descriptive data on the study population during the two years of follow-up. Information provided in this section includes descriptions of the study population, parasitological and haematological indices, indices indicative of fever (temperature and C-reactive protein levels) and patterns of clinic attendance.

4.2: Number of study participants by age and sex.

As described earlier (section 3.2.4), study participants were followed up weekly for a period of two years and newborns from the selected households recruited throughout the study period. Table 4.1 shows the number of people in the different age groups half way through the study period (June 2000).

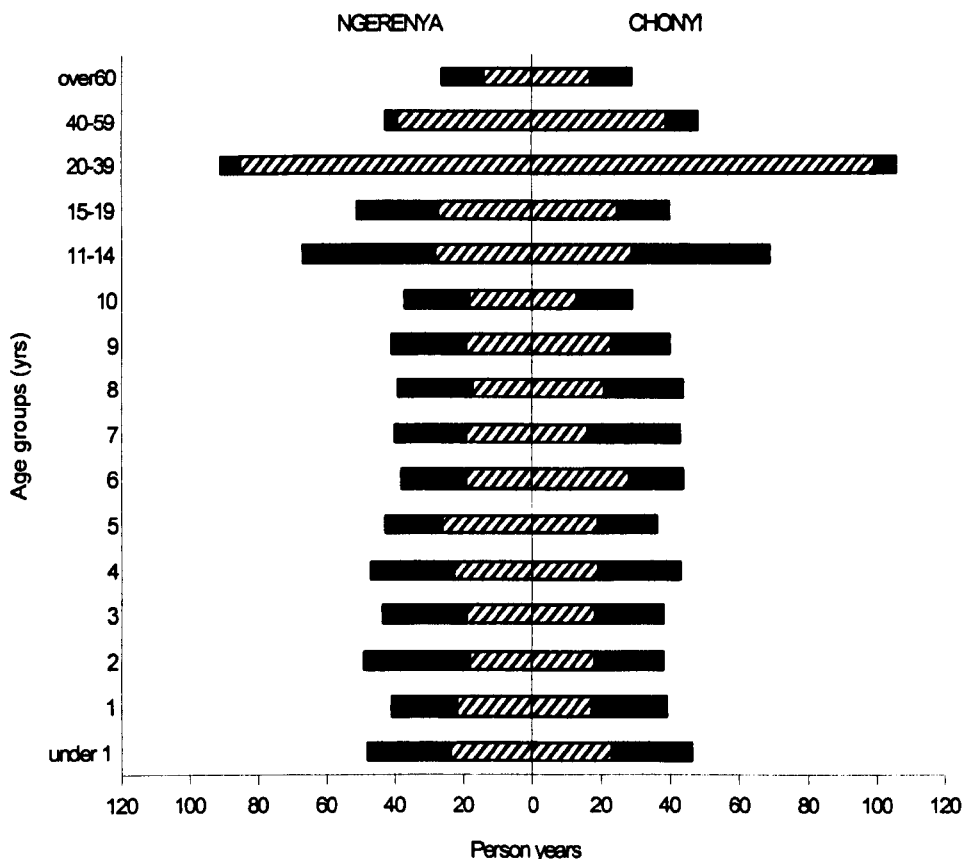
**Table 4.1: Sex and age distribution of study participants from Chonyi and Ngerenya
in June 2000**

Age in years	NGERENYA			CHONYI		
	Female	Male	Total	Female	Male	Total
Under 1	28	25	53	24	25	49
1	25	21	46	19	23	42
2	20	36	56	25	22	47
3	20	27	47	19	21	40
4	24	25	49	20	25	45
5	28	19	47	20	18	38
6	22	20	42	30	18	48
7	20	21	41	17	27	44
8	19	23	42	22	24	46
9	21	23	44	24	18	42
10	20	20	40	14	17	31
11-14	30	42	72	29	42	71
15-19	31	27	58	27	16	43
20-39	95	8	103	107	6	113
40-59	44	5	49	43	10	53
≥ 60	17	13	30	19	12	31
Total	464	355	819	459	324	783

Figure 4.1 shows the person years in each of the age groups for the whole study period.

About 1,500 people participated in the study of whom 60% were under the age of 10 years. There were at least 40 people in each of the age groups except among those >60 years of age and in children 10 years of age from Chonyi. There were very few men between the ages of 20-59 year (18 in Ngerenya and 22 in Chonyi). This was because men of this age bracket within the study area tended to migrate to the larger towns (Mombasa, Malindi and Kilifi) in search of work leaving their wives and children in the rural areas where maintenance was cheaper.

Figure 4.1: Age and sex distribution of person-years among study participants from Chonyi and Ngerenya from May 1999 – May 2001 (Black bars represent males and hatched bars females).



The overall female to male sex ratio was 1.3 to 1 and 1.4 to 1 in Ngerenya and Chonyi respectively. However in the 20 to 39 year old age group, the sex ratio was 12 females to

one male in Ngerenya and 18 females to one male in Chonyi. In the 40 to 59 year old age group, the sex ratio was 9 females to 1 male in Ngerenya and 4 females to 1 male in Chonyi. Between the ages of 20-59, fewer men were recruited into the study for reasons discussed earlier (section 4.2 above). However, many more women within this age range were recruited. This was due to a decision made during the study period to recruit all the mothers of children less than 10 years of age who had been recruited into the study. These women had to be present when the children were visited on a weekly basis and they also brought the children to the study clinics whenever they were ill, therefore the data from the women would not require extra resources or effort to acquire.

By the age of 60 years, the sex ratio had reverted to 1.3 females to one male and 1.6 females to a male in Ngerenya and Chonyi respectively. This was probably due to the fact that by the age of 60 years, most of the men have retired from their town jobs and gone back to their rural homes.

A total of 115 new births occurred in the 52 selected households from Chonyi between the period of May 1999 and May 2001. There were 43 births in 1999, 52 in the year 2000 and 20 by May 2001. There were a total of 158 new births in the 72 selected households within Ngerenya from September 1998 to May 2001. There were 11 births in 1998, 66 in 1999, 54 in 2000 and 27 by May 2001. Thus, there was approximately one birth/household/year.

4.3: Follow-up

Weekly follow-up started in September 1998 in Ngerenya and continued to May 2001. In Chonyi, the follow-up was from May 1999 to May 2001. Unless stated, the data described in the following sections will be that collected simultaneously in Chonyi and Ngerenya within the two year period from May 1999 to May 2001.

4.3.1 Amount of follow-up

A weekly follow-up visit was considered successful if the individual study participant was found at home or visited the clinic and an axillary temperature taken. There were a total of 86,168 person week visits conducted in Chonyi over the period of two years and out of these, 92% were successful. In Ngerenya, there were a total of 90,343 person weeks of follow-up, of which 88% were successful. The age distribution pattern of person weeks of follow-up was similar in the two study areas.

4.3.2 Success of follow-up

Eight percent (8%) of the weekly visits made in Chonyi and 12% of those made in Ngerenya were not successful during the two years of the study. Most of these unsuccessful or 'missed visits' occurred during the planting and harvesting periods when the women went to the fields to farm often accompanied by their younger children. Occasionally, temporary shelters were put up in the farms depending on how far the farms were and the women in a homestead would take turns to go there. There was a rise in the number of school-going children absent in the homesteads during the school holiday months of April, August and December. This was mainly because these children went to stay with their fathers in the towns or visit other relatives. Another common cause of unsuccessful visits was mourning ceremonies. If a member of a homestead died, the family would have a mourning period of one week during which time the fieldworkers did not visit the homestead. A number of children were also admitted to various hospitals in Kilifi (though mainly in KDH) for varying lengths of time and were therefore not found at home during the weekly visits and these were also counted as 'missed visits'.

There were a total of 60 (7%) people from both study areas lost to follow-up during the entire study period. A total of 39 people migrated from the study areas while 18 died. The majority of the migrants were people moving to new homes either by marriage or women

taking their children to join their husbands in the big towns. One person refused to participate in the study after joining a religious group that believed in healing by the power of prayer only without the use of conventional or herbal medicines. Another two people refused to participate in the study because they were unhappy about the taking of numerous blood samples during the course of the study.

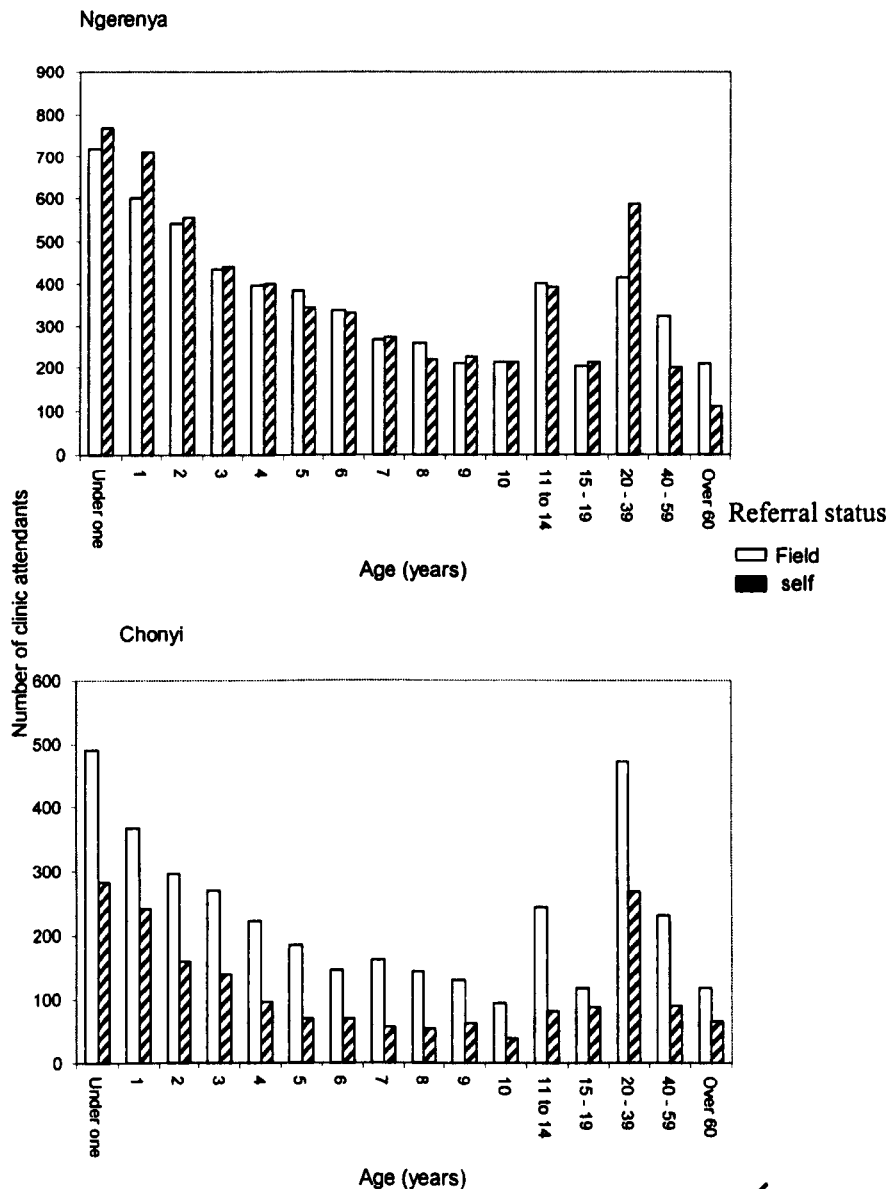
During the period of the study, there were seven deaths in Chonyi, six of which occurred at home and one occurred at the KDH. The deaths included two children under one year of age, one of five years of age, a 30 year old and three people > 60 years of age. The cause of five of the seven deaths was unknown. One of the children under one year of age died of an unknown febrile illness whereas a 30 year old died of a respiratory illness. In Ngerenya, there was a total of 11 deaths, six of which occurred at home and five at the KDH. Two of the deaths were of children under a year of old, two in one year olds and four among children aged 2 - 8 years. There were three deaths in adults >60 years of age. The cause of five of the 11 deaths was unknown whereas the rest of the deaths were caused by an array of reasons which included: tuberculosis, car crash, encephalitis, febrile illness, lower respiratory tract infections, a heart condition and post surgical problems.

4.4 Study clinic attendances

The study participants made a total of 11,925 clinic visits from Ngerenya compared to 5,533 clinic visits from study participants from Chonyi in the two years of simultaneous follow-up. Those attending the clinic were classified as either field or self-referrals. Field referrals were those that attended the clinic after a visit by the fieldworker and were also termed actively detected cases. Self-referrals were those that attended the clinic or were brought to the clinic spontaneously because either they felt unwell or their guardians thought they were unwell without waiting to be referred by the fieldworker. These were termed passively detected cases. In Ngerenya, 50% (5, 932) of the clinic visits were field-

referrals and the other half were self-referrals whereas in Chonyi 67% (3,681) of the visits were field-referrals and 33% (1,852) were self-referrals. Figure 4.2 compares the numbers of field and self-referrals in the two study areas by age.

Figure 4.2: The age pattern of field and self referred clinic attendants among study participants from Chonyi and Ngerenya from May 1999 to May 2001.



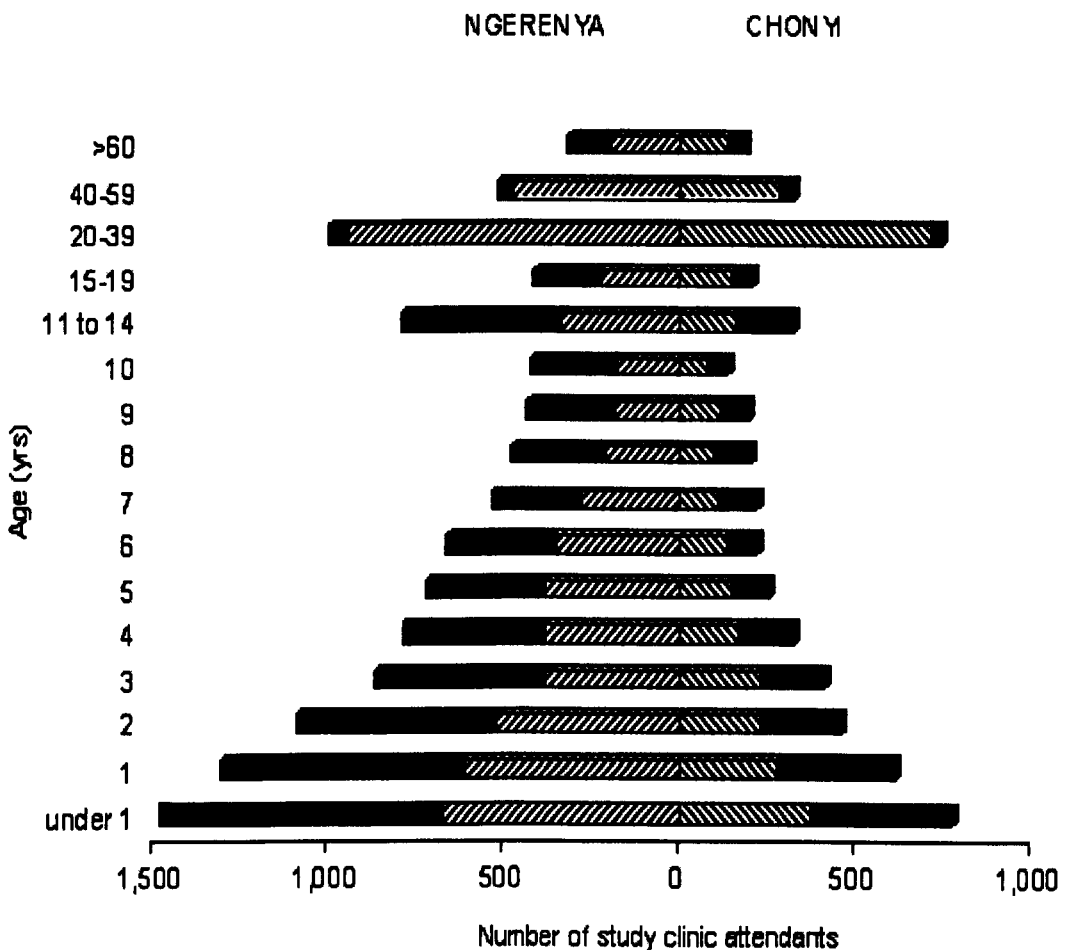
Across all age groups in Ngerenya except in those over 20, there appeared to be an equal distribution of field and self-referrals. Whereas there was a higher proportion of self-referrals among those 20-39 years old in Ngerenya, there appeared to be a higher proportion of field to self-referrals among those ≥ 40 years old from Ngerenya. In Chonyi

however, there were a higher number of field-referrals than self-referrals across all the age groups.

4.4.1 Total clinic attendance by age and sex

Figure 4.3 shows the age and sex distribution of the clinic attendees. Despite the greater number of clinic attendances among study participants from Ngerenya, the age and sex distribution of clinic attendees was the same in both areas.

Figure 4.3: Age and sex distribution of the study participants who attended the clinic from Chonyi and Ngerenya from May 1999 to May 2001 (Black bars represent males and hatched bars females)



The largest number of clinic attendants in both study areas was among children under a year old who comprised 14% and 13% of all clinic attendances from Chonyi and Ngerenya respectively. About 51% of the clinic attendances were children under five years of age whereas 70% were under the age of 10 years. There were more women than men in the 20-50 year old age groups attending the study clinic which reflected the age-sex patterns of the study population as shown earlier on Figure 4.1.

For each of the study participants that attended the study clinic, a hospital surveillance form was filled by the study clinicians (Appendix VII). The analysis that follows is a description of the various clinical symptoms and signs and also the diagnosis made by the clinicians. Only the symptoms reported by the patient spontaneously were analysed whereas those recorded after prompting by the clinician were ignored.

4.4.2 Commonest symptoms and diagnosis among patients attending the study clinic

Overall, the commonest symptoms reported were history of fever (54%), cough (37%), headache (20%), abdominal pain (12%) and vomiting (9%). The commonest clinical sign was a measured fever (20%), hot on palpation (17%) and cough heard (10%). Symptom presentation however differed with age.

4.4.2.1 Commonest symptoms and clinical signs by age and area

Proportions of the commonest (occurring in $\geq 10\%$ of those presenting to the clinic) symptoms and clinical signs by age and study area are shown on Table 4.2. Symptom presentation by age in the two study areas appears to be the same and is presented as averages for both areas. Over 60% of those children under the age of five years attending the clinic had a history of fever but only 26% were hot on palpation. About 44% of the patients under the age of five years that attended the clinic reported with a cough though the clinician recorded hearing a cough in only 14% of them.

Table 4.2: Distribution of the commonest clinical signs and symptoms by age in clinic attendants from Chonyi and Ngerenya in the period May 1999-May 2001.

Symptom/clinical signs	Frequency distribution	
	Ngerenya	Chonyi
≤ 5 years of age		
Symptoms	n=6,300	n=2,814
History of fever	62%	72%
Cough	42.7%	44.7%
Diarrhoea	14%	13%
Vomiting	12.1%	12.8%
Clinical signs		
Measured fever (Axillary temperature $\geq 37.5^{\circ}\text{C}$)	24.2%	28%
Hot on palpation	21.1%	24.9%
Cough heard	12.4%	14.8%
6 – 14 years of age		
Symptoms	n=3,353	n=1,277
History of fever	58.3%	53.6%
Cough	32.9%	34.5%
Headache	21.3%	37.2%
Abdominal pain	12.3%	15.2%
Vomiting	10.6%	9.2%
Shivering	7%	12.1%
Clinical signs		
Measured fever (Axillary temperature $\geq 37.5^{\circ}\text{C}$)	19.1%	18.4%
Hot on palpation	17.6%	15.8%
≥15 years of age (adults)		
Symptoms	n=2,272	n=1,442
Headache	32.3%	42.8%
History of fever	23.3%	20%
Cough	22.9%	26.4%
Abdominal pain	22.2%	22.2%
Chest pain	16.6%	23.3%
Backache	11.2%	14%
Joint pains	8.2%	10.4%
Aching all over	10.8%	8.9%
Shivering	7.1%	12.1%
Clinical signs		
Cough heard	7.3%	7%

Fifty-five percent (55%) of children between 6-14 years of age attending the clinic, reported with a history of fever, about 16% were hot on palpation but only 18% had a measured fever. In this age group, 34% reported having a cough while the clinician

recorded hearing a cough in 25% of all patients. About 21% of those 6-14 years of age presenting to the clinic from Ngerenya had a headache compared to 37% from Chonyi ($\chi^2=42.1, p<0.001$).

The most common clinical symptom among adults attending the clinic was headache. There were again more adults in Chonyi (43%) that reported to the clinic with headache than there were from Ngerenya (32%) ($\chi^2 =122, p<0.001$). A measured fever or being hot on palpation were not common clinical signs in adults, being found in <10% of those presenting to the clinic.

Fifty-four percent (54%) of all clinic attendants from both study areas presented with a history of fever. This was the commonest clinical symptom overall and is a commonly used to identify malaria cases. Later in chapter six, a detailed analysis of the symptoms and signs associated with malaria is conducted and an attempt is made to identify the most suitable clinical signs and symptoms that would be used for malaria diagnosis.

4.4.2.2 History of fever

The following section is an analysis of the proportions who presented with a history of fever by age and the proportions among those with a history of fever who had a raised body temperature or parasitaemia. Figure 4.4 shows the proportions of clinic attendants with a history of fever by age. Over 54% of the clinic attendants from Chonyi with a history of fever were children under four years of age, while 16% were ≥ 10 years of age. Similarly, in Ngerenya, about 44% of the study participants with a reported history of fever were under four years of age, while 20% were ≥ 10 years of age.

Figure 4.4: Proportions of the total clinic attendants from Chonyi and Ngerenya with a history of fever in the period May 1999 to May 2001.

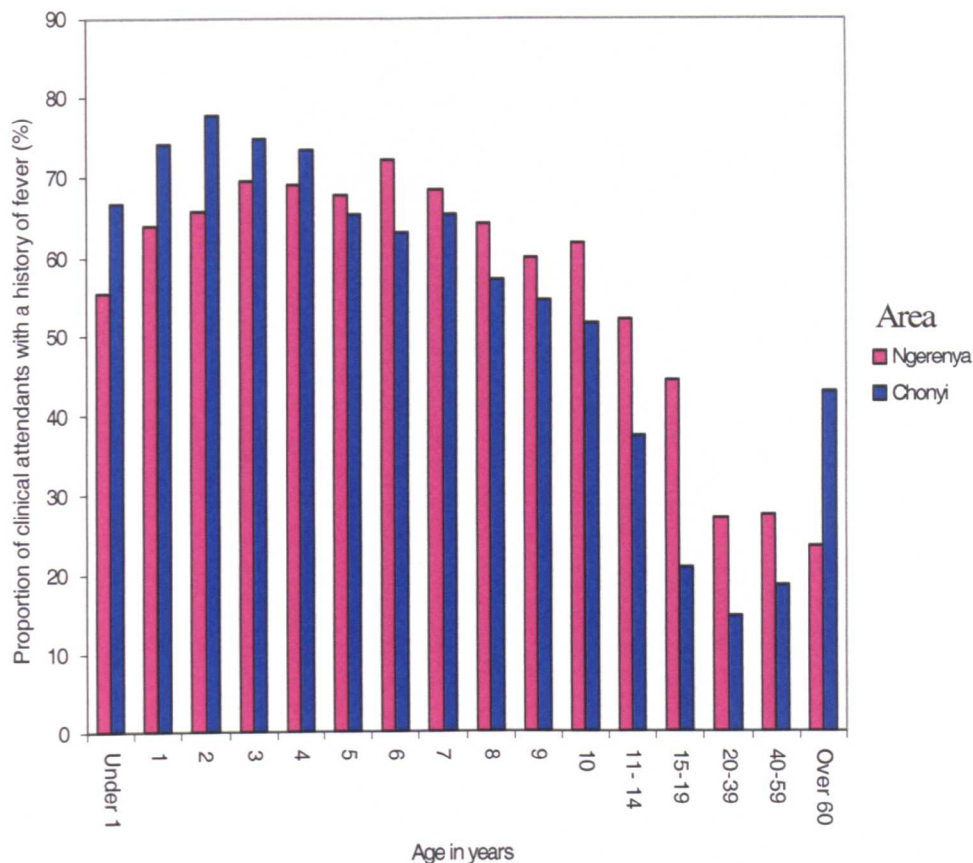


Table 4.3 shows the proportion of those that attended the clinic with a history of fever that were febrile (a measured axillary temperature $\geq 37.5^{\circ}\text{C}$) or had a positive slide on examination. Proportions were calculated only in those people that attended the clinic with a history of fever. There was evidence of a higher proportion of children two years and younger from Chonyi that were febrile and parasitaemic compared to those children of the same age in Ngerenya (40.9% Vs 37.2%, $\chi^2=4.9$, $p=0.03$). Between the ages of three to ten years of age, there was evidence of a higher proportion of children from Ngerenya presenting to the study clinic with a history of fever that were febrile compared to Chonyi (33.2% Vs 27.7%, $\chi^2=12.4$, $p<0.001$). There was no evidence of a difference in the proportion who presented to the clinic with a history of fever that were febrile in study

participants aged 20-59 years of age from Chonyi and Ngerenya (11.9% Vs 8.4%, $\chi^2=1.6$, $p=0.2$).

Between the ages of two to fourteen years of age, about 50% of those presenting to the clinic with a history of fever had parasitaemia. The highest proportions with parasitaemia (>60%) were found among those three to five years of age from Chonyi compared to five to six years of age from Ngerenya.

Table 4.3: Proportions of study participants that were febrile (axillary temperature $\geq 37.5^\circ\text{C}$) or parasitaemic among those reporting to the study clinic with a history of fever from both study areas in the period May 1999-May 2001.

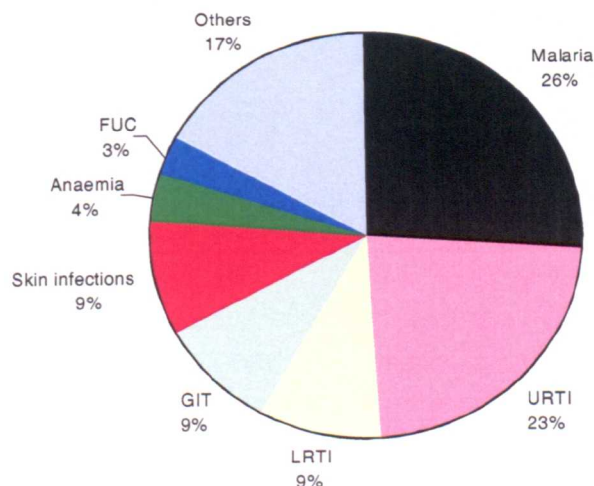
Age group (in years)	Proportion with fever (%)		Proportion with parasitaemia (%)	
	Chonyi	Ngerenya	Chonyi	Ngerenya
Under 1	40.5	34.3	34	16.8
1	45.7	42.1	45.2	31.7
2	35.4	34.7	55.9	44.6
3	30.3	35.4	65	54.1
4	26.9	36.2	67.6	59.2
5	24.1	35.6	62.9	63.2
6	32.6	33.9	56.7	61.6
7	32.6	28.5	56.9	56.8
8	24.8	30.2	59.9	57.9
9	18.9	29.2	54.3	58.5
10	26.5	28.6	51.6	50.7
11 to 14	26.4	24.6	49.3	52.2
15-19	11.9	17.6	27.7	40.1
20-39	11.1	9.9	20.4	22.4
40-59	13.6	5.6	15.2	14.2
Over 60	6.4	14	24.6	9.1

Fever and parasitaemia are the main features that are used in malaria definition in epidemiological studies. The use of raised temperature and parasite density in deriving malaria case definitions is described in detail in chapter five.

4.4.2.3 Diagnosis made during the study period

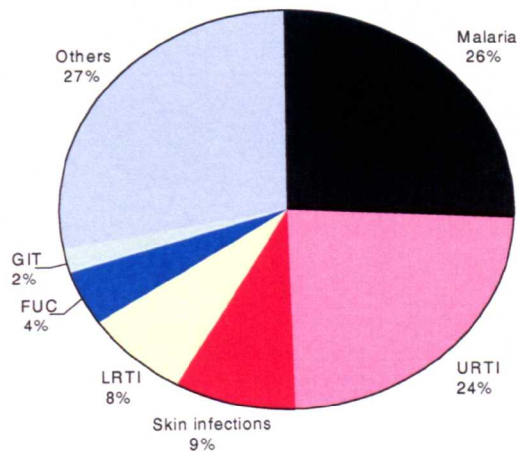
Since age-specific symptomatology did not differ much in the two areas, the data was merged to identify the most common diagnosis made at the study clinic. Figure 4.5 to 4.7 are a series of pie charts showing the various diagnoses made in the three age groups identified earlier on as under five, 6-14 years and those ≥ 15 years of age. The commonest diagnosis in all age groups was upper respiratory tract infections and malaria. A patient was diagnosed with malaria if they presented to the clinic with clinical symptoms or signs of fever or complained of the same and had an accompanying *P. falciparum* parasitaemia on examination. Any level of parasitaemia was indicative of malaria. All other diagnosis was based on laboratory investigations and clinician judgement of the likely diagnosis for disease presentation. Figure 4.5 shows the diagnosis made among patients less than five years of age from both study areas in the two-year period of follow-up.

Figure 4.5: Proportions with different diagnosis in children <5 years old from Chonyi and Ngerenya that attended the study clinic in the period May 1999-May 2001. (URTI – Upper Respiratory tract infections, LRTI – Lower respiratory tract infections, GIT- Gastro-enteritis, FUC – Fever of unknown cause).



Twenty-six percent (26%) of the children were diagnosed as having malaria and 23% as having upper respiratory tract infections. These accounted for about half of the diagnoses made in this age group. Lower respiratory tract infections, gastro-enteritis and various skin infections accounted for 9% of the diagnosis each. About 31% of the diagnosis was therefore as a result of respiratory tract infections. Anaemia represented only 4% of the diagnosis. Figure 4.6 shows the diagnosis made at the study clinic among children aged 6-14 years of age from both Ngerenya and Chonyi in the period May 1999-May 2001.

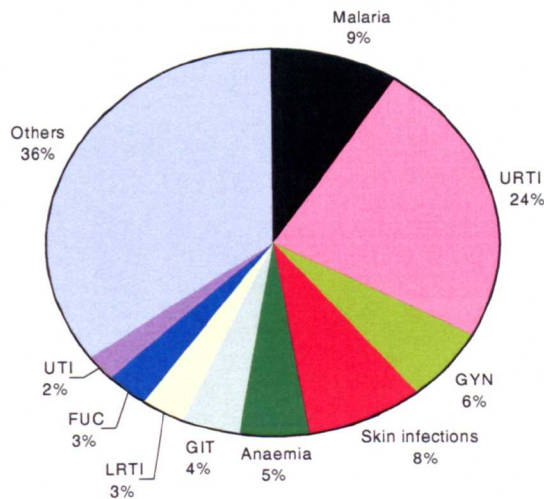
Figure 4.6: Proportions with different diagnosis in children 6 – 14 years old from both Ngerenya and Chonyi that attended the study clinic in the period May 1999-May 2001. (URTI – Upper Respiratory tract infections, LRTI – Lower respiratory tract infections, GIT- Gastro-enteritis, FUC – Fever of unknown cause)



About a quarter of the diagnosis was malaria and another quarter upper respiratory infections, which was very similar to the description of the children under five years of age. Upper and lower respiratory tract infections contained about 32% of all the diagnosis which was similar to the fraction among children under the age of five years. Helminths and skin infections occurred at the same rate as that of children less than five years of age.

There were however fewer cases of gastro-enteritis diagnosed in this age group than among children under five years of age and there were a larger variety of diagnosis hence the 'others' group was larger than that in the under five year old group. Figure 4.7 shows the diagnosis made in adults from both Chonyi and Ngerenya at the study clinic during the two years of follow-up.

Figure 4.7: Proportions with different diagnosis in adults (≥ 15 years old) from both Chonyi and Ngerenya that attended the study clinic in the period May 1999-May 2001. (URTI – Upper Respiratory tract infections, LRTI – Lower respiratory tract infections, GIT- Gastro-enteritis, FUC – Fever of unknown cause, GYN – Gynaecological problems, UTI- Urinary tract infections)



Only 9% of adults attending the clinic were diagnosed with malaria, which was lower than that found in the younger age groups. Among adults, the most commonly diagnosed condition was upper respiratory tract infections followed by malaria and the third was gynaecological problems (mainly sexually transmitted diseases) that took up 6% of the diagnoses. Anaemia accounted for more of the diagnosis than among children 6-14 years of age. The only similarity between the adults and younger study participants were the numbers of people with respiratory tract infections who accounted for 27% of all the

diagnosis made. There was a wide array of diagnosis made among adults that individually accounted for <2% of the diagnosed conditions and these were merged together as 'others'.

The next section describes the various physiological and laboratory parameters that were measured in the course of the study. This will include discussions of the temperatures that were taken routinely, parasite rates from the cross-sectional surveys, haemoglobin levels, C-reactive protein levels and white blood cell counts.

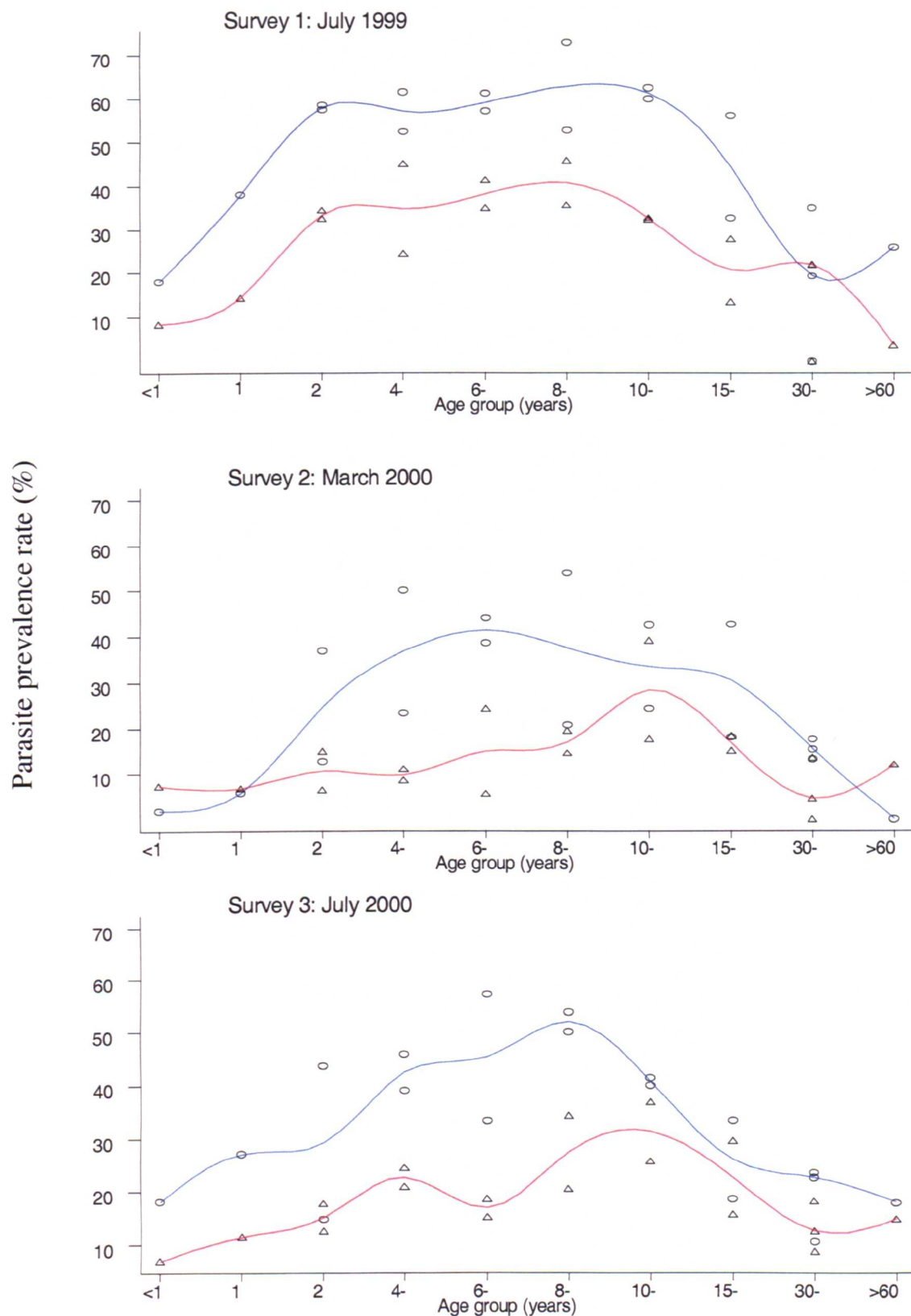
4.5 Parasite prevalence

The data presented in this section were from the six cross-sectional surveys carried out simultaneously in both study areas during both the dry and wet seasons as demonstrated on Figure 3.9. Blood smears were made from all the study participants whether they were healthy or unwell. The data that follows is a description of the parasite rates during these surveys.

4.5.1 *P. falciparum* parasite prevalence by age

Figure 4.8 is a series of charts that show the age prevalence curves of parasitaemia in Chonyi and Ngerenya for each of the six surveys. Parasite prevalence rates were highest among children compared to adults. There were very low parasite prevalence rates in those >40 years of age. The lowest parasite prevalence rates were found in those under a year old and those over 60 years of age. There was a reduction in the parasite prevalence rates within the population from Chonyi over time from 1999 to 2001. Figure 4.9 shows the overall parasite prevalence rates in all age groups for all surveys and those conducted during the wet and dry seasons separately.

Figure 4.8: Six cross-sectional surveys of age-parasite prevalence in the two study areas. Blue line represents Chonyi and the red line Ngerenya.



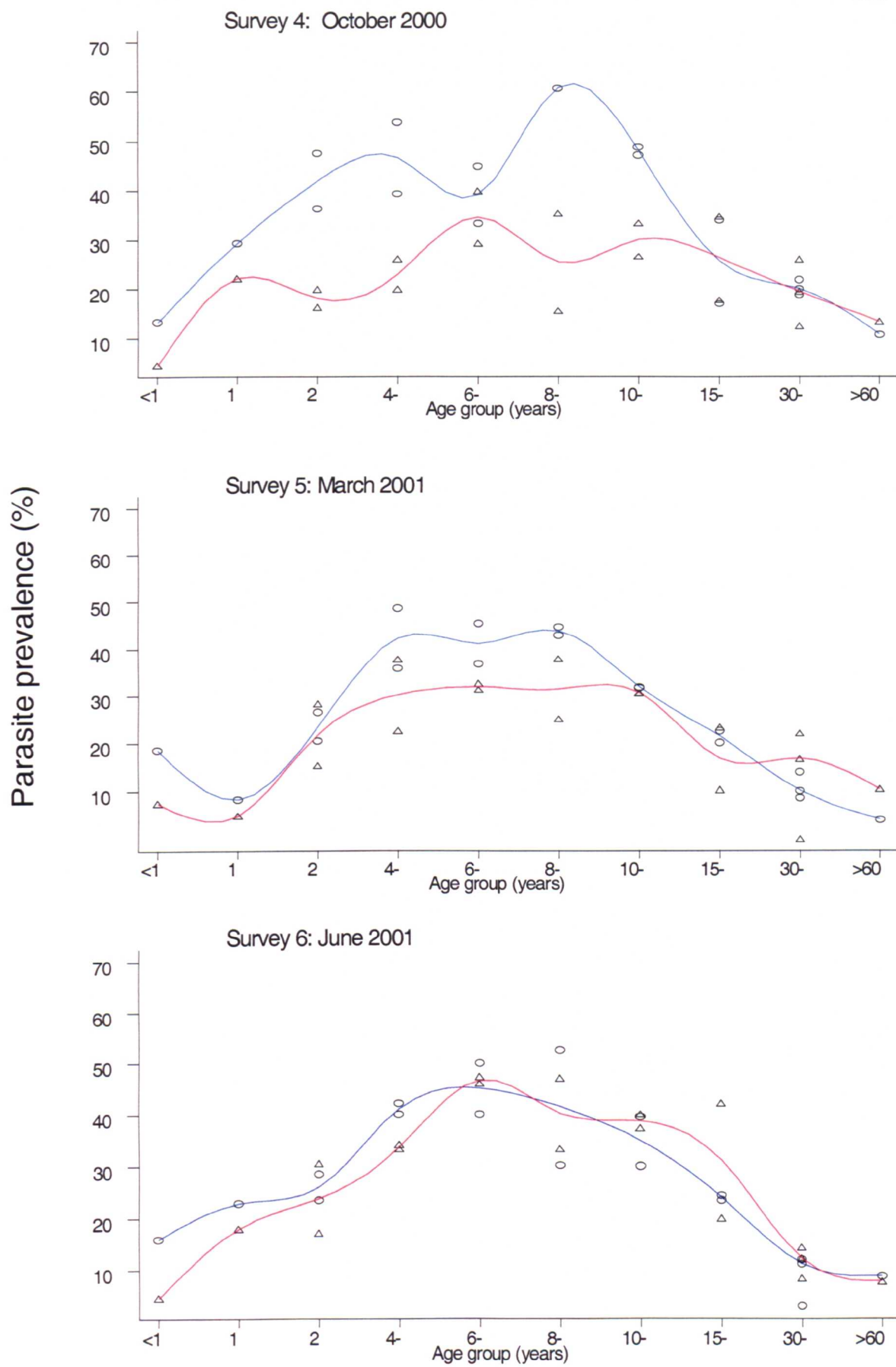
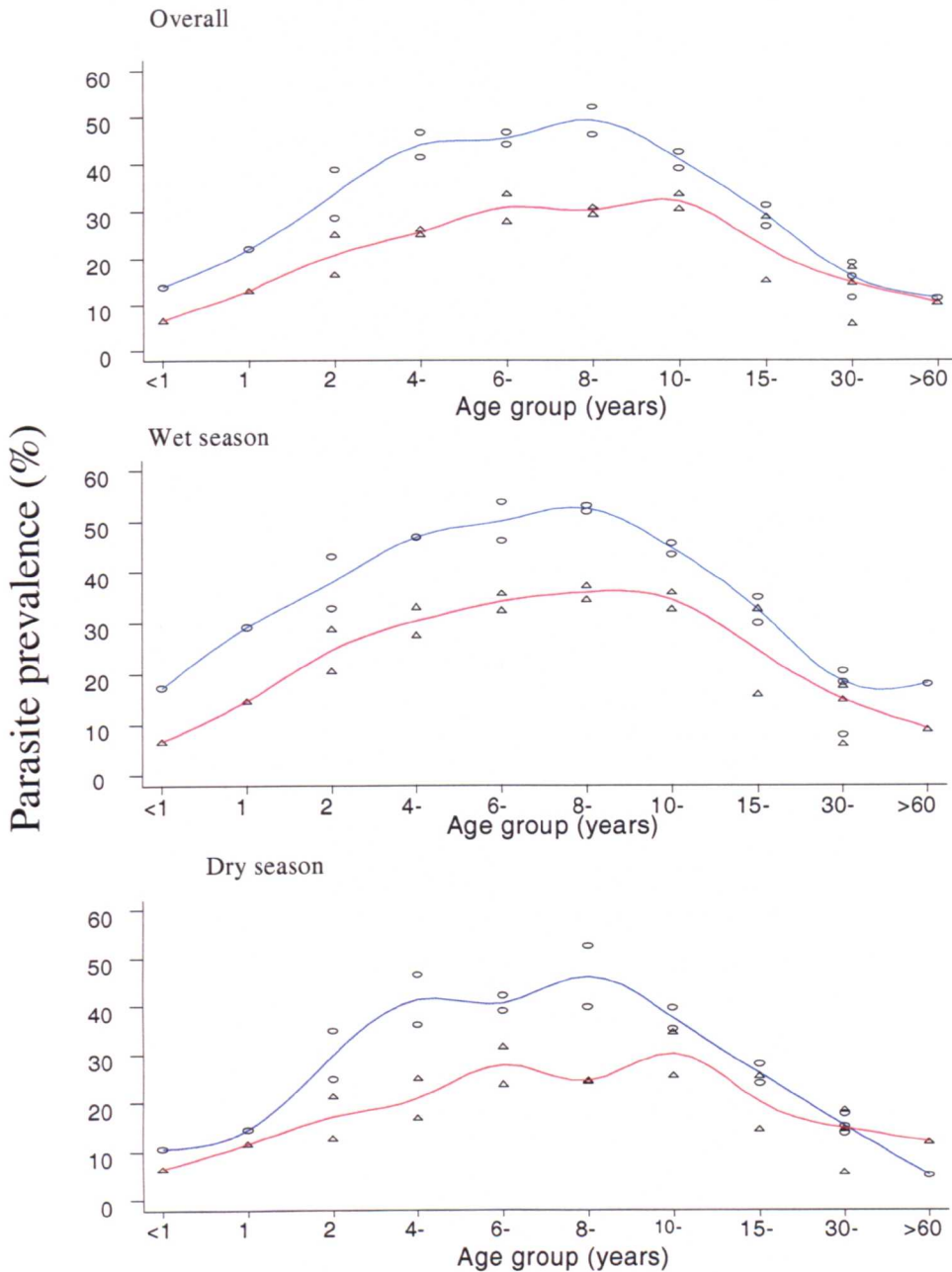


Figure 4.9: Overall and seasonal parasite prevalence rates in two study areas.

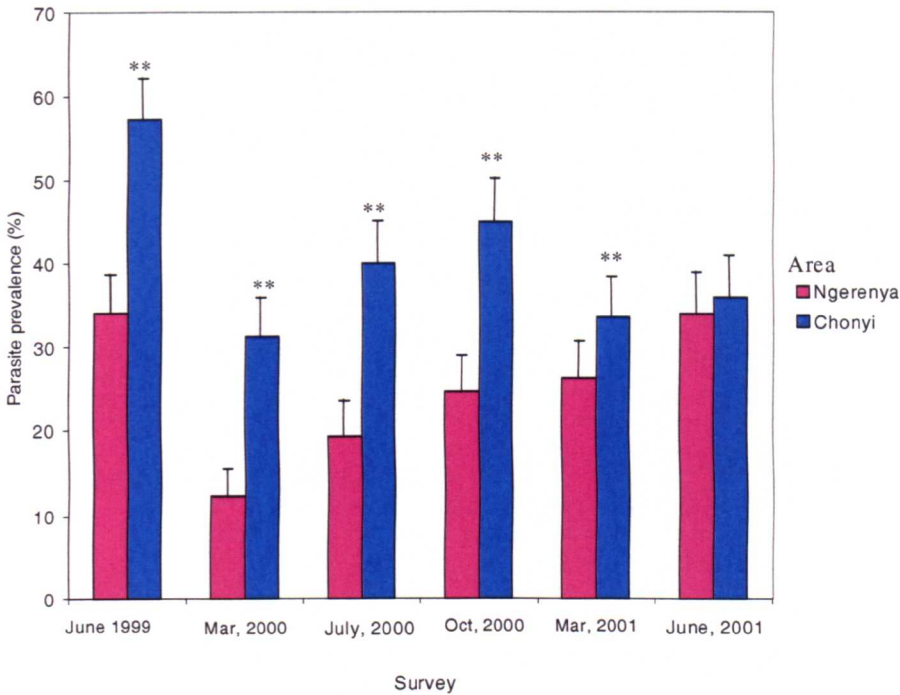
Blue line represents Chonyi and the red line Ngerenya.



Across all age groups, there was a consistently higher parasite prevalence rate in Chonyi compared to Ngerenya except among those 30 years old and above. There were lower parasite prevalence rates during the months of March and October. This was associated with the lack of rains at this time of the year (Section 3.1) that results in fewer breeding sites and a reduction in transmission intensity.

Parasite prevalence in the 1-9 year old group ranged from 34-57% in Chonyi and 12-34% in Ngerenya. Parasite prevalence among children aged 1-9 years have often been used as markers of endemicity and referred to often in this discussion. Figure 4.10 shows the parasite prevalence rates among children 1-9 years of age from Chonyi and Ngerenya.

Figure 4.10: Parasite prevalence rates among children aged 1- 9 years from Chonyi and Ngerenya from the six cross-sectional surveys.

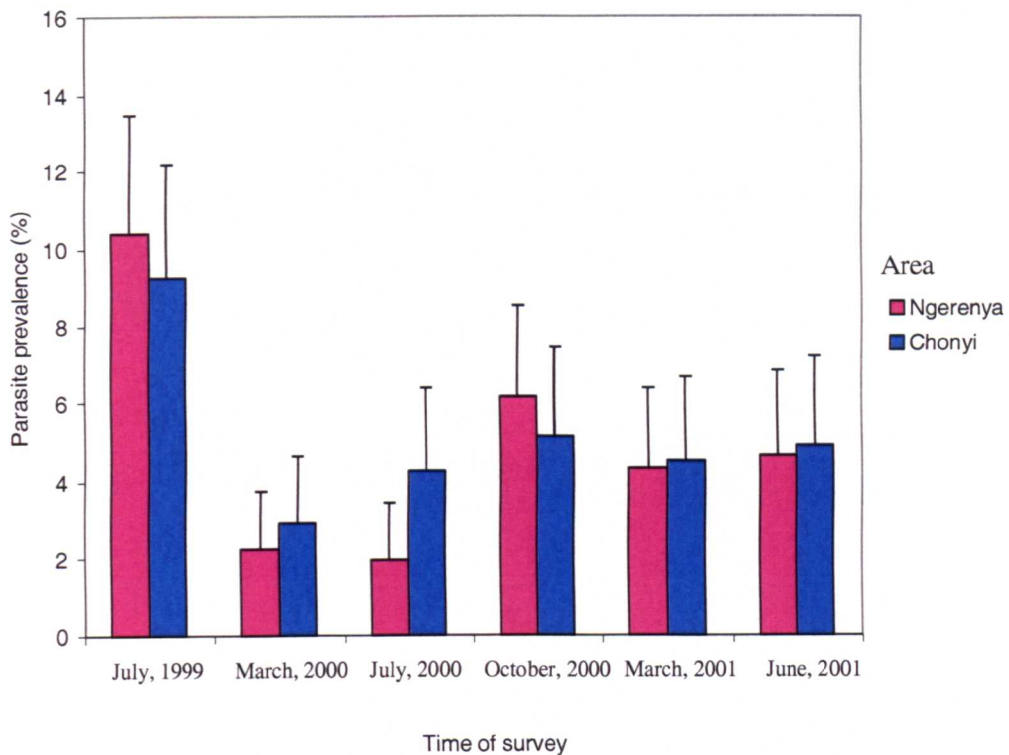


Key: ** Shows when there is a difference in the parasite prevalence rate among children 1-9 years of age from Chonyi and Ngerenya that is statistically significant ($p < 0.05$).

The chi-squared test was used to compare the proportions in the two study populations and it was found that in all except the last survey, there was a higher parasite prevalence rate among children 1-9 years old from Chonyi compared to Ngerenya. During the last survey, there were equal proportions of children in either area with a positive slide (33.8% in Ngerenya compared to 35.7% from Chonyi, $\chi^2 = 0.28$, $p = 0.6$). It is possible that the active detection of malaria cases over the years may have led to the reduction in the infection rates.

The next section considers the rates of the sexual stages of *P. falciparum* known as gametocytes. The rates of the sexual stages were estimated for the children aged 1-9 years of age and comparisons made between proportions of the sexual stages in the two areas as demonstrated on Figure 4.11.

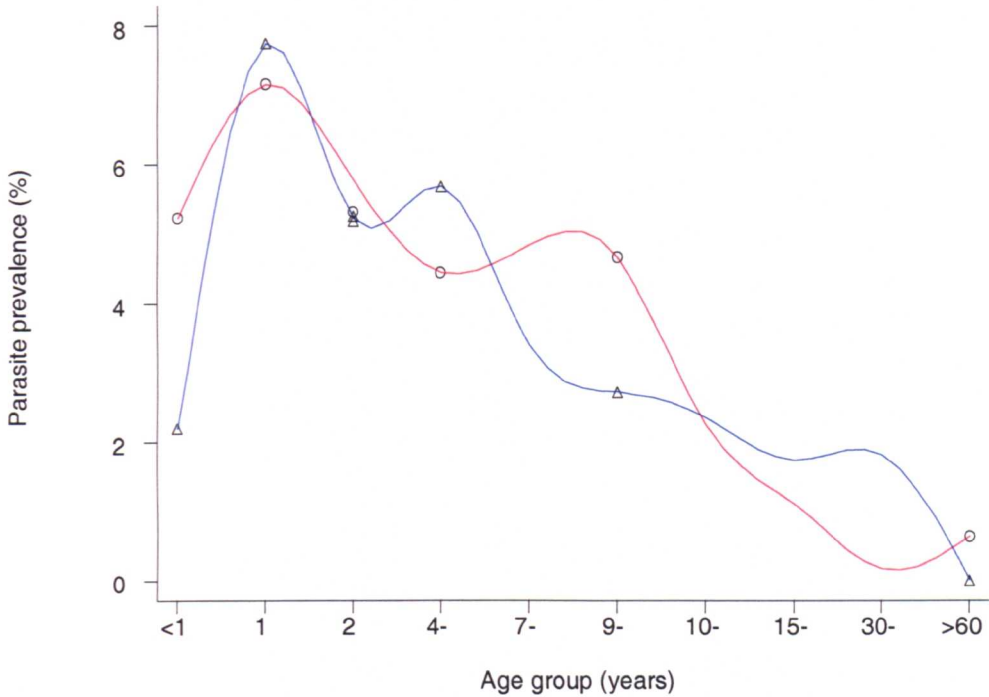
Figure 4.11: Gametocyte prevalence rates among children aged 1- 9 years from Chonyi and Ngerenya from the six cross-sectional surveys.



Key: ** Shows when the difference in the gametocyte rate among children 1-9 years of age from Chonyi and Ngerenya was statistically significant ($p < 0.05$).

There were no statistically significant differences in the proportions with gametocytes among the children in this age group in either area. From all six surveys, the average prevalence of gametocytes by age was as shown in Figure 4.12. The highest gametocyte rates were found in those <10 years of age in both study areas. After the age of 10 years, the gametocyte prevalence rate was less than 2%.

Figure 4.12: Average *P. falciparum* gametocyte parasite prevalence by age between July 1999 to June 2001. Blue line represents Chonyi and the red line Ngerenya.

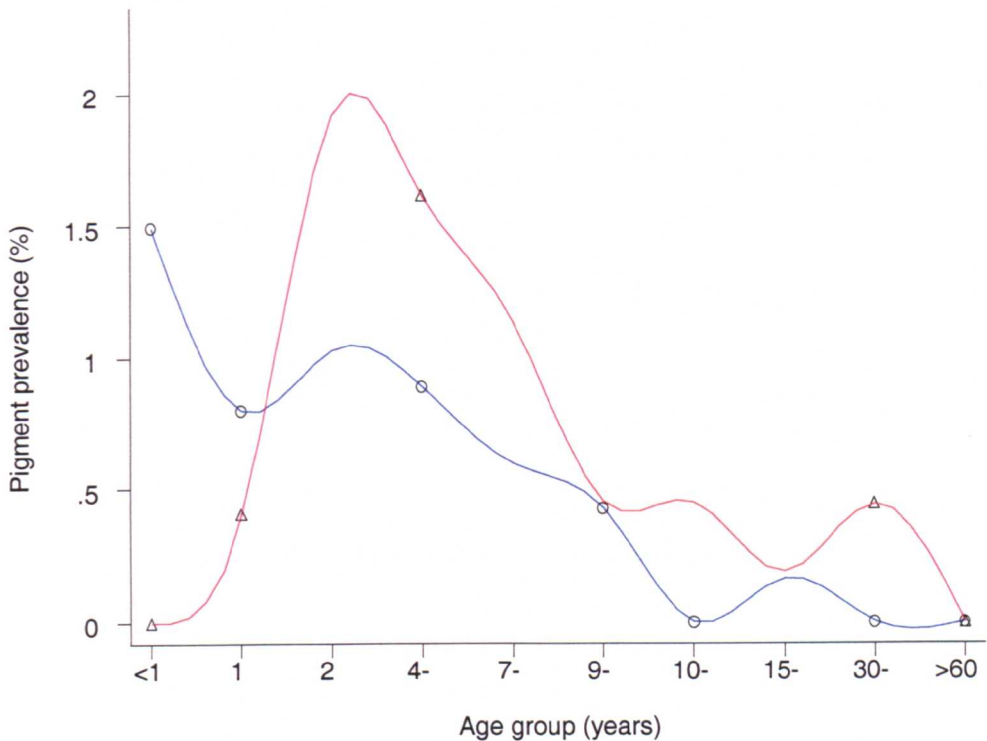


The overall gametocyte prevalence from all six surveys was 3.4% in both Chonyi and Ngerenya. In children between 1 and 9 years of age, the gametocyte parasite prevalence rate was 5.2% in Chonyi and 5% in Ngerenya whereas in those under one year of age, the rate was 5.3% in Chonyi and 2.2% in Ngerenya.

The presence or absence of pigment was noted from each smear made during the cross-sectional surveys. Parasite pigment or haemozoin is a by-product of haemoglobin digestion by trophozoites within the red blood cells and is associated with late stage parasites. Pigment was found in 0.5% of the study participants from Chonyi and 0.8% of those from Ngerenya. About 31% of those with pigment had gametocytes. Figure 4.13 shows the

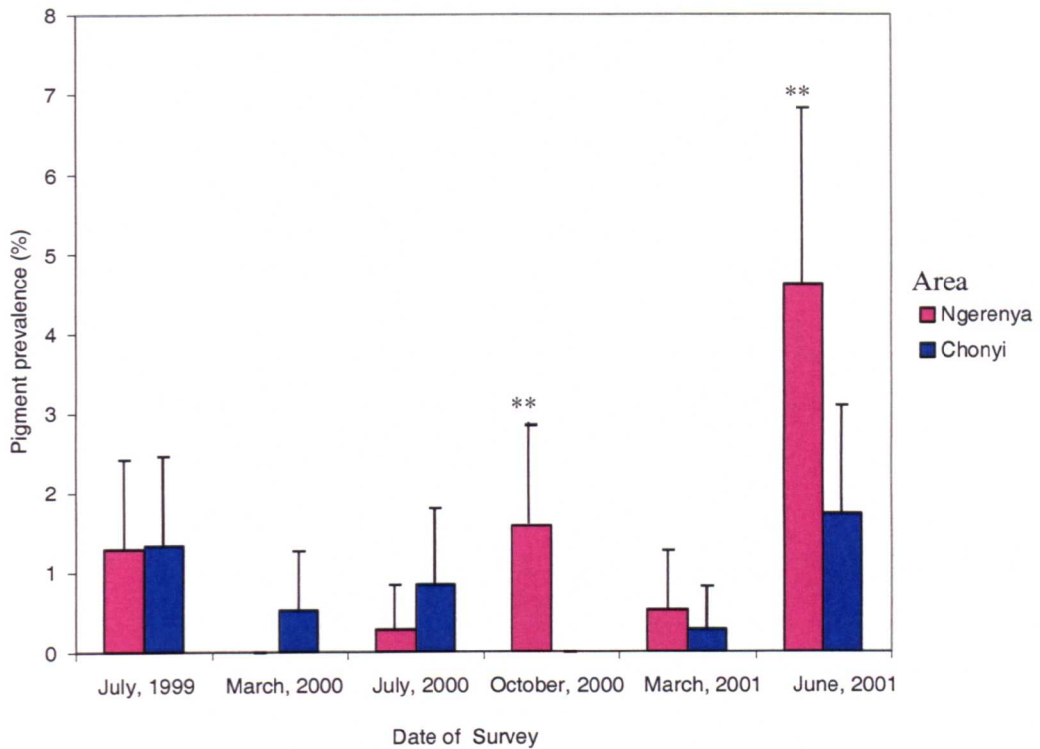
pigment prevalence rate in all age groups in Chonyi and Ngerenya as an average of all six cross-sectional surveys.

Figure 4.13: Prevalence of malaria pigment by age in surveys conducted between July 1999 and June 2001. Blue line represents Chonyi and the red line represents Ngerenya.



In Chonyi 1.5% of the under one had pigment compared to none in Ngerenya. Figure 4.14 shows the pigment prevalence rates among children 1-9 years of age from Chonyi and Ngerenya during six cross-sectional surveys. There was evidence of a higher pigment prevalence rate among children 1-9 years of age from Ngerenya than Chonyi (1.35% Vs 0.79%, $\chi^2=3.28$, $p=0.07$), however, this difference was of borderline significance.

Figure 4.14: Pigment prevalence rates among children 1-9 years of age in Chonyi and Ngerenya from six-cross-sectional surveys.



Key: **- statistically significant difference in proportion with pigment from Chonyi and Ngerenya ($p < 0.05$)

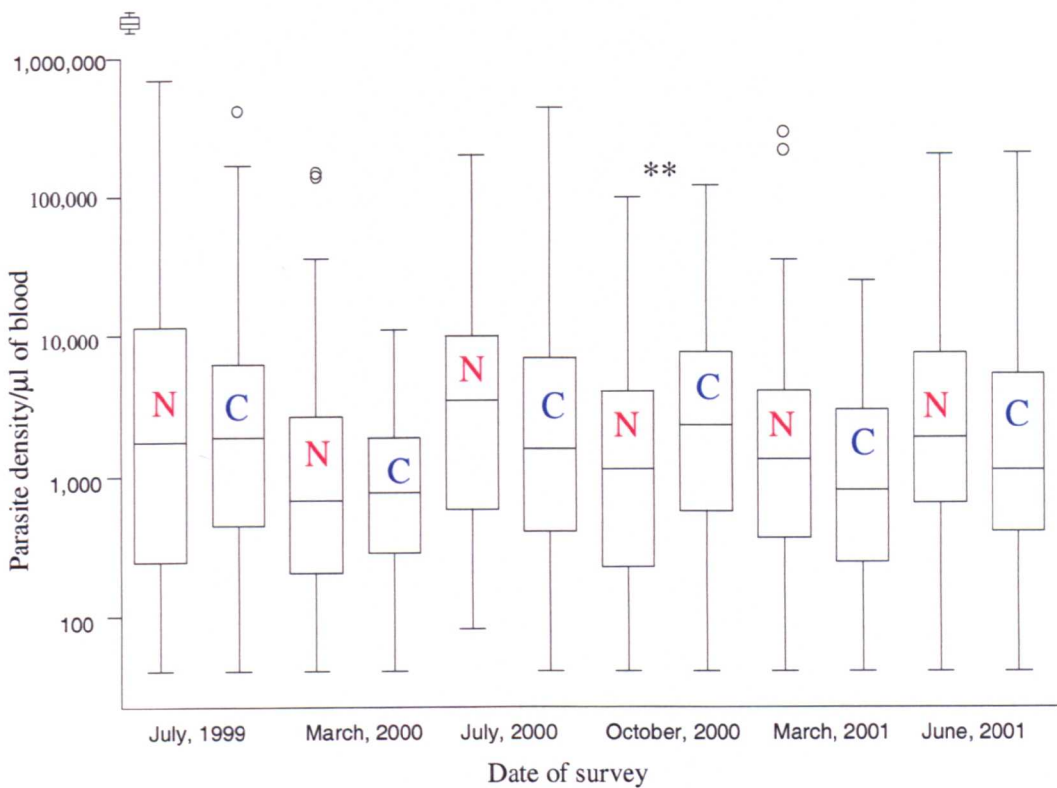
In only two of the six surveys was there a statistically significant difference between the pigment prevalence rates among children 1-9 years of age from Chonyi compared to Ngerenya. There was evidence of a statistically higher proportion of children with pigment among children from Ngerenya compared to children from Chonyi during the October 2000 survey (1.59% Vs 0%, $\chi^2=5.63$, $p=0.02$) and the June 2001 survey (4.61% Vs 1.73%, $\chi^2=4.63$, $p=0.03$).

4.5.2 *P. falciparum* geometric mean parasite density

Figure 4.15 shows the median and interquartile ranges of parasite density among children aged 1-9 from Chonyi and Ngerenya during the six cross-sectional surveys. The median

parasite density among those that were parasite positive was 1,320 parasites/ μ l (IQR: 400 – 5,240) of blood among 1-9 years old children from Chonyi compared to 1,520 parasites/ μ l (IQR: 320 – 6,760) among the same age group from Ngerenya.

Figure 4.15: Box plot showing median (central line), 25%, 75% quartile (box width), upper and lower limits (T) and outliers (dots) of geometric mean parasite density among children 1-9 years of age from Chonyi and Ngerenya during the six cross-sectional surveys. ‘N’ refers to Ngerenya and ‘C’ to Chonyi .



Key: **- Statistically significant difference between the median parasite density in Chonyi compared to Ngerenya ($p < 0.05$).

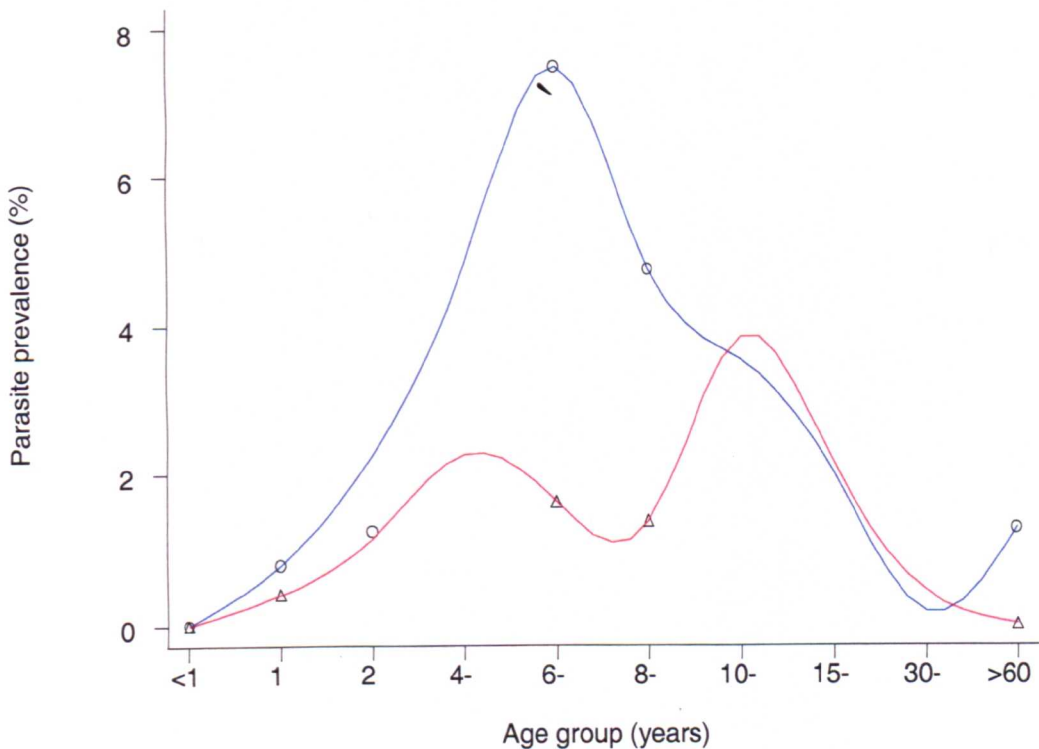
The highest geometric mean parasite densities were in those under three years of age for both areas. Only in the survey conducted in October 2000 was there evidence of a higher median parasite density among those that were parasite positive in Chonyi [2,300 parasites/ μ l (IQR: 560 – 7,430)] compared to Ngerenya [1,140 parasites/ μ l (IQR: 220 – 3,980)] ($p = 0.02$). There was however no evidence of a difference in the median parasite

season. The variation in parasite densities among children according to season appeared to be higher in Ngerenya compared to Chonyi.

4.5.3 Parasitaemia due to other *Plasmodium* species

Two other species of *Plasmodium* are found in Kilifi district: *P. malariae* and *P. ovale*. Their occurrence was rare during all cross-sectional surveys in both areas. The overall *P. malariae* parasite prevalence rate in the six surveys conducted was 2.9% and 1.7% in Chonyi and Ngerenya respectively. Figure 4.17 shows age-specific *P. malariae* prevalence rates in the two study areas.

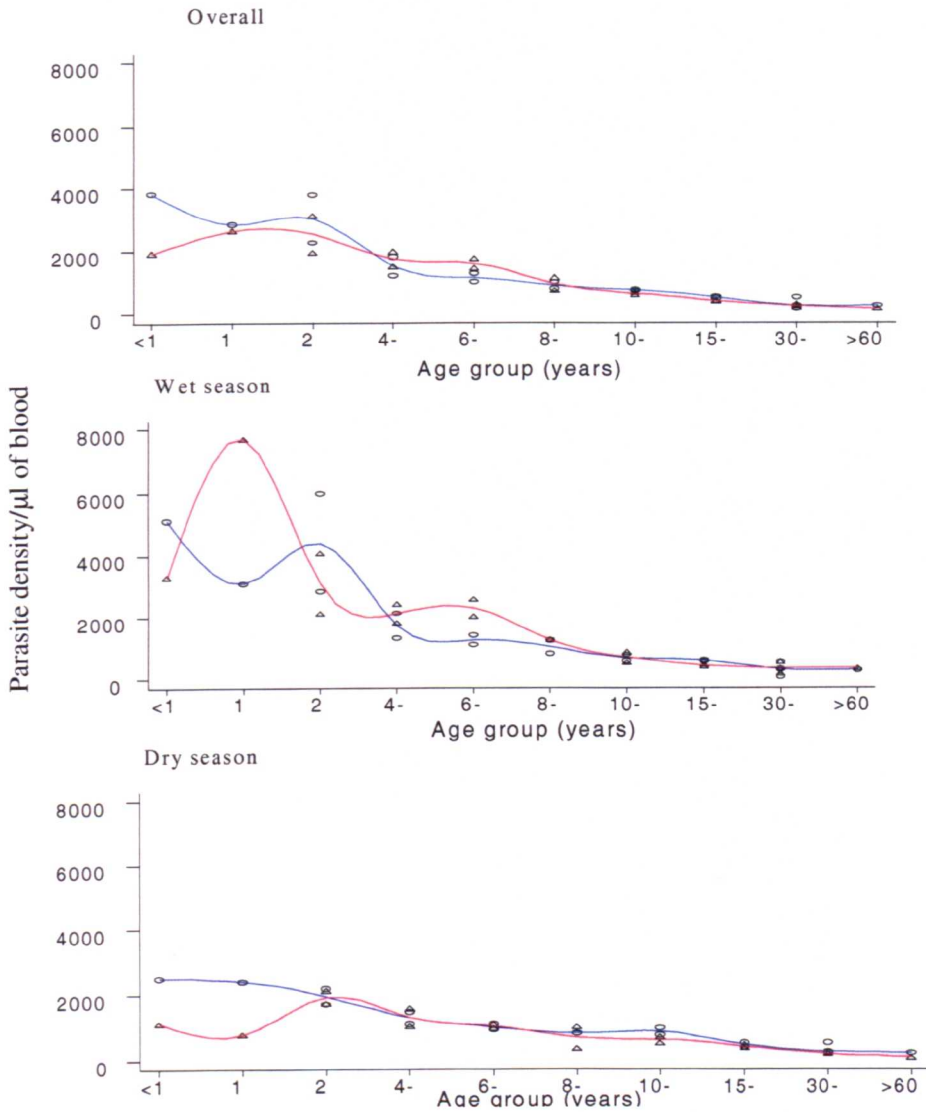
Figure 4.17: Average *P. malariae* prevalence by age from all six surveys conducted in the period July 1999-June 2001. The red line represents Ngerenya and the blue line Chonyi.



There was no *P. malariae* parasitaemia detected in those >40 years of age in Ngerenya but in Chonyi, there was a slight increase among those >60 years of age despite the drop to

density among children ages 1-9 years of age from Chonyi and Ngerenya in any of the other surveys conducted in the area. Figure 4.16 shows the geometric mean parasite density overall for all six surveys and during the wet and dry season separately.

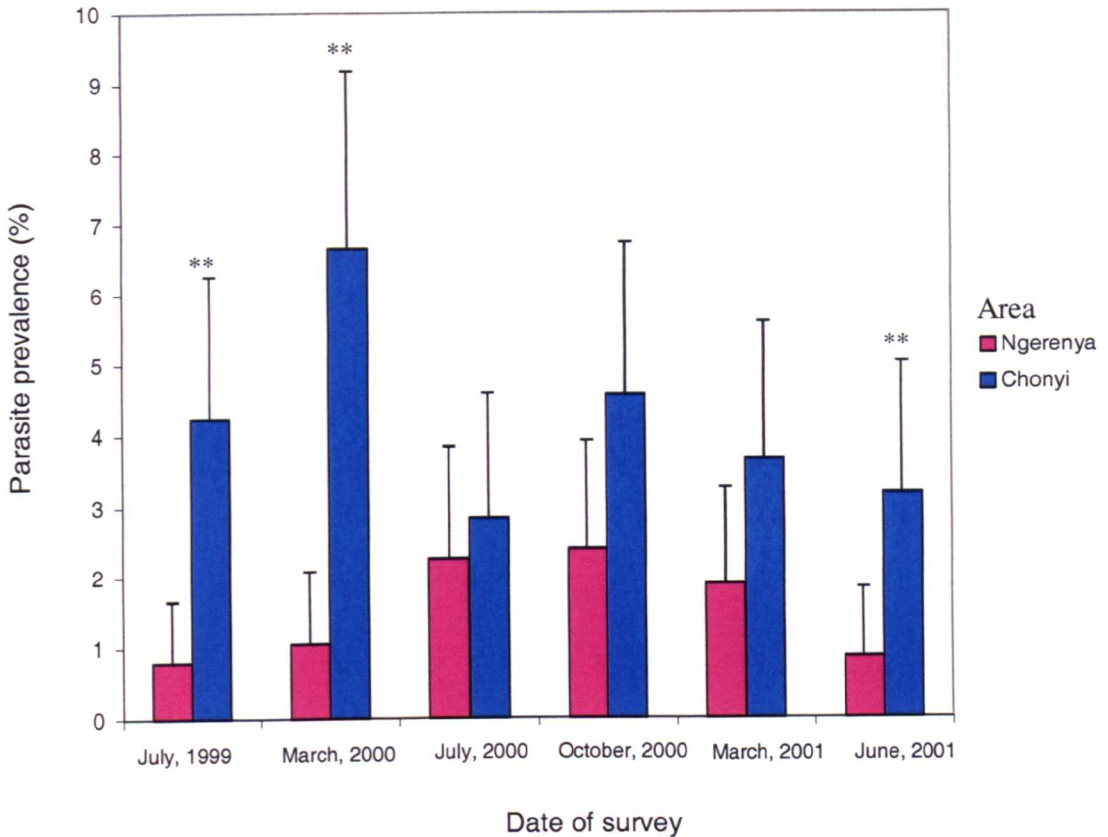
Figure 4.16: Geometric mean parasite density overall and during the wet and dry season by age in the two study areas. Blue line represents Chonyi and the red line represents Ngerenya.



From this chart, it appears that the parasite densities were higher in the children under 10 years of age during the malaria transmission season compared to the dry season, however, the parasite densities among those over 10 years of age did not vary with transmission

zero at the age of 40. Children under one year of age from both study areas did not have *P. malariae* parasitaemia but the parasite prevalence in those between 1-9 years of age was 4.2% in Chonyi and 1.5% in Ngerenya. Figure 4.18 shows the differences in the overall parasite prevalence in each of the surveys among children aged 1-9 years of age.

Figure 4.18: *P. malariae* parasite prevalence rates among children aged 1-9 years of age from Chonyi and Ngerenya in six cross-sectional surveys.



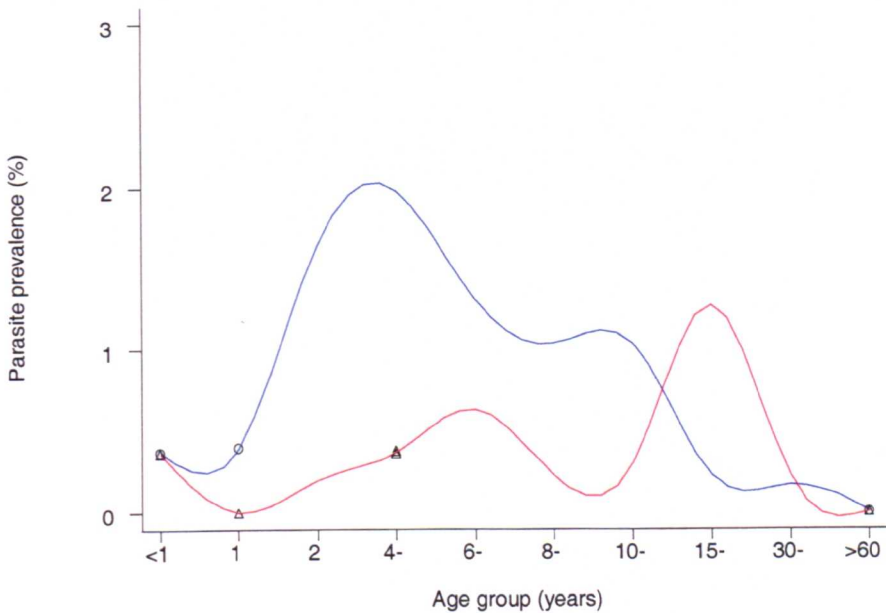
Key: **- statistically significant difference in the parasite prevalence between Chonyi and Ngerenya ($p < 0.05$).

There was a consistently higher rate of *P. malariae* parasite prevalence rates in Chonyi compared to Ngerenya, and statistically significant higher prevalence during the surveys conducted in the July 1999 survey (4.24% Vs 0.78 %, $\chi^2=9.44$, $p=0.002$), March 2000 (6.67% Vs 1.05%, $\chi^2=16.22$, $p < 0.001$) and June 2001 (3.18% Vs 0.86%, $\chi^2=4.71$, $p=0.03$). Two of the surveys (July and June) were conducted in the months with the highest malaria

transmission whereas one of the surveys (March) was conducted in a month where there is low malaria transmission.

The overall *P. ovale* parasite prevalence from the six surveys conducted was 0.9% and 0.4% in Chonyi and Ngerenya respectively. The age-specific parasite prevalence rates are shown on Figure 4.19. The parasite prevalence was 0.4% among those under a year old in both areas.

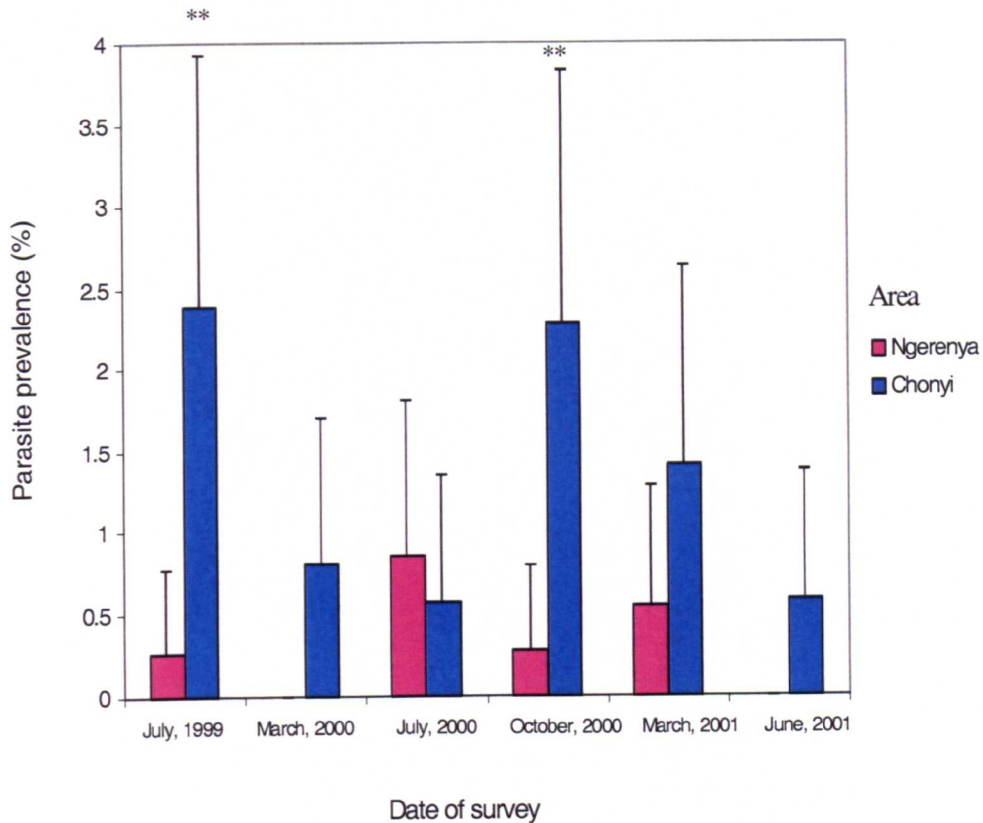
Figure 4.19: Average *P. ovale* prevalence by age from all six surveys conducted in the period July 1999-June 2001. The red line represents Ngerenya and the blue line represents Chonyi.



The highest prevalence (3%) was found in children 5 years of age from Chonyi. The age specific *P. ovale* parasite prevalence was below 1% in all the age groups in Ngerenya except the 11-14 year olds where there was a peak of almost 2%. *P. ovale* parasite prevalence rates were 1.4% and 0.3% in children 1-9 years of age in Chonyi and Ngerenya

respectively. Figure 4.20 demonstrates the *P. ovale* parasite prevalence rates among children 1-9 years old from Chonyi and Ngerenya.

Figure 4.20: *P. ovale* parasite prevalence rates among children aged 1-9 years of age in all six cross-sectional surveys from Chonyi and Ngerenya.



Key: ** - statistically significant difference between Chonyi and Ngerenya ($p < 0.05$).

There was a consistently higher *P. ovale* parasite prevalence rate in Chonyi compared to Ngerenya and this difference was statistically significant in the surveys conducted in July 1999 (1.54% Vs 0.26 %, $\chi^2=6.67$, $p=0.01$) and October 2000 (1.56% Vs 0.27%, $\chi^2=6.04$, $p=0.01$).

Mixed infections were not common in either study area. The prevalence of mixed *P. malariae* and *P. falciparum* infections was 0.5% in Chonyi and 0.2% in Ngerenya whereas

the prevalence of mixed *P. ovale* and *P. falciparum* infections was 2.1% in Chonyi and 1% in Ngerenya. The prevalence of mixed *P. falciparum*, *P. ovale* and *P. malariae* infections was 0.09% in Chonyi and 0.05% in Ngerenya. There were no mixed infections that involved *P. malariae* and *P. ovale*. Table 4.4 demonstrates the differences between the observed and the expected numbers of mixed infections in the two study areas.

Table 4.4: Differences between the observed and expected numbers of mixed infections from Chonyi and Ngerenya

Type of mixed infection	CHONYI			NGERENYA		
	Observed	Expected	p-value	Observed	Expected	p-value
<i>P.falciparum</i> & <i>P.malariae</i>	76	37	<0.001	21	8	0.02
<i>P.falciparum</i> & <i>P.ovale</i>	18	12	0.3	5	2	0.5
<i>P.falciparum</i> & <i>P.malariae</i> & <i>P.ovale</i>	4	0.5	0.4	1	0.03	0.9

Key: * - the Chi-squared test used if expected numbers were larger than 5, if the number expected was less than 5, then the fishers exact test was used.

There were more cases of mixed *P. falciparum* and *P. malariae* infections than was expected in both study areas. However, the number of mixed infections that included *P. falciparum* and *P. ovale* infections and all three infections together (*P. falciparum*, *P. malariae* and *P. ovale*) were not any more than was expected for this population.

4.5.4 Relationships between parasitaemia at cross-sectional surveys and malaria treatment.

Since repeat cross-sectional surveys were conducted among the same study participants, there was the possibility that being treated for malaria in between the surveys would alter the parasitaemia status in the subsequent cross-sectional survey. Being parasitaemic at any survey was found to be associated with the being parasitaemic in the subsequent survey as shown on Table 4.5.

In Chonyi, those that had a parasitaemia in any survey were about three to five times more likely to be parasitaemic in the subsequent survey after controlling for age and treatment

between surveys ($p < 0.001$). In Ngerenya, those that had parasitaemia in any survey were about two to five times more likely to have parasitaemia in the subsequent survey after controlling for both age and treatment between surveys ($p < 0.05$).

Table 4.5: Unadjusted odds ratios of parasitaemia status in one survey compared to the subsequent survey in both Chonyi and Ngerenya in the period July 1999- June 2001.

Cross-sectional survey	Chonyi	Ngerenya
*March 2000	4.2 (2.8 – 6.3) $p < 0.001$	1.6 (1 – 2.6) $p = 0.05$
July 2000	4.7 (3.2 – 6.8) $p < 0.001$	3.2 (1.9 – 5.1) $p < 0.001$
October 2000	3.3 (2.3 – 4.7) $p < 0.001$	4.3 (2.9 – 6.5) $p < 0.001$
March 2001	3.2 (2.2 – 4.6) $p < 0.001$	3.9 (2.7 – 5.9) $p < 0.001$
June 2001	5.3 (3.6 – 7.8) $p < 0.001$	5.2 (3.5 – 7.7) $p < 0.001$

NB: *- Comparing this survey with previous survey, March 2000 compared to July 1999, July 2000 compared to March 2000 and so on

Table 4.6 shows the odds of being parasitaemic after receiving malaria treatment since the previous survey. The first survey (July 1999) was unique in that by then the Chonyi cohort had been under surveillance (both active and passive case detection) for only nine weeks whereas the Ngerenya cohort had been under surveillance for over 40 weeks therefore, more malaria treatments had been offered to the study participants from the Ngerenya cohort compared to the Chonyi cohort.

In Ngerenya, contrary to what was expected, those treated for malaria between a preceding survey and the subsequent survey were more likely to be parasitaemic than those that did not receive treatment during this period. Those treated for malaria were at least two times more likely to be parasitaemic in the subsequent survey compared to those not treated after

controlling for age and parasitaemia in the preceding survey. In all but one survey (March 2000), there was evidence that this difference was statistically significant ($p < 0.01$).

Table 4.6: Relationship between malaria treatments on parasitaemia in subsequent cross-sectional surveys conducted in both Chonyi and Ngerenya in the period July 1999 – June 2001.

*Cross-sectional survey	Chonyi	Ngerenya
July 1999	1.18(0.83 – 1.69) p=0.3	1.72 (1.22 – 2.43) p<0.000
March 2000	0.43 (0.29 – 0.65) p<0.000	1.09 (0.68 – 1.74) p=0.7
July 2000	1.2 (0.74 - 1.9) p=0.5	1.72 (1.13 – 2.63) p=0.01
October 2000	0.68(0.47 – 0.99) p=0.05	1.66 (1.1 – 2.48) p=0.01
March 2001	0.83 (0.54 – 1.27) p=0.4	2.18 (1.48 – 3.21) p<0.000
June 2001	1.57 (0.83 – 2.96) p=0.2	2.5 (1.56 – 4.03) p<0.000

NB: *- Treatments occurred before these cross-sectional survey

Results given as unadjusted odds ratio with the confidence interval and p-values

In half of the surveys in Chonyi, those that were treated for malaria in the period after the preceding survey were less likely to have parasitaemia in the subsequent survey after controlling for age and being parasitaemic in the preceding survey. For the other half of the survey, those treated after preceding surveys were more likely to be parasitaemic in the subsequent survey however, there was no evidence of a statistically significant association. The surveys in which treatment was found to be protective against parasitaemia were those in which the subsequent survey was in the dry season which are periods of low transmission. This included the two surveys in March and the October survey. Treatments for malaria in the wet season months of June and July when malaria transmission was high either did not make a difference to the subsequent parasitaemia or lead to an increase in the likelihood of being re-infected.

We also examined whether there was an increased risk of being treated for malaria in the subsequent period of follow-up if the person was parasitaemic at the cross-sectional survey. For this analysis, data of those with fever during the cross-sectional survey was removed. Analysis was conducted separately for those under five and over five as the highest incidence of disease in both areas were in children under five years of age as described on Table 4.7.

Table 4.7: Relationship between parasitaemia and malaria treatment in subsequent follow-up period in both Chonyi and Ngerenya in the period July 1999 – June 2001.

Survey after which odds for treatment were calculated	Ngerenya		Chonyi	
	< 5 years old	≥ 5 years old	< 5 years old	≥ 5 years old
July, 1999	5.9 (2.2 – 15.6) p<0.001	2.4 (1.6 – 3.6) p<0.001	2.7 (1.3 – 5.6) p=0.006	1.6 (1.1 – 2.3) p=0.01
March, 2000	4.2 (1.4 – 12.5) p=0.004	1.3 (0.77 – 2.1) p=0.3	2.8 (1.2 – 6.5) p=0.01	1.7 (0.9 – 2.9) p=0.05
July, 2000	7.3 (1.9 – 28.3) p<0.001	1.4 (0.9 – 2.3) p=0.3	1.6 (0.8 – 3.1) p=0.2	1.2 (0.8 – 1.9) p=0.04
October, 2000	3.9 (1.7 – 8.9) p<0.001	1.2 (0.8 – 1.9) p=0.3	3.8 (1.9 – 7.6) p<0.001	3.3 (2.1 – 5.4) p<0.001
March, 2001	4.5 (2.1 – 9.8) p<0.001	1.6 (0.9 – 2.9) p=0.09	4.7 (2.1 – 10.5) p<0.001	2 (0.8 – 5.1) p=0.1

In both Chonyi and Ngerenya, there was evidence of an increased risk of being treated for malaria if one was parasite positive during the preceding cross-sectional among age groups and including all surveys. However, this difference was not statistically significant among all the children over five years of age in Ngerenya except in the first survey (July, 1999) whereas among children of the same age group in Chonyi, the differences were statistically significant during all surveys except the last one (June, 2001).

4.6 Temperature readings.

This section describes axillary temperatures taken in the field during active surveillance for the two years of follow-up and does not include temperatures taken at the study clinic

but includes temperatures taken during cross-sectional surveys. A total of 79,268 axillary temperatures were taken in Chonyi and 79,441 taken in Ngerenya during this period.

4.6.1 Average temperatures by age

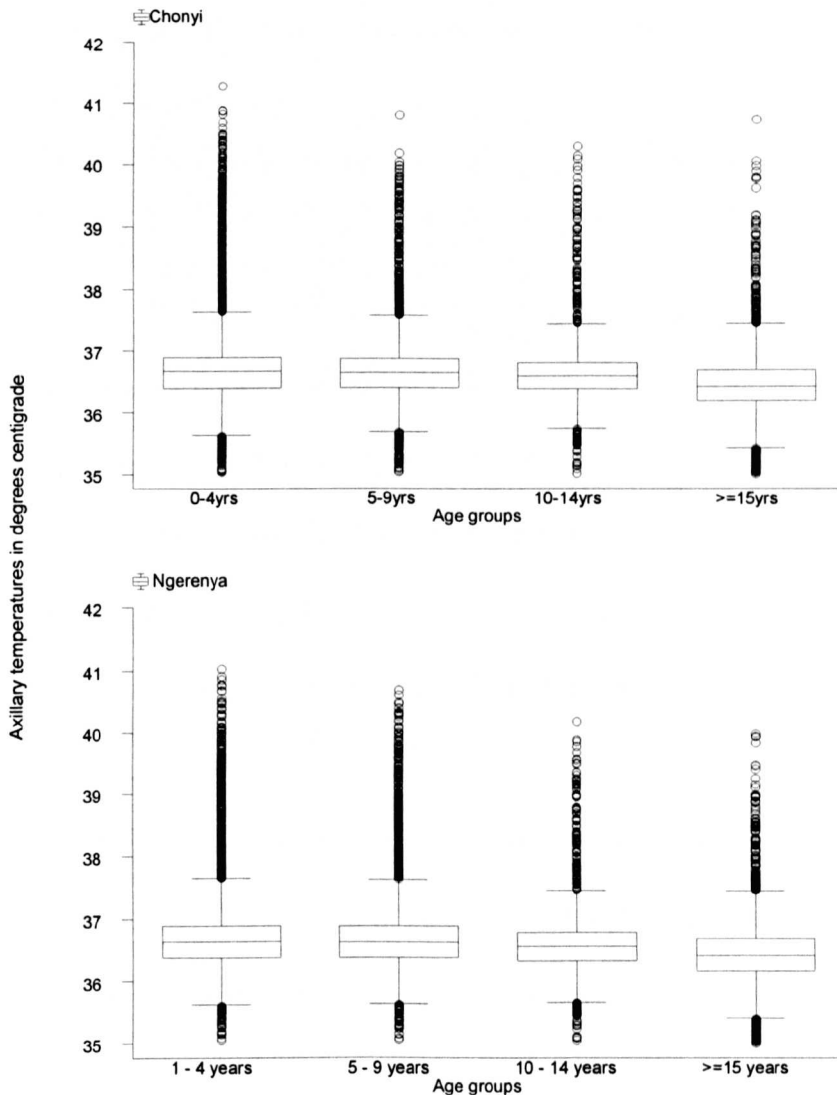
The average axillary temperature among study participants from Chonyi area was $36.6 \pm 0.4^{\circ}\text{C}$ compared to $36.6 \pm 0.5^{\circ}\text{C}$ among study participants from Ngerenya. The average temperatures by age among study participants from the two study areas are described in Table 4.8 as ranging from 36.7°C in children under the age of four years to 36.4°C in people ≥ 15 years of age. There were no differences in the mean temperatures in both areas, neither was there a difference in the mean temperatures among males and females in the different age groups.

Table 4.8: Mean axillary temperatures among children in the different age groups from active surveillance in Chonyi and Ngerenya during the period May 1999-May 2001.

Age group	Mean axillary temperature (\pm standard deviation)	
	Chonyi	Ngerenya
≤ 4 years	$36.69 \pm 0.5^{\circ}\text{C}$	$36.69 \pm 0.5^{\circ}\text{C}$
5 – 9 years	$36.65 \pm 0.4^{\circ}\text{C}$	$36.67 \pm 0.5^{\circ}\text{C}$
10-14 years	$36.59 \pm 0.4^{\circ}\text{C}$	$36.57 \pm 0.4^{\circ}\text{C}$
≥ 15 years	$36.43 \pm 0.4^{\circ}\text{C}$	$36.43 \pm 0.4^{\circ}\text{C}$

Figure 4.21 shows the range of axillary temperatures among study participants from the two study areas by age. In both study areas, there was a small reduction in the mean axillary temperatures with age. There was also a reduction of the number of those with high axillary temperatures ($>40^{\circ}\text{C}$) with age in the two study areas, the range of temperatures in older children and adults was narrower than that of younger children.

Figure 4.21: Box plot showing median (central line), 25%, 75% quartile (box width), upper and lower limits (T) and outliers (dots) of axillary temperatures taken during active surveillance in Chonyi and Ngerenya during the period May 1999-May 2001.



4.6.2 Measured fever

More than 98.2 % and 97.6% of all axillary temperatures taken during the active surveillance in Chonyi and Ngerenya respectively measured $<37.5^{\circ}\text{C}$ though the proportions in the different age groups varied as demonstrated on Table 4.9. Among children less than four years of age 96.2 % in Chonyi and 95.1 % in Ngerenya had an axillary temperature $<37.5^{\circ}\text{C}$. In both study areas, the proportion febrile (axillary

temperature $\geq 37.5^{\circ}\text{C}$) ranged from a maximum of 3.8% in those under four years of age to 0.7% in adults.

Table 4.9: Proportions in the different axillary temperature grades by age in the two study areas from active surveillance for the period May 1999-May 2001.

Temperature	Age group							
	0-4 year old		5-9 yr old		10-14 yr old		>15 yr old	
	Chonyi	Ngerenya	Chonyi	Ngerenya	Chonyi	Ngerenya	Chonyi	Ngerenya
<37	82.2%	82.6%	85.8%	83.9%	88.6%	90.8%	93.6%	93.6%
37-37.4	14.1%	13.5%	12.6%	13.2%	10.4%	7.9%	5.8%	5.7%
37.5-37.9	1.3%	1.4%	0.6%	1.1%	0.4%	0.5%	0.3%	0.3%
38-39	1.6%	1.5%	0.7%	1.2%	0.4%	0.6%	0.2%	0.3%
>39	0.8%	1%	0.3%	0.6%	0.2%	0.2%	0.1%	0.1%
*Total febrile	818	920	343	607	118	162	134	142

Key: Febrile cases were those with axillary temperature $\geq 37.5^{\circ}\text{C}$

A total of 1,831 fevers were detected through active case detection among the Ngerenya study participants of which, 698 (38 %) were between $37.5-37.9^{\circ}\text{C}$, 733 (40 %) were between $38-39^{\circ}\text{C}$, while 400 (22 %) were $>39^{\circ}\text{C}$. A total of 1,413 fevers were detected by active case detection among study participants from Chonyi of which, 542 (38 %) were between $37.5-37.9^{\circ}\text{C}$, 574 (41 %) were between $38-39^{\circ}\text{C}$, while 297 (21 %) were $>39^{\circ}\text{C}$. More than 60% of all the fevers that were detected by active case detection were $> 38^{\circ}\text{C}$. About 63% and 58% of all fevers were in children under five year of age in Chonyi and Ngerenya respectively while 9% and 8% were in those ≥ 15 years old from Chonyi and Ngerenya respectively.

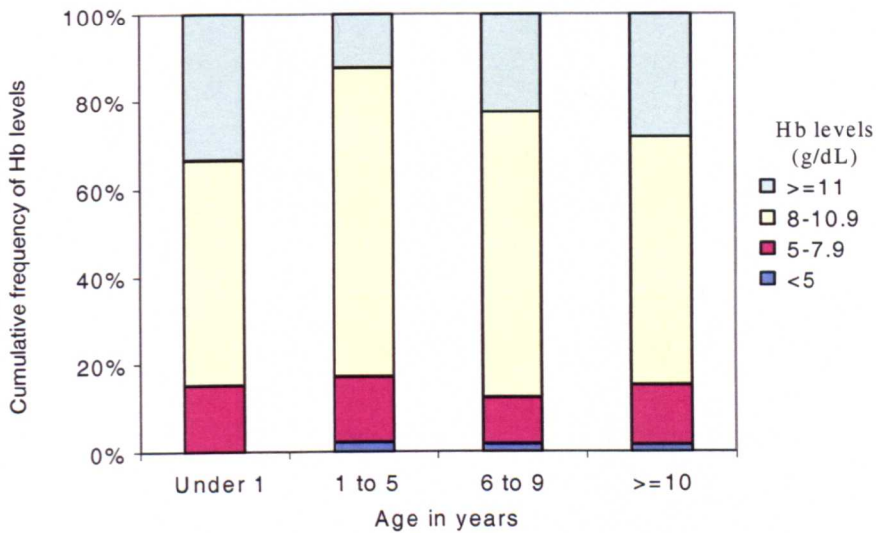
4.7 Haemoglobin status

4.7.1 Mean haemoglobin concentrations

Only one cross-sectional haemoglobin (Hb) survey was conducted among 541 people from Ngerenya in September of 1998. However, most of the Hb data available was obtained from clinical data, which was therefore not representative of the study population. A capillary blood sample was taken for measuring Hb and white blood cell counts at the

same time that the slide was made from people reporting to the study clinic with a history of fever. Figure 4.22 shows the cumulative frequency of Hb levels in all age groups from the single cross-sectional survey conducted in Ngerenya. The geometric mean Hb levels among those under five years of age was 8.9g/dL, it was 9.2g/dL among those six to nine years of age and 9.5g/dL among those that were 10 years and above. The haemoglobin levels were divided into <5g/dL indicative of severe anaemia, <8g/dL indicative of moderate anaemia and <11g/dL that was indicative of mild anaemia while Hb levels >11/dL were normal according to the WHO guidelines.

Figure 4.22: Cumulative frequency of haemoglobin levels by age from a single cross-sectional survey conducted in Ngerenya (Aug-Sept, 1998)

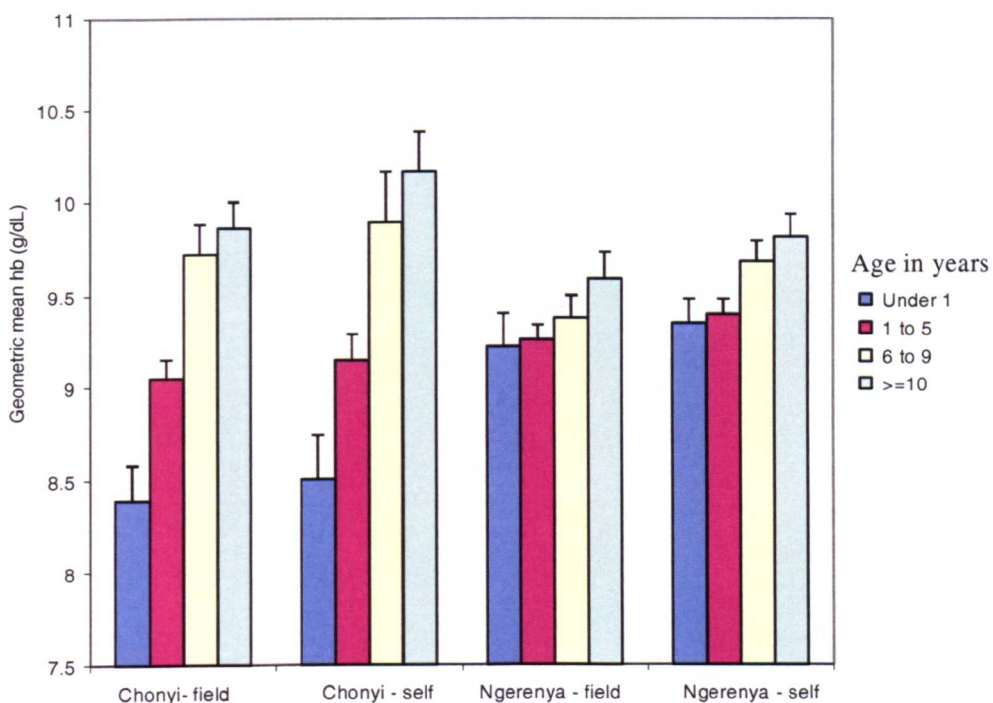


None of the children under one year of age had severe anaemia whereas 2.1% of children between one to five years of age and 1.6% of all those > 5 years of age did. Fifteen percent (15%) of children under six years of age had moderate anaemia compared to 10.6% among children aged 6-9 years old and 13.6% among those ≥ 10 years old. The majority of study participants had Hb levels of between 8-10.9%, which was considered to be mildly anaemic. The proportions were 51.5% among children under a year old, 70.6% among children aged one to five years, 65% among 6-9 year olds and 56.5% among those ≥ 10

years of age. Thirty-three percent (33%) of children under a year old had Hb levels ≥ 11 /dL, compared to 12.4% of those aged 1-5 years, 22% of those 6 to 9 years and 28.3% of those ≥ 10 years.

The next sections will be a description of data collected from the study clinic unless otherwise stated. Figure 4.23 shows the geometric mean Hb levels in the different age groups in the two study areas according to whether this person was a field or self-referral.

Figure 4.23: Geometric mean haemoglobin levels by age among study clinic attendants that were either field or self-referrals in the period May 1999-May 2001.



KEY:

Chonyi-field –Field-referrals from Chonyi attending the clinic

Chonyi-self – Self-referrals from Chonyi attending the clinic

Ngerenya-field – Field-referrals from Ngerenya attending the clinic

Ngerenya-self – Self-referrals from Ngerenya attending the clinic

The overall geometric mean haemoglobin level for Chonyi was 9.32 g/dL (95% CI 9.27-9.38) while in Ngerenya, the overall geometric mean haemoglobin level was 9.45g/dL

(95% CI 9.41-9.49). There was evidence that the median Hb levels in Ngerenya were higher than those from Chonyi ($p=0.02$). The lowest Hb levels were among children under a year old in both study areas followed by those one to five years of age.

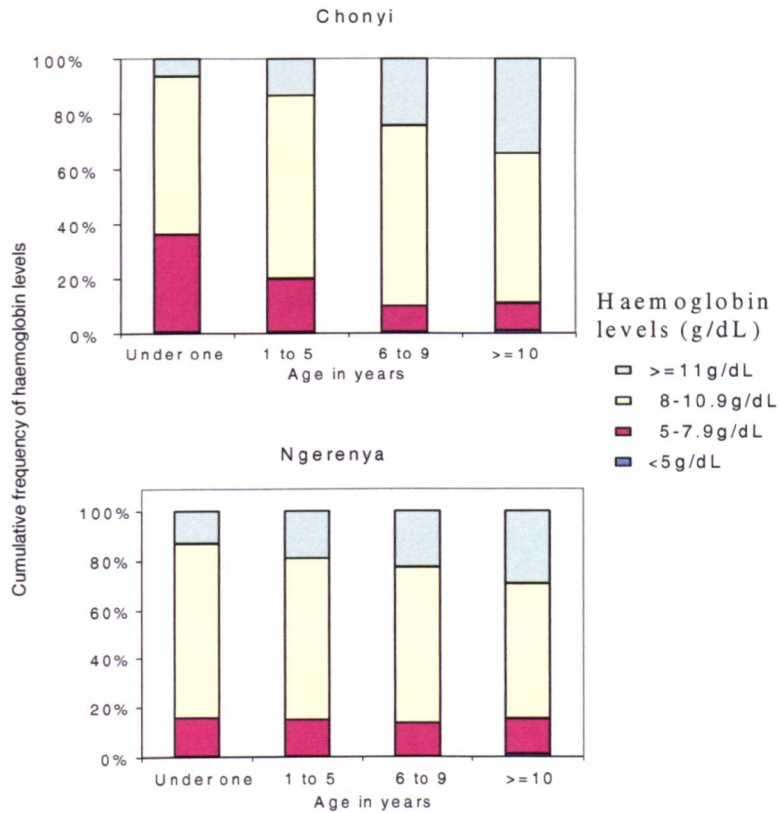
Although the haemoglobin levels were higher among those that were self referrals than field referrals in Ngerenya ($p<0.001$), there were no differences between those that were field or self-referrals in Chonyi ($p=0.1$). There were however similar age patterns in the Hb levels when comparing those that were field and self-referrals. The data from both field and self-referrals were therefore combined in all the future analysis.

Figure 4.24 shows the proportion within different haemoglobin levels in the different age groups among those that attended the study clinic. Comparisons between proportions with different haemoglobin levels were compared in both study areas. There was no evidence of a difference in the proportion with Hb levels $<5\text{g/dL}$ in the two study areas across all the age groups. There was however evidence of a higher proportion with Hb levels between $5\text{-}7.9\text{g/dL}$ in Chonyi compared to Ngerenya among those under a year old (35.3% Vs 15.2% , $\chi^2=118.1$, $p<0.001$) and those $1\text{-}5$ years old (19.2% Vs 14.5% , $\chi^2=12.6$, $p<0.001$). This relationship was however reversed in the older age groups where there was evidence of a higher proportion with Hb levels between $5\text{-}7.9\text{g/dL}$ in Ngerenya compared to Chonyi (13.4% Vs 9.5% , $\chi^2=6.6$, $p=0.01$).

There was evidence of a higher proportion of children under a year old with a Hb level $8\text{-}10\text{g/dL}$ in Ngerenya compared to Chonyi (71.2% Vs 57.6% , $\chi^2=38.9$, $p<0.001$), however, in all the other age groups, there were no differences in the proportion of people with this haemoglobin level in the two study areas. There was evidence of a higher proportion of children in Ngerenya with Hb levels $\geq 11\text{g/dL}$ among all the children under 6 years of age

($p < 0.001$), however there was no evidence of a difference in this proportions among children 6-9 years of age ($p = 0.5$).

Figure 4.24: Cumulative frequency of haemoglobin levels by age among those that attended the study clinic from Chonyi and Ngerenya in the period May 1999-May 2001.

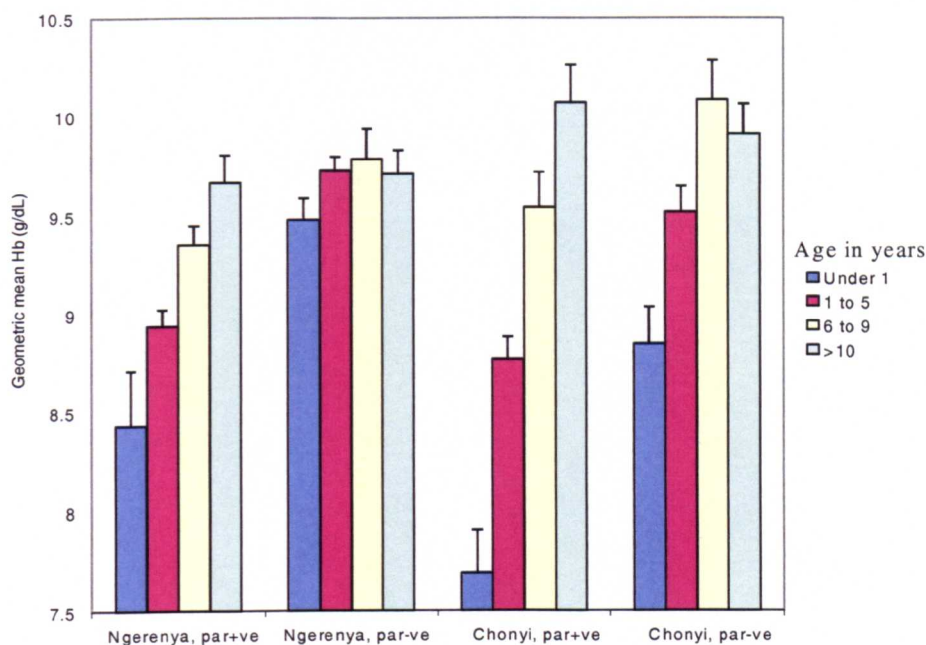


Among those ≥ 10 years of age, there was evidence of a higher proportion from Chonyi with Hb levels ≥ 11 g/dL compared to that from Ngerenya (34.1% Vs 29.4%, $\chi^2 = 4.88$, $p = 0.03$).

This section has illustrated differences in haemoglobin levels in the two areas according to age, but how much of the difference in the haemoglobin levels is a result of parasitisation?

The next section compares geometric mean parasite densities in the different age groups among those that are parasite positive and negative as illustrated on Figure 4.25.

Figure 4.25: Geometric mean Hb levels by age and parasite status among those attending the study clinic from Chonyi and Ngerenya in the period May 1999 –May 2001.



When comparing median Hb levels among those that were parasite positive in both study areas, there were lower Hb levels among children under six years of age from Chonyi compared to those from Ngerenya ($p < 0.001$). However, there was no evidence of a difference in the Hb levels among children aged 6-9 years old that were parasite positive in Ngerenya compared to Chonyi ($p = 0.08$) whereas there was evidence of a higher Hb level among those over 10 years of age that were parasite positive in Chonyi compared to Ngerenya ($p = 0.02$). There was evidence of a higher median Hb level among children under six years of age that were parasite negative from Ngerenya compared to Chonyi ($p < 0.001$) however this relationship reversed after the age of 6 where there was evidence of a higher Hb level among those that were parasite negative from Chonyi compared to Ngerenya ($p < 0.04$).

When comparing proportions within the same study area among those that were parasite negative and positive, there was evidence in both study areas of a higher median Hb level among children aged less than 10 that were parasite negative compared to those that were parasite positive ($p < 0.001$). However, among those ≥ 10 years, there was no evidence of a difference in the median Hb levels among those that were parasite positive compared to those that were parasite negative within the same study area ($p > 0.4$).

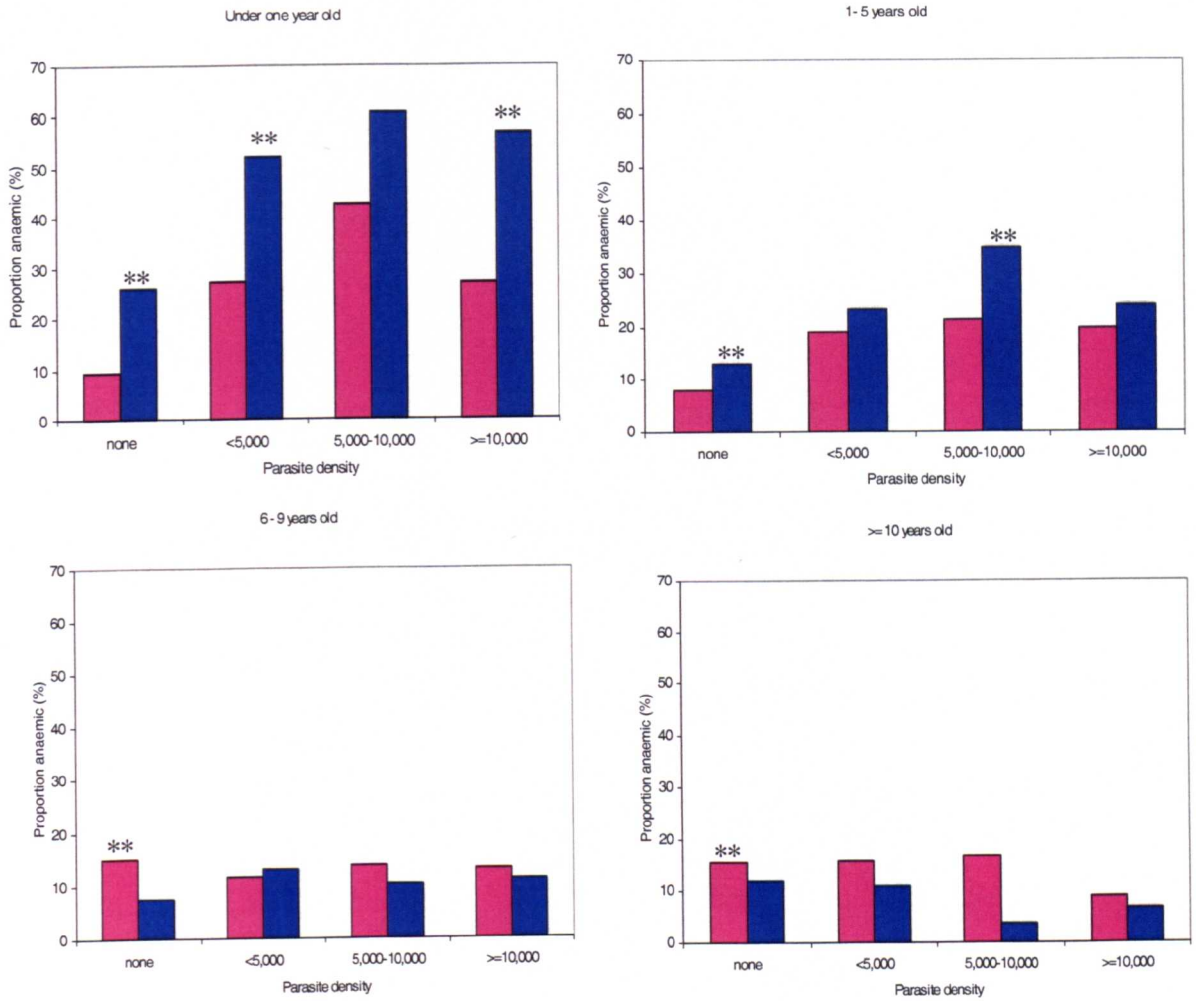
It may therefore be possible that the density of the parasitaemia may affect haemoglobin levels. In the next section, I look at the proportions with various parasite densities that had anaemia. Since there were few children with severe anaemia, I shall look at the combined proportions of those with severe and moderate anaemia and call those anaemia (anaemia=severe+moderate, anaemia=Hb<8g/dL). Figure 4.26 shows the proportions with anaemia among those with varying parasite density in the four age groups from Chonyi and Ngerenya.

Among those under a year old, there was evidence of a higher proportion with anaemia in children from Chonyi compared to those from Ngerenya except in those children with parasitaemia between 5,000-10,000 parasites/ μ l of blood. Among those 1-5 years of age, there was evidence of a higher proportion with anaemia among those from Chonyi compared to those from Ngerenya. The differences were however significant only among those with a parasitaemia of 5,000-10,000 parasites/ μ l of blood ($p = 0.2$) and borderline significance among those with a parasitaemia $\geq 10,000$ parasites/ μ l of blood ($p = 0.08$).

Among those over 6 years of age, there was evidence of a higher proportion with anaemia among those with no parasitaemia from Ngerenya compared to those from Chonyi

($p < 0.04$). Among those over 6 years of age however, there was no evidence of a difference in the proportion anaemic with varying parasite densities in the two areas ($p > 0.1$).

Figure 4.26: Proportions with anaemia (hb<8g/dL) by parasite density from Chonyi and Ngerenya. Red bars represent Ngerenya and blue bars Chonyi.



Key: ** - Statistically significant differences in the proportion anaemic comparing Chonyi and Ngerenya ($p < 0.05$).

4.8 Measures of C-reactive protein (CRP)

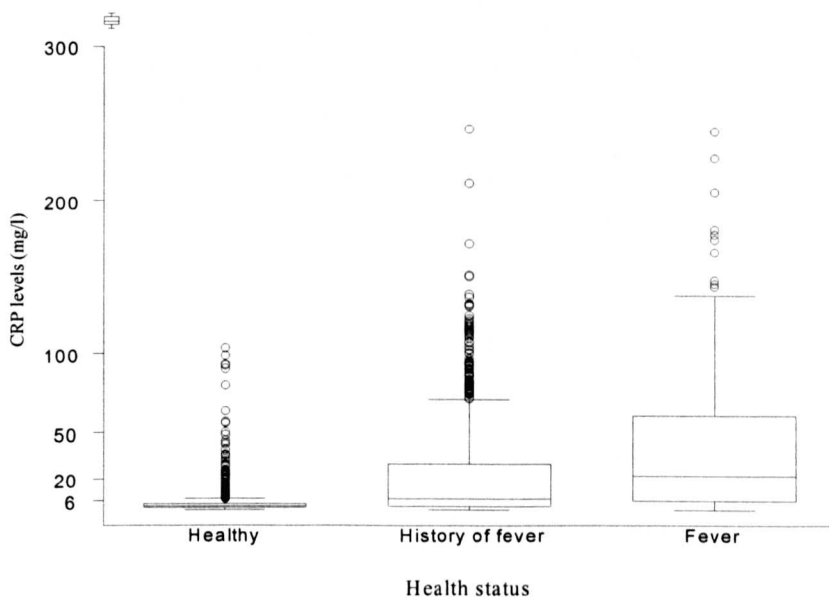
CRP measurements were taken from finger prick blood samples taken from study participants of all age groups using the ELISA technique as described in section 3.2.7. CRP levels for healthy study participants of all age groups were collected during a cross-

sectional survey carried out in October 2000. Samples for CRP measurements were also collected for a period of nine months from July 2000 to May 20001 among study participants that presented to the clinic complaining of fever. The later group provided CRP levels from people with a history of fever but with no raised body temperature and those with a raised body temperature (axillary temperature $\geq 37.5^{\circ}\text{C}$).

4.8.1 Average levels of CRP

CRP levels were classified into three categories according to the health status of the study participants as those that were healthy, those who complained of a fever but had no raised body temperature (history of fever) and those that were febrile described on Figure 4.27.

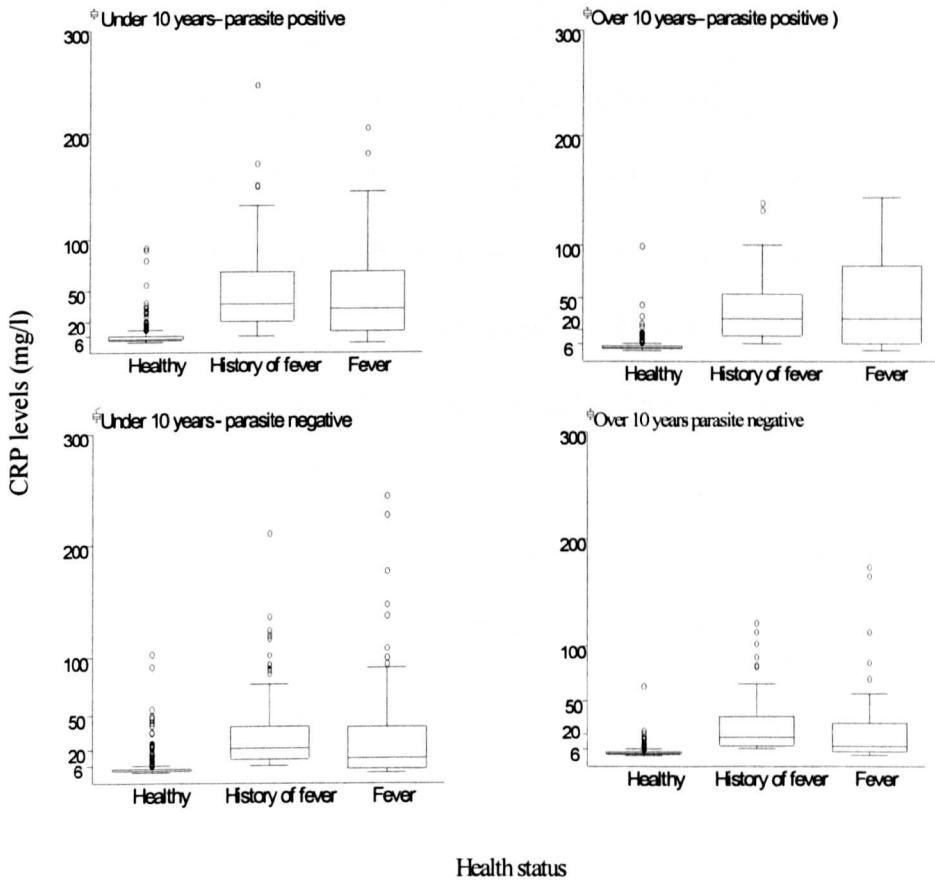
Figure 4.27: Box plot showing the median (central line), 25%, 75% quartile ranges around the median (box width), upper and lower limits (T) and outliers (dots) of CRP levels according to whether the person was healthy, had a history of fever or febrile.



The median CRP level in those that were healthy was 2.27 mg/L (IQR: 1.48 – 3.69) and those that had a history of fever had a median value of 21.9 mg/L (IQR: 5.83 – 60.43) while those febrile had a median CRP value of 27.78 mg/L (IQR: 14.16 – 55.59). When the groups were compared, there was evidence of a lower CRP level when comparing those

that were healthy and those with a history of fever ($p < 0.001$) and those with a measured fever ($p < 0.001$) and also when comparing those with a history of fever and those that were febrile ($p < 0.001$). This may however have been different if parasite positivity and age were considered as illustrated on Figure 4.28.

Figure 4.28: Box plot showing median (central line), 25%, 75% quartile ranges around the median (box width), upper and lower limits (T) and outliers (dots) of CRP levels among those parasite positive or negative among those <10 years and those ≥ 10 years of age.



Among the healthy, whatever the age group and parasitaemia status, the median CRP was < 3 mg/L and the upper inter-quartile range (IQR) did not go beyond 5mg/L. In those that had a history of fever, the median CRP was > 30 mg/L in those that were parasite positive whatever the age compared to 16 mg/L in those that were ≥ 10 years of age and 21 mg/L in

those that were <10 years of age that were parasite negative. Among those that had a measured fever, the median CRP level was >30 mg/L in those that were parasite positive whatever the age group. However, the median CRP level was 8 mg/L in those ≥10 years of age and 13 mg/L in those <10 years of age among the parasite negatives.

In both age groups, there was evidence of a higher CRP value among those that were parasite positive compared to those that were parasite negative whether they were healthy ($p<0.001$), had a history of fever ($p<0.001$) or had a raised body temperature ($P<0.03$).

4.8.2 Raised CRP levels

A cut-off of 6 mg/L was chosen as a cut-off for raised CRP levels. This cut-off has been used before in other studies (Hurt *et al.*, 1994) and it was also observed in this study that most of the healthy people had CRP levels below this cut-off. About 11.9% (159/1336) of those that were well (no history of fever or measured fever) during the cross-sectional survey had a CRP level greater than 6 mg/L while 52.5% (508/967) of those with a history of fever and 74.1 % (286/386) of those that were febrile had a CRP level >6 mg/L.

After controlling for age, those with parasitaemia were 2.4 times more likely to have raised CRP levels (6mg/L) than those without parasitaemia [Adjusted odds ratio 2.4 (CI: 1.1 – 5.4, $p<0.001$)]. Among children under 10 years old with fever, children with or without parasitaemia were 14 times more likely to have a raised CRP than healthy children ($p<0.001$). Among those over 10 years of age, those without parasitaemia were 16 times more likely to have raised CRP [Adjusted odds ratio 16.1(CI: 6.9 – 37.1, $p<0.001$)] whereas those with parasitaemia were 25 times more likely to have raised CRP [Adjusted odds ratio 25.2(CI: 7.6 – 83.1, $p<0.001$)] compared to their healthy counterparts.

4.9 Conclusions and discussions

There were several differences demonstrated between study participants from Chonyi and Ngerenya that may be associated with the level of transmission, these include differences in parasite prevalence rates and differences in haemoglobin levels among children of different age groups.

There was a higher prevalence of *P. falciparum* parasitaemia among children 1-9 years of age in Chonyi compared to Ngerenya (40.6% Vs 24.5%, $\chi^2 = 121.3$, $p < 0.001$). However, there was an equal *P. falciparum* gametocyte prevalence rate in the two areas but a higher pigment rate among those from Ngerenya compared to Chonyi (1.6% Vs 0.8%, $\chi^2 = 3.3$, $p = 0.07$) though the difference was of borderline significance. The higher parasite rate in Chonyi compared to Ngerenya was expected as study participants from Chonyi are exposed to a higher entomological inoculation rate than those from Ngerenya.

There was also a higher prevalence rate of *P. malariae* and *P. ovale* among children 1-9 years of age from Chonyi compared to Ngerenya [(*P. malariae* = 4.2% Vs 1.5%, $\chi^2 = 28.4$, $p < 0.001$) & (*P. ovale* = 1.3% Vs 0.3%, $\chi^2 = 14.1$, $p < 0.001$)]. This again demonstrates that the study participants from Chonyi are exposed to more mosquito bites than those from Ngerenya. There was a higher observed mixed *P. falciparum* and *P. malariae* infection prevalence in both study areas compared to what was expected. There were however no differences between the observed and expected mixed infections with *P. falciparum* and *P. ovale* or the combination of all three species (*P. falciparum*, *P. malariae* and *P. ovale*). A study was conducted by Lowe (1997) in northern study area (containing the Ngerenya study area), and he found that there was a higher than expected number of mixed infections of both *P. falciparum* and *P. malariae* as well as *P. falciparum* and *P. ovale*. This study did not show an increased observed rate of the later mixed infection however, in this study

and Lowe's study, there were no mixed *P. malariae* and *P. ovale*. Lowe (1997) also found that the presence of *P. falciparum* and *P. malariae* was associated with a protective effect against clinical episodes of malaria.

Among children ≤ 5 years of age, there was a higher proportion with haemoglobin levels $<8\text{g/dL}$ in Chonyi compared to Ngerenya (24% Vs 15.3%, $\chi^2 = 70.6$, $p < 0.001$). This relationship was present irrespective of parasitaemia status at the time of examination. However, this relationship was reversed in older children. Among children 6-9 years of age, there was a higher proportion with a haemoglobin level $<8\text{g/dL}$ among children from Ngerenya compared to those from Chonyi (13.9% Vs 10.2%, $\chi^2 = 5.4$, $p = 0.02$). However, among these older children the relationship was statistically significant only among those children that did not have any parasitaemia. It appears that haemoglobin levels were not altered as much by increasing parasite density in older children as they were in the younger children. Younger children are more prone to anaemia in the face of malaria parasitaemia than older children and therefore it's likely that younger children exposed to higher transmission are likely have lower haemoglobin levels than those exposed to lower transmission.

CHAPTER FIVE

DEFINING AND QUANTIFYING NON-SEVERE MALARIA IN KILIFI

5.1 Introduction

Malaria control strategies currently in place (e.g. bednets) and future interventions (e.g. malaria vaccines) that are aimed at reducing morbidity and mortality need careful evaluation in order to assess their effectiveness. To measure morbidity, a clear definition of clinical malaria is needed (Armstrong-Schellenberg *et al.*, 1994). In areas where malaria is not endemic, clinical malaria would be defined as a history of fever accompanied with peripheral parasitaemia. However, in malaria endemic areas sometimes close to 80% of the general 'asymptomatic' population between 1-9 years of age have peripheral parasitaemia (Greenwood *et al.*, 1987). In such areas, the presence of parasitaemia and clinical symptoms does not necessarily imply clinical malaria (Greenwood *et al.*, 1987). The occurrence of fevers due to other conditions may be confused with clinical malaria due to the accompanying parasitaemia. On the other hand, febrile childhood illnesses such as influenza and measles may lower the parasite density to non-detectable levels even when these diseases are accompanied by clinical malaria (Rooth and Björkman, 1992). All these factors contribute to the complication of malaria diagnosis in endemic areas.

Malaria case definitions in the past have relied on a history of fever and a peripheral parasitaemia above a certain threshold (Armstrong-Schellenberg *et al.*, 1994). This was based on the assumption that high level parasitaemias are more likely to cause fever than lower level parasitaemias as described in section 2.3.2.2.1. However such cut-offs may lead to a degree of misdiagnosis that is not quantifiable (Rougement *et al.*, 1991). The fraction of fevers attributable to malaria can be used to calculate the number of fevers that would be

eliminated if malaria was eradicated (Greenwood *et al.*, 1987). This classical method of calculating the fraction of fevers attributable to parasitaemia compares the proportion febrile that have parasitaemia and the proportion afebrile with parasitaemia. In areas of high malaria endemicity, this method fails as most of the afebrile population will have parasitaemia (Armstrong-Schellenberg *et al.*, 1994). To overcome this, Smith *et al.* (1994) have used logistic regression to model the risk of fever with parasite density as described in section 2.3.2.2.3. This method can also estimate the sensitivity and specificity of various parasitaemia cut-offs as used in case definitions. Using logistic regression models, definitions have been derived for malaria among people in different age groups in areas of differing malaria transmission as described in Table 5.1. These definitions are derived from various studies designed either as descriptive studies or intervention trials (Armstrong-Schellenberg *et al.*, 1994; Smith *et al.*, 1994; Alonso *et al.*, 1994; D'Alessandro *et al.*, 1995a; Menendez *et al.*, 1997; McGuinness *et al.*, 1998; Bloland *et al.*, 1999; Vounatsou *et al.*, 2000; Whitworth *et al.*, 2000).

These studies were conducted in areas across Africa with differing transmission (EIR's ranging from 8.5 to 300) and were conducted using varying methodology. Most of the studies were a mixture of cross-sectional surveys and passive or active case detection of fevers. In one study only cross-sectional data were used (Armstrong-Schellenberg *et al.*, 1994) whereas in another only health facility data were used (Whitworth *et al.*, 2000). There appeared to be differences in the malaria case definitions with age in the studies where age-specific definitions were computed (Smith *et al.*, 1994; McGuinness *et al.*, 1998; Bloland *et al.*, 1999, Vounatsou *et al.*, 2000). However all estimates of sensitivity and specificity were at least 80% when reported.

Table 5.1: Sensitivities and specificities of various malaria case definitions by age in various sites within Africa.

Reference	Study area	Study type	EIR	Age	Parasites/ μ l of blood +axillary temperature $\geq 37.5^{\circ}\text{C}$	Specificity (%)	Sensitivity (%)
Armstrong-Schellenberg <i>et al.</i> , 1994	The Gambia	Weekly visits + cross-sectional surveys		Under 10yrs	$\geq 5,000$	90	90
Smith <i>et al.</i> , 1994	Tanzania (Ifakara)	Cross-sectional surveys only	300	Under 6yrs Under 1 yr	$\geq 5,000$ Any parasitaemia	80 97	80 100
VAlonso <i>et al.</i> , 1994	Tanzania (Idete)	Passive detection and intermittent weekly visits +cross-sectional surveys	300	1-5 years	$> 20,000$	82	83
V D'Alessandro <i>et al.</i> , 1995a	The Gambia	Twice weekly visits + Cross-sectional surveys	150	6 – 11 months	6,000	86	86
Menendez <i>et al.</i> , 1997	Tanzania (Ifakara)	Passive case detection + Cross-sectional surveys	300	Under 1 year	Any parasitaemia	88	100
McGuinness <i>et al.</i> , 1998	Ghana (Prampram)	Local health centre + Fortnight visits	8.5	Under 1yr 1-2yrs	≥ 100 $\geq 3,400$	90 80	90 80
Bloland <i>et al.</i> , 1999	Kenya – Siaya	Monthly visits	270	≤ 14 years	$\geq 4,000$	82.3	81.1
Whitworth <i>et al.</i> , 2000	Uganda	Local clinic – routine 3 monthly visits		Adult – HIV-ve Adult – HIV+ve	$> 1,250$ $> 1,250$	98 98	51 84
Vounatsou <i>et al.</i> , 2000	Tanzania (Idete)	Dispensary cases + Cross-sectional surveys	300	Under 6 months 6 months-1 yr	Any parasitaemia $\geq 10,000$	80	

NB: EIR= Infective bites per person per year

All descriptive studies except: 'v' - SPf66 vaccine trial and 'l' - Iron supplementation trial

The main aim of this chapter is to attempt to define and quantify clinical malaria in two areas with differing transmission among people of different age groups in Kilifi.

5.2 Materials and methods

This section describes the logistic regression approach to the calculation of attributable fractions and sensitivities and specificities of various parasite density cut-offs as used in malaria definitions. The later part of this section defines the terms that were used to quantify malaria within the study area.

5.2.1 Data collection

Data collected through longitudinal and cross-sectional surveys conducted in Ngerenya and Chonyi from May 1999 to May 2001 were used in this analysis. The people recruited into the study were visited weekly for the duration of the study with regular cross-sectional surveys in the dry and wet season as described in sections 3.24 and 3.25. Axillary temperatures were taken from all the study participants during two years of weekly visits. Smears were made from all of the study participants with a history of fever or a measured fever either in the field or in the study clinic. All records were double entered into a database (FoxPro[®] version 2.5) and both entries cross-checked for errors.

5.2.2 Data analysis

Data analysis was conducted using STATA software, version 7.0 Most of the data analysis was age-stratified. Age of the individual changed with time during the course of the study and the age at the time of data collection was used in most of the analysis except in the calculation of the period prevalence and cumulative total episodes per person, where age at entry to the study was used instead.

5.2.2.1 Calculation of attributable fractions

An individual with an axillary temperature $\geq 37.5^{\circ}\text{C}$ reported either through active or passive case detection was defined as a case. If an individual had a fever on two consecutive weeks, only the first episode was considered as a case and the second week's data discarded from the analysis. An individual who was well and afebrile at the time of the cross-sectional survey was defined as a control.

Both the classical and the logistic regression methods were compared in the calculation of the fraction of fevers attributable to parasitaemia in Chonyi and Ngerenya. The classical attributable fraction was calculated using equation 2.6 as described in section 2.3.2.2.2. (Armstrong-Schellenberg *et al.*, 1994). Logistic regression was used to calculate the fraction of fevers due to parasitaemia as demonstrated in equation 2.7

$$\text{Log } P / (1 - P) = \alpha + \beta x^{\iota}$$

Where 'P' is the risk of fever ' α ' (log odds of fever among those with no parasitaemia), ' β ' (increase in log odds of fever with unit increase in x^{ι}) were computed conditional on the parameter ' ι '. The parameter ' ι ' was defined as the power function of parasitaemia that best described the fever risk as a continuous function of parasite density (x). The maximum likelihood estimates of the three parameters ' α ', ' β ' and ' ι ' were calculated and are shown on Table 5.2. Initially these estimates were calculated for the two areas separately but it was observed that the difference was mainly within the age groups and not between the areas and therefore data from the two areas was combined to derive these estimates.

Table 5.2: Maximum likelihood estimates for the parameters ‘ α ’, ‘ β ’ and ‘ ι ’ that were used to estimate attributable fractions by logistic regression.

Age group	ι	α (SE)*	β (SE)*
Under 1	0.3	0.267 (0.81)	0.1384 (0.019)
1 to 5 yrs	0.4	-0.5177 (0.044)	0.0442 (0.002)
6 to 9 yrs	0.8	-1.2018 (0.0598)	0.0013 (0.00008)
10 to 14 yrs	0.7	-1.8166 (0.086)	0.0044 (0.00037)
≥ 15 yrs	0.6	-2.2469 (0.077)	0.01172 (0.001111)

Key: *- SE = Standard error

Each of the estimates varied with age, which demonstrated the change in the fever, parasite density relationship with age in this study area.

5.2.2.2 Estimating sensitivity and specificity of parasite density cut-offs of malaria case definitions

Using the method described by Smith *et al.* (1994), various parasite density cut-offs were considered as case definitions. In deriving sensitivity and specificity estimates of various malaria case definitions, a case was defined as a fever accompanied by a parasite density above a cut-off ‘ c ’, and the total cases of malaria using the cut-off were ‘ n_c ’ (total number of fevers with a parasite density above the cut-off, ‘ c ’). A variable was derived for each of the cut-offs ($d_{c,i}$) categorized as ‘ $d_{c,i}$ ’ =1 if an individual ‘ i ’ had a parasite density above the cut-off or ‘ $d_{c,i}$ ’ =0 if the parasite density in individual ‘ i ’ was below the threshold. The proportion of cases that was attributable to parasitaemia using a particular threshold ‘ c ’ was termed as ‘ λ_c ’ and was calculated as:

$$\lambda_c = 1/n_c \sum_i d_{c,i} (R_i - 1) R_i$$

R_i being the relative risk of fever as described in section 2.3.2.2.3. The number of fevers with parasitaemia above the cut-off ‘ c ’ were ‘ $n_c \lambda_c$ ’ while the number of cases incorrectly classified (non-fevers with parasite density above the cut-off) were ‘ $n_c(1-\lambda_c)$ ’. Since the actual number

of fevers attributable to parasitaemia were ' $N\lambda$ ' and the numbers of non-malaria fevers were ' $N(1-\lambda)$ ', it was possible to calculate the sensitivity and specificity of each parasite density cut-off for detecting malaria attributable fevers as described: Sensitivity = $n_c\lambda_c / N\lambda$ and Specificity = $1 - n_c(1-\lambda_c) / N(1-\lambda)$. Using sensitivity and specificity curves, the parasite density cut-off for defining malaria with the lowest number of false positive and false negatives was obtained. Age was expected to modify malaria case definitions and therefore age-specific parasite density cut-offs were derived.

5.2.2.3 Comparing case definitions using different data sets

As demonstrated in Table 5.1, various methods of data collection are used in the attempt to derive malaria definitions in various sites across Africa. Since there were various data sets collected for this study, these different methods were compared. Comparisons were made between age-specific attributable fractions and parasite cut-offs for malaria case definitions. Table 5.3 is a summary of the three data sets, the differences in the definition of cases and controls, the amount of data and period of data collection. The first data set described in these comparisons as the 'full' data set is that described in section 5.2.2.1. A case was defined as an axillary temperature $\geq 37.5^\circ\text{C}$ while a control was an individual with no raised body temperature or history of fever during the cross-sectional surveys.

The second data set involved the use of raised C-reactive proteins (CRP) as a proxy marker of fever alongside raised body temperatures. This data set involved all individuals from whom CRP measures were available. CRP samples were collected from a single cross-sectional survey and random samples collected from people with a history of fever during an eleven month period from July 2000 to May 2001. In this data set, a case is defined as an individual with an axillary temperature $\geq 37.5^\circ\text{C}$ or raised CRP levels (described as $\text{CRP} > 6\text{mg/L}$

according to section 4.8.2). A control was an individual without a raised body temperature and with CRP levels below 6mg/L during the cross-sectional survey. There were 1,336 controls (670 from Ngerenya and 666 from Chonyi), 386 with an axillary temperature $\geq 37.5^{\circ}\text{C}$ (221 from Ngerenya and 165 from Chonyi) and 508 with a CRP $> 6\text{mg/L}$ (281 from Ngerenya and 227 from Chonyi).

The third set of data set comprised data from the six cross-sectional surveys conducted from the period July 1999 to June 2001 as described on section 3.2.5. About 98% of the people were afebrile during the cross-sectional surveys.

Table 5.3: Description of various data sets and the definitions of case and control for each set of data.

Data set	Case				Controls			
	Definition	Period of data collection	Amount of data collected		Definition	Period of data collection	Amount of data collected	
			Cho	Nge			Cho	Nge
Full data set	Axillary temperature $\geq 37.5^{\circ}\text{C}$	May 1999-May 2001	1,668	2,351	Afebrile + well	Six x-sect surveys	3,113	2,803
CRP data	Axillary temperature $\geq 37.5^{\circ}\text{C}$ &/or CRP $> 6\text{mg/L}$	July 2000-May 2001	392	502	Afebrile &/or CRP $\leq 6\text{mg/L}$	One x-sect survey (Oct, 2000)	666	670
Cross-sectional survey data	Axillary temperature $\geq 37.5^{\circ}\text{C}$	Six x-sect surveys	125	162	Afebrile + well	Six x-sect surveys	4,269	4,260

Key: Cho – Chonyi ; Nge – Ngerenya; x-sect - Cross-sectional

Age attributable fractions of fever were calculated using the two smaller data sets and compared to those using the full data set. A non-parametric test (Kruskal-Wallis χ^2 tests) were used to compare the attributable fractions in the three sets of data as the ANOVA assumptions of normal distribution and equal variance were not met from this data set. Sensitivities and

specificities of case definitions derived using these data were calculated and compared to those derived from the full data set.

5.2.2.4 Quantifying malaria and fever in the two study populations

This section describes various methods used to quantify malaria and fever within the study population. Two estimates were used to quantify disease; the incidence rate and period prevalence. Cumulative numbers of episodes per person for all study participants and the time to first episode for newborns were also measured.

5.2.2.4.1 Incidence rate

Since the study participants were visited on a weekly rather than a daily basis, some fevers associated with parasitaemia may have been missed. Therefore incidence rate estimates in this analysis were lower than that which would have been calculated if the study involved daily rather than weekly visits. Incidence was estimated using fever or malaria cases detected by both active and passive case detection.

$$\text{Incidence rate} = \frac{\text{Number of episodes of disease during the study period}}{\text{Total number of person years of risk}}$$

The incidence rate was therefore the number of detected episodes of fever or malaria per person year. If fever and malaria occurred in two consecutive weeks in the same person, then only the first episode was counted as a malaria case. Poisson regression was used to estimate the association between malaria incidence and study area after controlling for age using Incidence Rate Ratios (IRR).

5.2.2.4.2 Period prevalence

In this study, the period prevalence was defined as the proportion of people that had malaria (at least once) during the period of the study. Age at entry to the study was used and only one episode per person was counted. For example if the number of children aged one year were 'n' and 'd' of them got malaria at least once in the course of the study, then the period prevalence of malaria for those a year old would be 'd/n'. This is the proportion of people in a particular age group that had malaria in the course of follow-up. These proportions were compared using chi-squared tests.

5.2.2.4.3 Cumulative malaria episodes

Among the different age groups, there were varying numbers of total episodes that an individual experienced over the period of follow-up. Using age at entry, the cumulative number of episodes per person over the period of follow-up was calculated and summarized for the different age groups. The mean number of episodes in each age group in the two study areas was compared using t-tests to find out if any of the observed differences were statistically significant.

5.2.2.4.4 Time to first episode

All data from newborns recruited into the study was used in this analysis. Time to first episode in the two study areas was demonstrated using a Kaplan-meier survival curve. The logrank test was used to compare survival time in the two groups and to test the hypothesis that the two sets of newborns from Ngerenya and Chonyi were from the same population.

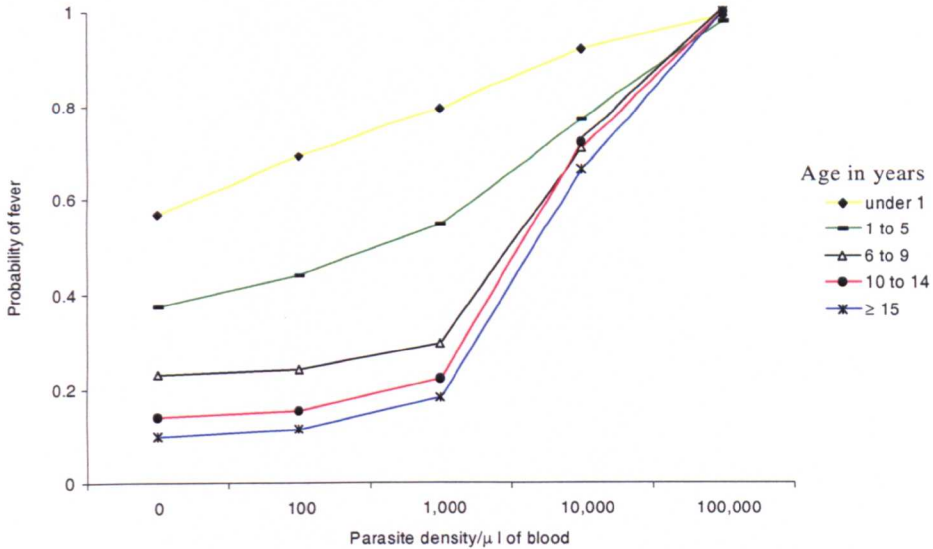
5.3 Results

In this section, I will describe the fraction of fevers attributable to parasitaemia in the different age groups, deriving case definitions and quantifying malaria in the two study areas. There will also be a comparison with data from people treated for malaria in a government dispensary in Ngerenya and study participants from the same area to see whether the age and seasonal patterns of malaria diagnosis in the local health centre were comparable to those within this study.

5.3.1 Defining malaria

During the two years of follow-up, 2,650 fevers were detected in Ngerenya and 1,924 in Chonyi through both active and passive surveillance. If there were no parasitaemia data for a fever either because the person failed to turn up at the clinic or because a person refused to have a smear taken, those fever data were removed from the analysis. In Ngerenya 128 (4.8%) fevers occurred <14 days apart and 171(6.5%) did not have parasitaemia data. In Chonyi, 64 (3.3%) fevers were <14 days apart and 192 (10%) did not have parasitaemia data. Therefore out of the fevers detected, 2,351 (88.7%) from Ngerenya and 1,668 (86.7%) from Chonyi were included in the analysis to calculate the malaria attributable fevers. For this 'full' data set, there were 2,803 and 3,113 controls from Ngerenya and Chonyi respectively. Figure 5.1 demonstrates the probability of fever with increasing parasite density in the different age groups from the two study areas.

Figure 5.1: The probability of fever with increasing parasite density in different age groups in Kilifi



The probability of fever in children under a year old was higher than all other age groups whatever the parasite density. The figure illustrates the pattern of increasing risk of fever with small increases in parasite density in those under six years of age and the tendency for the older age groups to have small changes in the probability of fever with low parasite densities but sharp increases in the rates of fever with high parasite densities.

5.3.1.1 Attributable fractions

Attributable fractions were calculated using both the classical and logistic regression methods. Using the classical approach, the overall fraction of fevers due to parasitaemia in Ngerenya was 49% and 41% for Chonyi. Calculation of the attributable fraction using the classical approach is demonstrated in Figure 5.2.

Figure 5.2: Classical approach to the calculation of the fraction of fevers attributable to parasitaemia in the sample population of Chonyi and Ngerenya.

Ngerenya			Chonyi		
	Fever	Control		Fever	Control
Parasitaemia +ve	1,400	566	Parasitaemia +ve	1,014	1,057
Parasitaemia -ve	951	2237	Parasitaemia -ve	654	2,056
	2,351	2,803		1,668	3,113

$P = 1400/2351 = 0.5955$	$P = 1014/1668 = 0.6079$
$OR = 1400 \cdot 2237 / 951 \cdot 566 = 5.82$	$OR = 1014 \cdot 2056 / 654 \cdot 1057 = 3.016$
$AF = 0.5955(5.82 - 1/5.82) = 0.49$	$AF = 0.6079(3.016 - 1/3.016) = 0.4063$

Key: P=proportion with parasitaemia among those that are febrile
OR= Odds ratio
AF = Attributable fractions

There was no evidence of a difference in the proportion of people with fever that had parasitaemia when comparing Chonyi and Ngerenya (59.6% vs 60.8%; $\chi^2 = 0.63$, $p=0.4$). However, there were more people with parasitaemia among the afebriles in Chonyi than there were in Ngerenya (34% vs 20%; $\chi^2 = 140.3$, $p < 0.001$). This difference resulted in the higher fraction of fevers attributable to parasitaemia in Ngerenya compared to Chonyi.

Using the logistic regression approach, the fraction of fevers attributable to parasitaemia in Ngerenya was 50.2% (95% CI: 48.6-51.7%) whereas in Chonyi it was 47.9% (95% CI: 46.1 – 49.4%). The fraction of fevers calculated using the classical approach and logistic regression approach for Ngerenya produced very similar estimates whereas for Chonyi, the estimate was lower using the classical approach than it was when using the logistic regression approach. Age comparisons of the fraction of fevers attributable to parasitaemia for the two areas were made using both the classical and the logistic regression approach in Table 5.4.

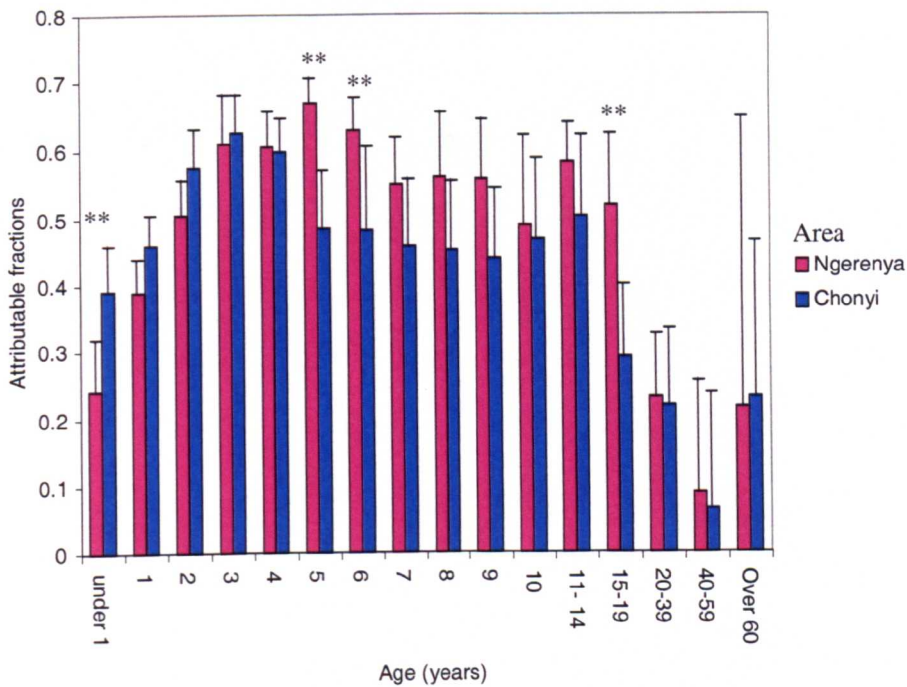
In Chonyi, the malaria attributable fractions of fever calculated using the logistic regression approach was higher than that calculated using the classical approach except in children a year old and younger. The differences in the malaria attributable fraction of fevers calculated using the logistic regression as compared to the classical approach suggests that the classical method under-estimates the fraction of fevers due to parasitaemia more in Chonyi than Ngerenya and this is probably due to the higher levels of asymptomatic parasitaemia in Chonyi compared to Ngerenya. The highest differences between the fractions of fever calculated by the two methods were found in those children aged 5-8 years. However, the differences were small (<3.3%) in children under five years of age. In children from Ngerenya malaria attributable fraction calculations using the classical approach resulted in higher estimates of the attributable fractions than the use of the logistic regression method.

Table 5.4: Age comparisons of fraction of fevers attributable to parasitaemia using both the classical and logistic regression approach in both Chonyi and Ngerenya.

Age group (in years)	Classical approach (%)		Logistic regression approach (%)		Difference (logistic- classical- %)	
	Chonyi	Ngerenya	Chonyi	Ngerenya	Chonyi	Ngerenya
Under one	38.9	24.6	39.1	24.2	0.2	-0.4
1	49.2	39.8	46	38.7	-3.2	-1.1
2	53.9	45.4	57.6	50.4	1.5	5
3	58.8	67	62.5	61.1	3.3	-5.9
4	57.7	63.1	59.7	60.6	2.0	-2.5
5	27.4	72.6	48.3	66.7	20.9	-5.9
6	36.5	65.2	48.1	62.7	11.6	-2.5
7	38.8	54.7	45.6	55	6.8	0.3
8	18.9	60.9	45.2	56.1	26.3	-4.8
9	36.2	58.7	43.9	55.7	7.7	-3.0
10	39.6	39.8	46.8	48.8	7.2	9.0
11 – 14	42.7	60.4	50	58.1	7.3	-2.3
15- 19	14.8	49.4	29.1	51.6	14.3	2.2
20 – 39	21.3	18.9	22	23.2	0.7	4.3
40 – 59	-2.6	4.2	6.5	8.9	9.1	4.7
Over 60	18.4	13.4	23.2	21.6	4.8	8.2

T-tests were used to compare the attributable fractions among people of the same age group for Chonyi and Ngerenya. Figure 5.3 compares the attributable fraction of fevers in the two study areas and shows the age groups for which the fraction of fevers due to malaria were different in the two areas. There were no differences in the overall attributable fraction of fevers for the two areas ($p=0.1$). Among those under a year old, there was evidence that there were more children with fevers due to parasitaemia in Chonyi than Ngerenya although the difference had borderline statistical significance ($p=0.05$).

Figure 5.3: Logistic regression estimates of fraction of fevers attributable to parasitaemia by age in Chonyi and Ngerenya.



Key:

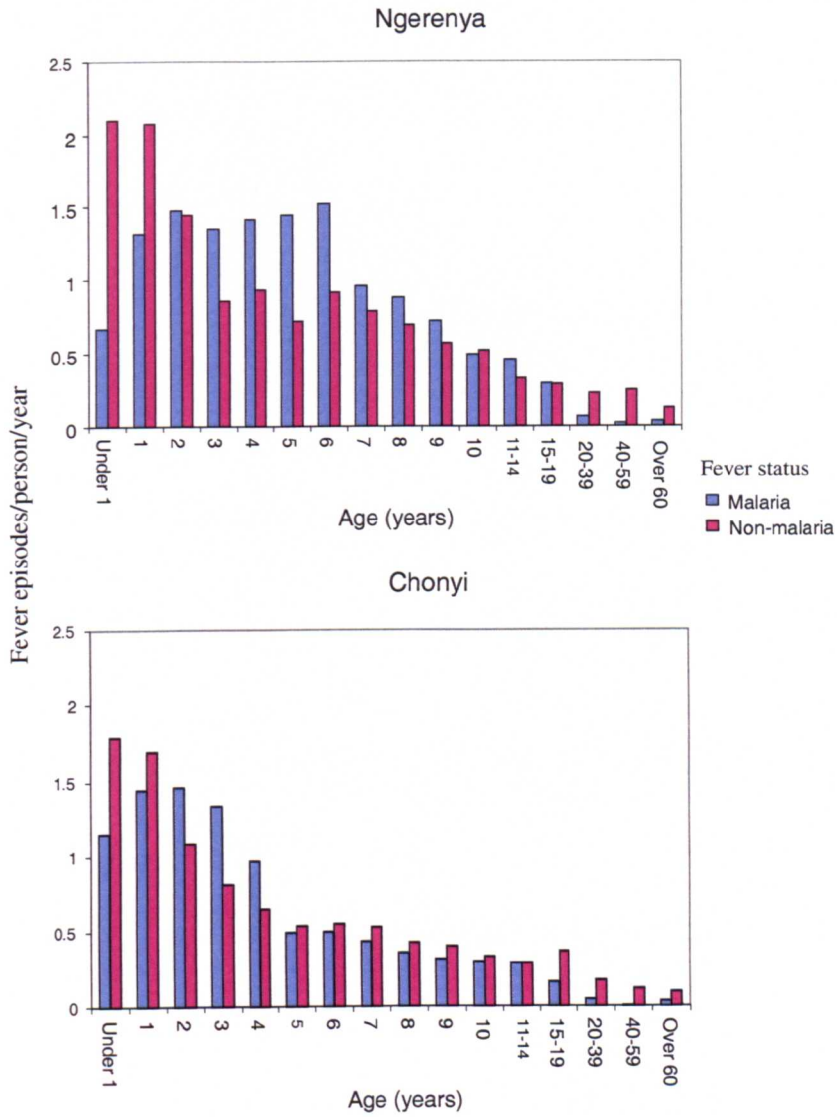
** = malaria attributable fractions that were statistically significantly different in the two areas (t-test, $p < 0.05$).

There was no evidence of a difference in malaria attributable fevers in the two areas between the ages of 1-4years. However, there was evidence of more fevers attributable to parasitaemia in Ngerenya than Chonyi among children 5-6years of age ($p<0.01$) and adults 15-20years of age ($p=0.01$).

Figure 5.4 shows the estimated numbers of malaria and non-malaria fevers by age among study participants from Chonyi and Ngerenya. The number of malaria fevers was calculated by taking all the observed fevers and applying the derived fraction attributable to parasitaemia, those fevers not attributable to parasitaemia were therefore defined as non-malaria fevers. There were differences in the numbers of non-malaria fevers in children ≤ 1 year old between the two study areas, there were more fever cases in Ngerenya than Chonyi. Considering that the age distribution of participants in both study areas was similar (section 4.1), there appears to be a higher burden overall of febrile illness conditions in children ≤ 1 year old in Ngerenya compared to Chonyi.

Between the ages of 2 to 11 years in Ngerenya and 2 to 4 years in Chonyi, there were more malaria than non-malaria fevers but after these ages, there appears to be almost equal numbers of malaria and non-malaria fevers in both study areas. After the age of 20 years in both study areas, most of the fevers were non-malaria.

Figure 5.4: Estimated numbers of malaria and non-malaria fevers by age among study participants from Ngerenya and Chonyi during two years of follow-up.



5.3.1.2 Comparison of attributable fraction estimates calculated using different data sets

Age-specific attributable fractions were calculated using the CRP data set and the cross-sectional data set (section 5.2.2.3). The contribution of individuals with a raised CRP level to the number of cases was significant in both study areas. There were 502 individuals defined as cases in Ngerenya, of whom 281 (56%) did not have a raised body temperature but had raised

CRP levels. Whereas in Chonyi, out of the 392 cases, 227 (58%) did not have a raised body temperature but had raised CRP levels.

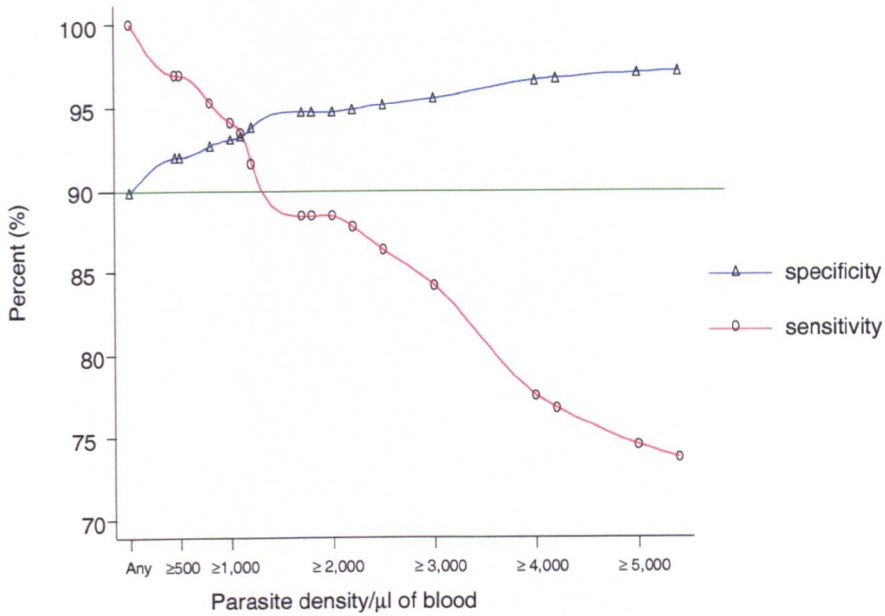
Due to the small numbers of fevers during cross-sectional surveys, there were wide confidence intervals on the age attributable fractions this was especially so in those >20 years of age. There were however few differences in the attributable fraction estimates when these two sets of data were compared to the 'full' data set. In Ngerenya, the malaria attributable fraction of fever estimate was lower when calculated using the cross-sectional data set compared to the CRP or 'full' data set in children three years of age ($p=0.05$) and in children six years of age ($p=0.03$). There was however no evidence of a difference in the estimates of the malaria attributable fraction of fevers in all three sets of data for all the other age groups.

In Chonyi, there was a difference in the attributable fraction of fevers due to malaria in those under year old with the 'full' data set having a lower estimate than the other two data sets ($p=0.005$). However, there were no other differences in the three data sets that were noted among people from Chonyi.

5.3.1.3 Sensitivities and specificities of various parasite density cut-offs

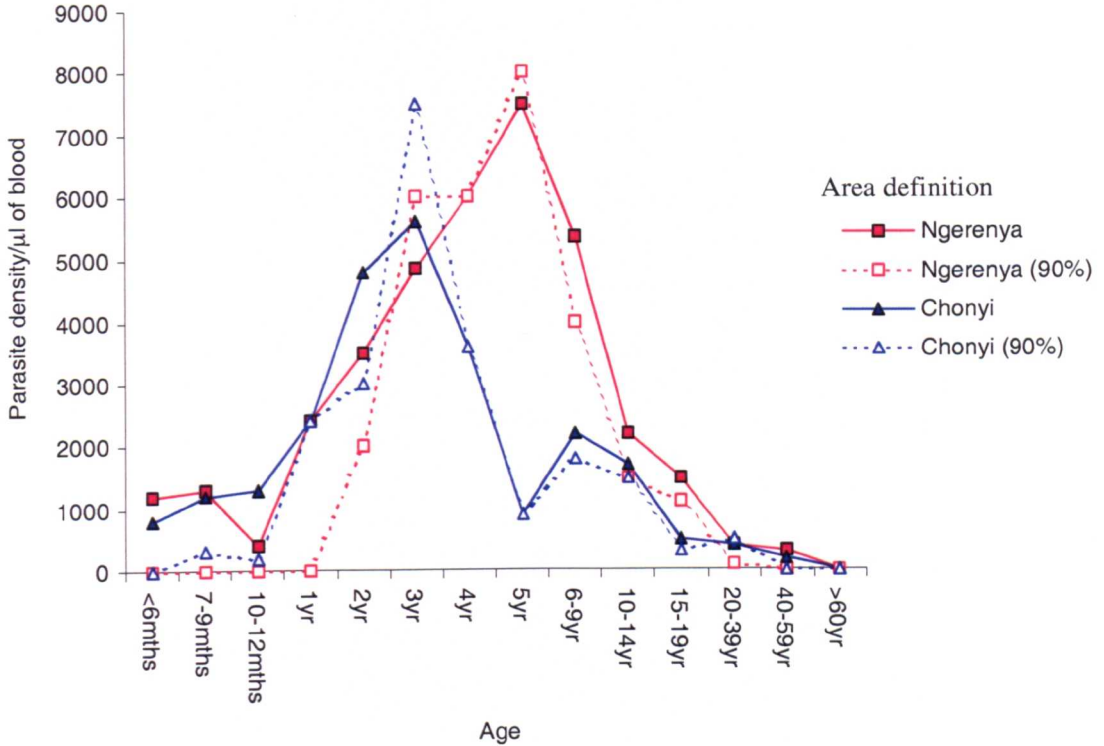
Malaria attributable fractions are useful in defining the amount of malaria at the population level but at the individual level, this will not give information as to whether a particular individual case of fever was due to parasitaemia or not. This requires case definitions that use fever and parasite density cut-offs. This section discusses parasite density cut-offs used in malaria definitions derived from logistic regression methods and their sensitivities and specificities. Figure 5.5 is an example of sensitivity and specificity estimates using different parasite densities cut-offs for defining malaria in children under a year old from Chonyi.

Figure 5.5: Sensitivity and specificity estimates of using different parasite density cut-offs for malaria case definitions for children under a year old from Chonyi.



From this figure, a parasite density cut-off of 1,100 if used to define malaria in children under a year old will have a sensitivity and specificity of 92%, which is the cut-off with the fewest false positives and false negatives. The green line shows the 90% mark and an alternative case definition would be one with a specificity of 90%. In this case of under one year olds in Chonyi, the use of fever accompanied by any level of parasitaemia as a case definition would result in a diagnosis with a specificity of 90% and a sensitivity of 100%. Sensitivity and specificity estimates were calculated for various ages groups in the two study areas and the data summarized on Figure 5.6. Two parasite density cut-offs have been selected. The best-case definition was a parasite density cut-off which when used in a malaria case definition had the lowest numbers of false negatives and false positives. An alternative was a parasite density cut-off which when used in a malaria case definition had a specificity of at least 90%.

Figure 5.6: Parasite density cut-offs in malaria case definitions by age in study participants from Chonyi and Ngerenya (solid lines refer to definitions with the highest sensitivity and specificity, dashed lines refer to definitions with a specificity $\geq 90\%$).



In Ngerenya, the parasite cut-off for defining malaria was 500-parasites/ μl of blood (with a sensitivity and specificity of 96%) in the under one year olds peaking to 7,500 parasites/ μl of blood (with a sensitivity and specificity of 91%) at the age of six years. The specificity of using any parasitaemia in malaria definitions remained at least 90% among those ≤ 1 year old. The parasite density cut-offs for malaria definition started to drop by the age of 6-9 years to less than 1,000 parasites/ μl of blood by the age of 20 years at which point any parasitaemia as a cut-off for definition had a specificity of at least 90%.

In Chonyi, the parasite density cut-off for malaria case definition at the age of six months was 1,100-parasites/ μ l of blood (with a sensitivity and specificity of 93%) and peaks to 5,600-parasites/ μ l of blood (with a sensitivity and specificity of 87%) at three years of age. The specificity of using any parasitaemia with a fever remains at least 90% for those below one year of age. The parasite cut-off for malaria case definition dropped after the age of three years to less than 1,000 parasites/ μ l of blood by the age of five years. As in Ngerenya, by the age of 20 years, the use of any level of parasitaemia in case definitions had a specificity of at least 90%.

Figure 5.6 also illustrates that the peak parasite cut-off for malaria case definitions occurred at a younger age group in Chonyi compared to Ngerenya. While it peaked at the age of five years in Ngerenya, it had already dropped to less than 1,000 parasites/ μ l of blood by the same age in Chonyi. These age-specific parasite density cut-offs for malaria definitions demonstrated in Figure 5.6 were compared to commonly used parasite density cut-offs. Using the gold standard of the number of fevers that were attributable to parasitaemia as derived using logistic regression as shown on Table 5.4, the absolute number of fevers that would be attributed to parasitaemia was calculated. For each of the other parasite cut-offs, the number of fevers attributable to parasitaemia was calculated for children ≤ 5 years old.

Table 5.5 shows that the use of fever with any parasitaemia as a malaria case definition would diagnose all fevers attributable to malaria in children under a year old in both areas. This definition would however have a low specificity in children over a year old and would diagnose 60-100 children that did not have fever attributable to parasitaemia as malaria cases, which equates to about a 16% over-estimate of disease episodes.

Table 5.5: The numbers of fevers attributable to parasitaemia that would be detected by using various parasite density cut-offs as part of the malaria case definition in children ≤ 5 years from Chonyi and Ngerenya.

Age group	Area	*Total MAF	Total fevers attributable to malaria using different definitions				
			∞ Age-specific	$\Phi > 40$	$\Omega 1,000$	$\Omega 2,500$	$\Omega 5,000$
Under one	Ngerenya	65 (54 – 76)	73	75	71	57	52
	Chonyi	109 95 - 127	113	126	114	102	86
1- 5 yrs	Ngerenya	634 609 - 668	627	734	689	657	610
	Chonyi	465 442 - 482	458	526	500	465	435

Key:

*- Estimate of the total fevers attributable to malaria with a range according to the confidence intervals of the malaria attributable fractions

∞ -Age-specific definitions as demonstrated in Figure 5.6

Ω -Commonly used parasite density cut-offs for case definitions

$\Phi > 40$ is the microscopy limit and therefore implies any level of parasitaemia

A definition using 1,000-parasites/ μ l of blood would capture all the fevers attributable to parasitaemia for children under a year old in Ngerenya. However, it would over-estimate malaria cases in both Chonyi and Ngerenya in children 1-5 years old. In Chonyi, a definition using 2,500-parasites/ μ l of blood would capture all the fevers attributable to parasitaemia in the two age groups in both areas. Use of a cut-off of 5,000-parasites/ μ l of blood as a malaria case definition would capture most of the fevers attributable to parasitaemia in children over a year old but would under-estimate fevers due to malaria in children under a year old.

From Table 5.5, two definitions for malaria may be used for both Ngerenya and Chonyi. Fever and any parasitaemia would be a useful definition in those under one year old from both study areas as it captures all the fevers attributable to parasitaemia. For children between one to five

years, a malaria definition of fever and a parasitaemia $\geq 2,500$ -parasites/ μl of blood could be used as it captures most fevers that are attributable to parasitaemia. From Figure 5.6, one would extrapolate that parasite cut-offs that would be useful in children under the age of a year old ought to be the same as those used in adults. Table 5.6 shows the sensitivity and specificity of using two definitions in people of different age groups from the two study areas.

Table 5.6: Sensitivity and specificity estimates of using two parasite density cut-offs in malaria case definitions in different age groups of people from Chonyi and Ngerenya.

Age	Cut-off Parasitaemia + axillary temps $\geq 37.5^{\circ}\text{C}$	Ngerenya		Chonyi	
		Sens. (%)	Spec. (%)	Sens. (%)	Spec. (%)
Under one	Any parasitaemia	100	95	100	90
1 to 5 yrs	$\geq 2,500$ par/ μl of blood	95	89	91	89
6 to 9 yrs	$\geq 2,500$ par/ μl of blood	95	88	91	92
10 to 14 yrs	$\geq 2,500$ par/ μl of blood	90	93	88	93
≥ 15 yrs	Any parasitaemia	100	87	100	82

Key: Sens - sensitivity Spec : specificity

If the definition of any parasitaemia and fever were used as a malaria case definition in those under a year old, about 5% false negatives would be identified. In those over 15 years of age, a definition of any parasitaemia and fever as a malaria case definition would result in a false negative rate of 15%. However in both age groups, all fevers attributable to malaria would be identified.

In those between 1 to 14 years, the use of a malaria case definition that includes fever and parasitaemia $\geq 2,500$ parasites/ μl would lead to a false positive rate of 5-10% and a false negative rate of about 7-18%. For epidemiological studies in Kilifi, these definitions could be used as, they provide adequate sensitivity and specificity estimates in the different age groups for the two areas.

5.3.1.4 Comparison with definitions derived differently

This section compares parasite density cut-offs that would be derived using two different data sets (as described in Table 5.3) and their sensitivity and specificity estimates. Table 5.7 shows the parasite density cut-offs that were derived as malaria case definitions in the different age groups using the two sets of data. Sensitivity and specificity of these case definitions within the data were also estimated.

Table 5.7: Parasite cut-offs and their sensitivity and specificity estimates when used as malaria case definitions in the different age groups in Chonyi and Ngerenya using the cross-sectional and CRP data sets.

Age in years	Cross-sectional data		CRP data	
	Ngerenya	Chonyi	Ngerenya	Chonyi
Under one	Any Sn-100, Sp-98	1,000 Sn-92, Sp-92	125 Sn-97, Sp-97	1,000 Sn-94, Sp-94
1 to 5	2,300 Sn- 89, Sp-89	3,250 Sn-89, Sp-89	3,450 Sn- 87, Sp-87	2,275 Sn- 88, Sp-88
6 to 9	1,120 Sn- 87, Sp-87	4,800 Sn-84, Sp-84	2,225 Sn-87, Sp-87	1,900 Sn- 87, Sp-87
10 to 14	2,000 Sn-90, Sp-89	400 Sn-93, Sp-93	2,250 Sn-90, Sp-90	1,500 Sn- 91, Sp-91
≥15	550 Sn-90, Sp-90	Any Sn-100, Sp-97	875 Sn-92, Sp-92	400 Sn-92, Sp-92

Key: Sn – Sensitivity Sp- Specificity

The parasite density cut-offs for defining malaria derived from the CRP data from Chonyi were very similar to those derived from the full data set. However, with the use of the CRP data, the peak definitions were found in the same age groups in both areas (1 to 5 years) unlike that found using the full data set. When using the cross-sectional data, the definitions derived in those 1 to 14 were different to those derived from the full data set. The age of the peak definitions were the opposite of that found from the full data set. In this case, the peak definition occurred earlier in Ngerenya than in Chonyi. This suggests that the type of data set

used from within the same study population was likely to yield different results. Table 5.8 demonstrates the sensitivity and specificity estimates of parasite cut-offs malaria case definitions as derived from the 'full' database if used on these sets of data.

Table 5.8: Comparison of the sensitivity and specificity estimates of the selected malaria case definitions using the cross-sectional and CRP data sets

Age in years	Cut-off Parasitaemia + axillary temps $\geq 37.5^{\circ}\text{C}$	Cross-sectional data				CRP data			
		Ngerenya		Chonyi		Ngerenya		Chonyi	
		Sen	Spe	Sen	Spe	Sen	Spe	Sen	Spe
Under one	Any parasitaemia	100	98	100	88	100	96	100	93
1 to 5	$\geq 2,500$ par/ μl of blood	87	89	91	90	90	85	87	88
6 to 9	$\geq 2,500$ par/ μl of blood	77	92	95	80	86	89	84	90
10 to 14	$\geq 2,500$ par/ μl of blood	90	89	78	98	88	92	87	96
≥ 15	Any parasitaemia	100	81	100	97	100	96	100	80

Key: Sen – Sensitivity Spe- Specificity

Whatever the data set that was used, the sensitivity and specificity estimates were $>80\%$ (except in the cross-sectional data). The results from the cross-sectional data were likely to yield different results due to the small numbers of fevers. For example among those 6-9 years of age, there were 38 fevers in Ngerenya and only 15 fevers in Chonyi.

As noted in section 5.3.1.2, more than half of those defined as cases in the CRP data base did not have raised body temperature but had raised CRP level, Table 5.9 shows the differences the additional CRP data would make to the total number of disease episodes.

Table 5.9: The numbers of cases attributable to parasitaemia that would be detected in children under the age of five years using different case definitions

Area	Case	Total cases attributable to malaria using different definitions		
		*Total MAF	^Φ >40	^Ψ 2, 500
Chonyi	Fevers only	53	62	56
	Fevers &/or raised CRP	106	131	102
Ngerenya	Fevers only	57	71	58
	Fevers &/or raised CRP	106	135	105

*- Estimate of the total fevers attributable to malaria with a range according to the confidence intervals of the malaria attributable fractions

Φ - >40 is the microscopy limit and therefore implies any level of parasitaemia

Ψ - malaria case definition derived in this study for children 1-14 years old

Between 44-53% additional cases would be estimated if raised CRP data was available in addition to measured raised body temperature whatever definition of a clinical episode of malaria is used.

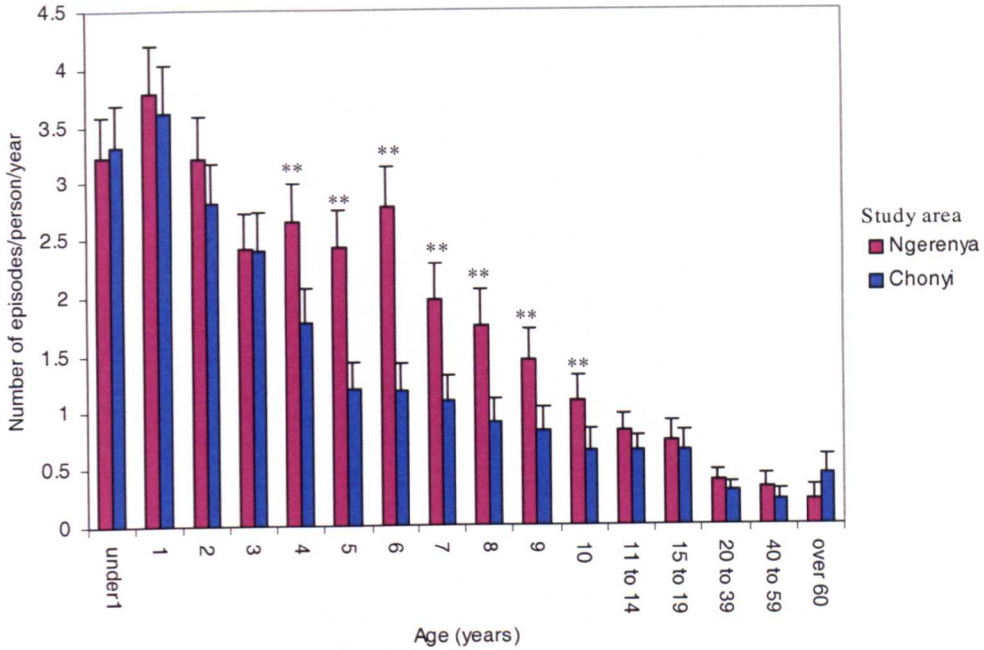
5.3.2 Quantifying malaria

This section deals with quantifying malaria in the two Kilifi study areas during the period of the study. The first section deals with quantifying malaria and the later section describes the relationship between the dispensary and study clinic diagnosis over time.

5.3.2.1 Rates of fever

A total of 2,650 and 1,924 fevers were detected in Ngerenya and Chonyi respectively by both active and passive case detection in the two years of follow-up from May 1999 to May 2001. Passive case detection accounted for 26% and 31% of all fevers detected in Chonyi and Ngerenya respectively. Figure 5.7 compares fever incidence by age in the two study areas and Poisson regression was used to compare fever incidence in the same age groups among study participants from the two study areas.

Figure 5.7: Incidence of fever in the different age groups among study participants from Chonyi and Ngerenya.



Key: ** - Difference in the incidence of fever in this age group was statistically significant ($p < 0.05$) between the two study areas.

There was evidence that study participants from Chonyi had a lower fever incidence rate compared to study participants from Ngerenya [IRR=0.72, (95%CI: 0.69 – 0.77), $p < 0.001$]. The highest incidence of fever (about 3.5 fevers/child-year) was found in children who were a year old in both study areas. The incidence of fever in the two study areas was the same up to the age of three years at which age, the incidence in both areas was 2.4 episodes/child-year. Between the age of four to 10 years, the incidence of fever was higher in Ngerenya than Chonyi with about one extra fever/child-year in Ngerenya compared to Chonyi ($p < 0.005$). Children in Ngerenya experienced at least 2 episodes of fever per child-year up to the age of seven and this dropped to 1 episode per child year by the age of 10 years. By the age of four years, children in Chonyi had less than 2 episodes of fever/child-year and this dropped to less

than 1 episode/child-year by the age of eight years. After the age of 10 years, the incidence of fever in the two study areas was the same.

Using the number of fever episodes/person/year, it is possible to estimate the total burden of fever (both malaria and non-malaria) for an individual up to the age of 60 years in the two study populations. An individual from Ngerenya would be expected to experience about 41 episodes of fever by the age of 60 years while an individual from Chonyi would be expected to experience about 31 episodes of fever by the age of 60 years. It therefore appears that overall, an individual from Ngerenya experiences a higher burden of fever in their lifetime compared to an individual from Chonyi.

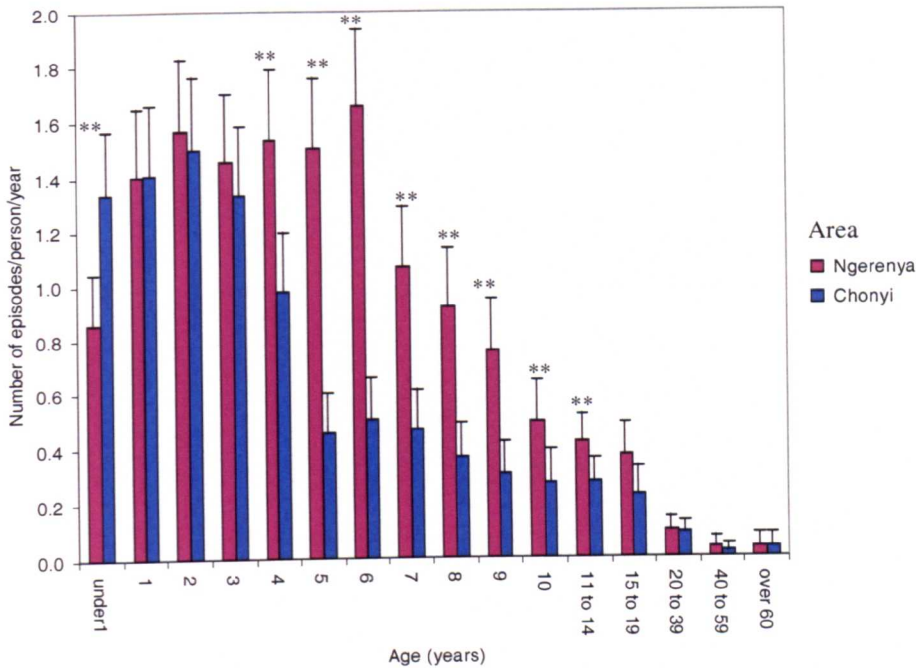
5.3.2.2 Rates of clinical malaria

The incidence of total number of malaria cases as calculated using the total attributable fevers or the use of parasite density cut-offs for malaria case definitions as described on Table 5.9 both yield the same results. Figure 5.8 however shows the age incidence of malaria using the parasite density derived case definitions. There was evidence of a lower clinical malaria incidence rate among study participants from Chonyi compared to study participants from Ngerenya [IRR=0.66, (95% CI: 0.61 – 0.72), $p<0.001$]. Only in children under the age of one year was the incidence of clinical malaria higher in Chonyi than Ngerenya. Children under a year of age in Chonyi had an incidence rate of clinical malaria 1.6 times higher than that of children in the same age group from Ngerenya [IRR=1.56, (95% CI : 1.18-2.06), $p=0.002$].

There were no differences in the incidence of clinical malaria in children from the two areas between the ages of 1 to 3 years. However, from the age of 4 to 14 years there was evidence of

a higher incidence of malaria in Ngerenya than Chonyi ($p < 0.03$). Incidence rates of clinical malaria after the age of 14 were similar in the two areas.

Figure 5.8: Age-incidence rates of clinical malaria using study derived malaria case definitions in Chonyi and Ngerenya

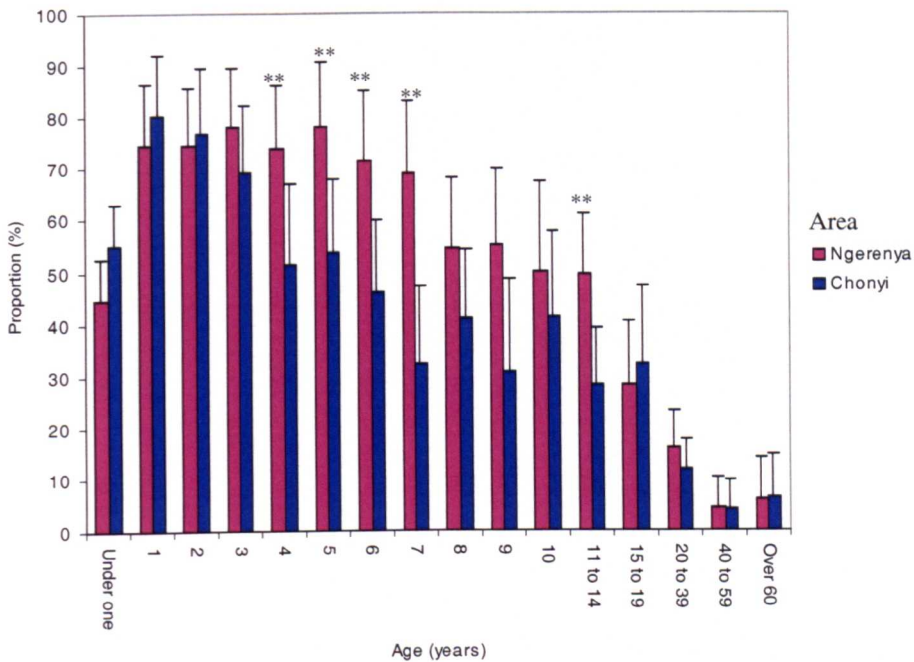


Key:**- Incidence rates that differ within the same age group in the 2 areas ($p < 0.05$).

Another way of quantifying clinical malaria was to calculate the period prevalence in each age group. Figure 5.9 shows the period prevalence of clinical malaria in the different age groups during the two years of follow-up where only one episode of clinical malaria was counted for each person. There was a higher proportion of children with at least one episode of clinical malaria in Ngerenya than Chonyi although the differences were of borderline statistical significance (54.97% Vs 44.65%, $\chi^2 = 3.3$, $p = 0.06$). It is worth noting though that newborns into each of the homes were recruited to the study at birth and therefore some of the children in this

age group may have been followed up for as long as two years and some for as little as a few weeks. This analysis did not account for the length of time that each individual was followed up. However, only among children two years and below was there a higher proportion of children with at least one episode of clinical malaria in Chonyi compared to Ngerenya.

Figure 5.9: Proportion of children with at least one episode of clinical malaria (period prevalence) in Chonyi and Ngerenya in the period May 1999 to May 2001.



Key: ** - represents differences in proportions in the two areas that were statistically significant (χ^2 test, $p < 0.05$).

Among children aged 4 to 7 years, there was evidence of a higher proportion of children with at least one episode of clinical malaria in Ngerenya compared to Chonyi. Between the ages of 8-10 years of age, there was no evidence of a difference in the proportions with clinical malaria in the two areas ($p > 0.2$). Surprisingly between the ages of 11-14 year, there was

evidence of a higher period prevalence in Ngerenya than Chonyi (49.28% Vs 28.36%, $\chi^2=6.25$, $p=0.01$). After the age of 14 years, there were no differences in period prevalence in the two study areas with age.

5.3.2.3 Cumulative episodes of clinical malaria

Using the age at entry to the study, the number of episodes experienced by each individual during the period of the study were counted and the data summarized by similar age groups. Figure 5.10 shows the cumulative frequency of multiple clinical malaria episodes for each age group in the two study areas.

No individual in Chonyi had more than nine clinical malaria episodes in the period of the study. However, in Ngerenya, between 0-12.5% of children under the age six years had ≥ 10 episodes during the course of follow-up with the highest number of repeat episodes occurring among those five years of age at the start of follow-up. Only children aged ≤ 3 years of age from Chonyi with malaria had between 6-9 episodes of malaria. Unlike in Chonyi, some children in Ngerenya as old as eight experienced between six to nine clinical malaria episodes in the course of follow-up. In Ngerenya, about 50% of children ages ≤ 6 years of age had more than 3 episodes of malaria. In Chonyi, 56% of the study participants that had clinical malaria in the course of the study had only one episode of clinical malaria whereas in Ngerenya, 38% of those with malaria had only one episode of clinical malaria.

Figure 5.10: Cumulative frequency of the number of clinical malaria episodes per person among study participants in the different age groups for the two-year period

May1999 – May 2001.

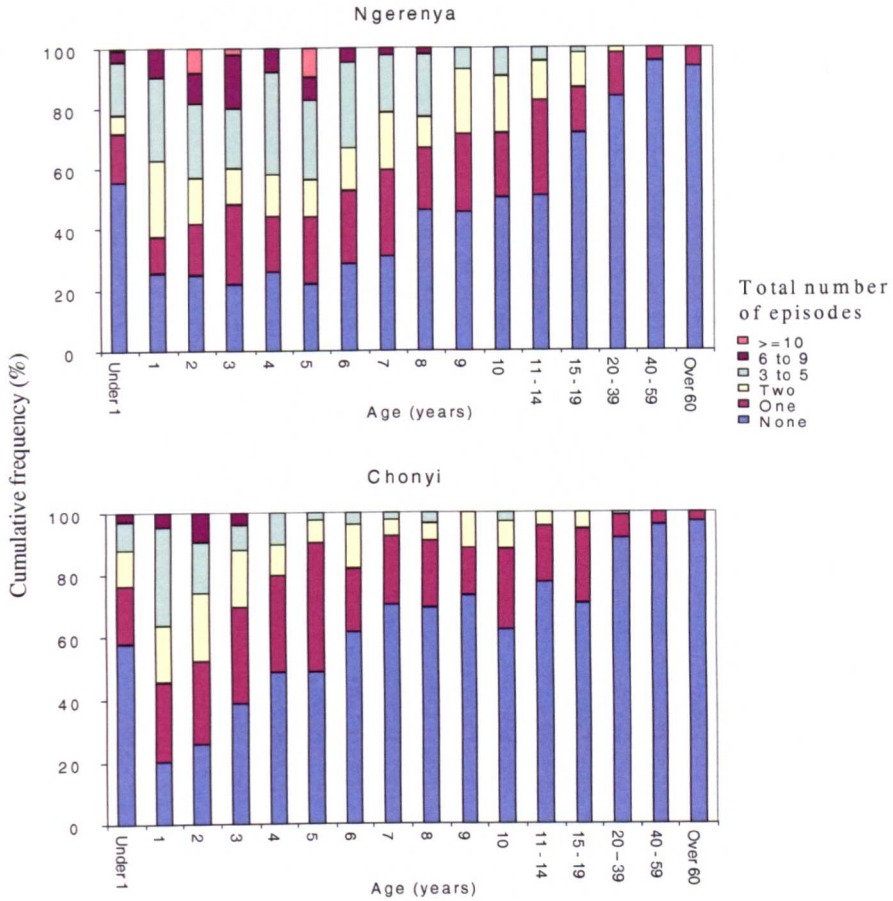
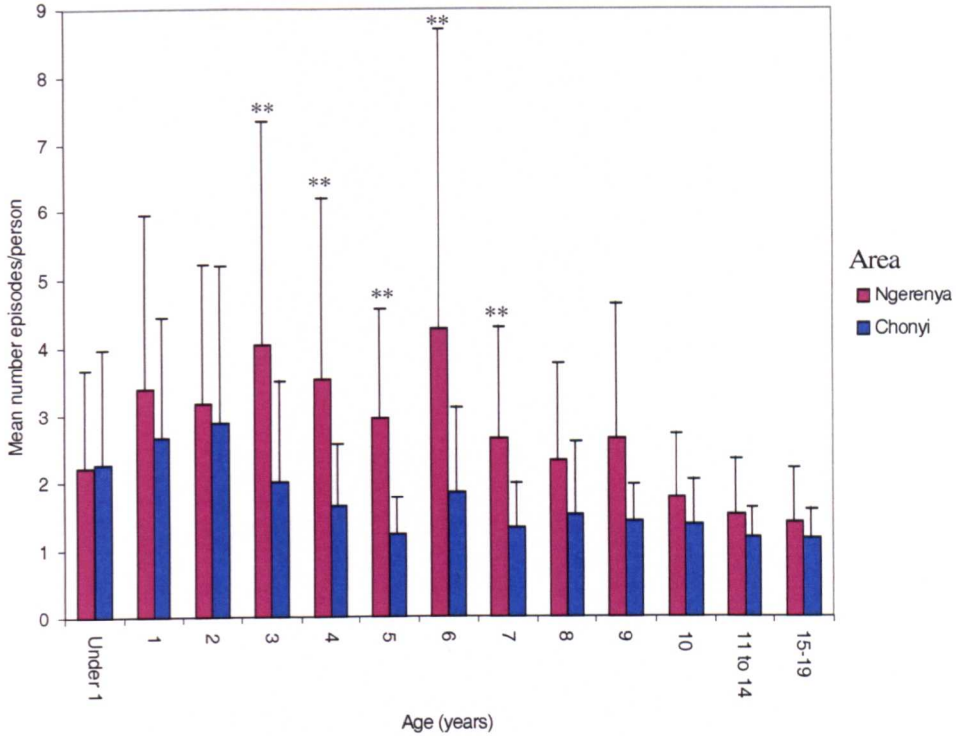


Figure 5.11 is an illustration of the mean number of clinical malaria episodes a person in each age group from the two areas was likely to experience. T-tests were used to test whether there were any differences in the mean number of episodes per age group in people from Chonyi and Ngerenya. There was evidence of a difference in the mean number of clinical malaria episodes per person between Chonyi and Ngerenya among children aged between 3 to 7 years of age. The confidence intervals appear to be wider for children from Ngerenya than children from Chonyi, which may mean there is more variation within individuals of each age group in Ngerenya compared to Chonyi.

Figure 5 11: The mean (and standard deviation) number of clinical malaria episodes per person by age in the two study areas



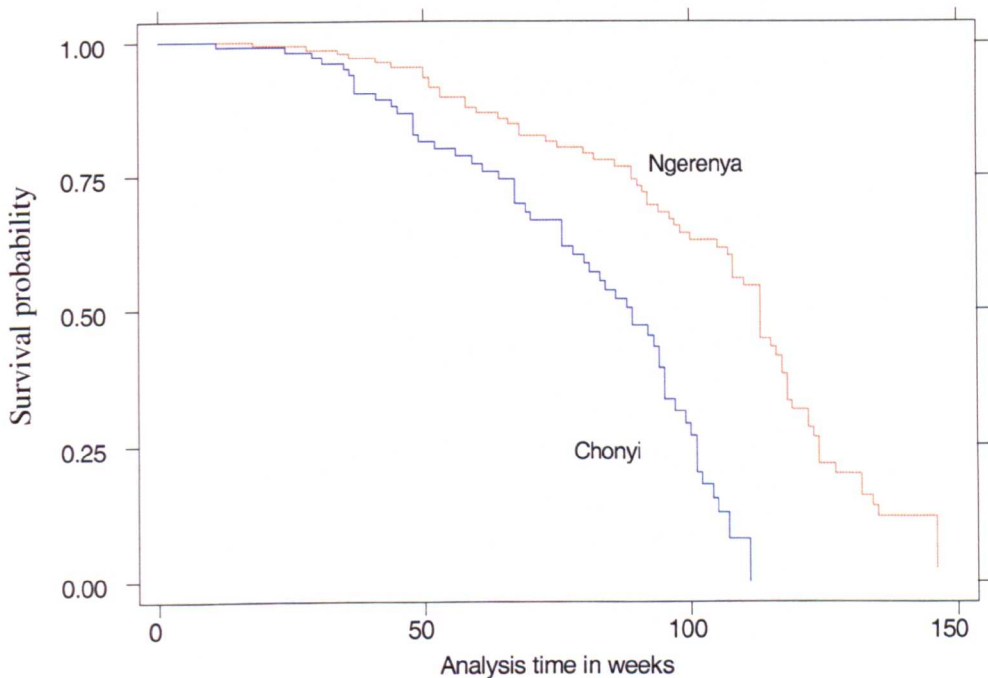
Key: ** - show that any differences are statistically significant t-test with $p < 0.05$.

An estimate of the total number of malaria episodes that an individual is likely to experience by the age of 60 years can be calculated by summing the annual incidence of clinical malaria for each age group. A person from Ngerenya is therefore likely to experience about 17 episodes of clinical malaria by the age of 60 years compared to 11 episodes of malaria for an individual from Chonyi. Just as with the lifetime experience of fever, there appears to be a higher burden of clinical malaria in individuals from Ngerenya compared to those from Chonyi.

5.3.2.4 Time to first episode of clinical malaria

Another way of looking at the age pattern of clinical malaria in the two areas was to look at the time to first malaria episode in newborns recruited into the study. The data used was that of children born from 1998 in Ngerenya and from 1999 in Chonyi and the time to first clinical malaria episode in the two areas calculated. Figure 5.12 shows the survival curve of time to first clinical malaria episode in the two areas. A clinical malaria episode in this case was the study definition of fever and any level of parasitaemia for those under a year old or fever and a parasitaemia $\geq 5,000$ -parasites/ μl of blood in children over a year old. Only a quarter of the children had clinical malaria within the first year of life in both study areas.

Figure 5.12: Kaplan-Meier survival analysis curve of time to first clinical malaria episode in newborns from Ngerenya and Chonyi.



The majority of the cases occur within the second year of life, where survival drops from 75% to less than 20% in Chonyi whereas in Ngerenya, survival went down from about 90% to 60%. Time to the first episode appears to be earlier in children from Chonyi than in children from Ngerenya. The log rank test was used to test the hypothesis that the children came from a similar study population. The results provide evidence that the two groups of children come from different populations ($\chi^2=34.4$, $p<0.0001$). Children from Chonyi have their first clinical malaria episode earlier than children from Ngerenya reflecting the higher intensity of malaria transmission in Chonyi compared to Ngerenya.

5.3.3 Non-severe clinical malaria by age in the local dispensary

The study used the presence of parasites on microscopy in a patient complaining of fever as an indication for treatment. However, none of the rural dispensaries in this area had microscopes and people were treated according to the judgment of the attending clinician. I therefore compared the patterns of presentation at the study clinic with those at the rural dispensaries that would normally have been used by the study participants. The populations that use this clinics are large and may not include the small group of people that were involved in the longitudinal study.

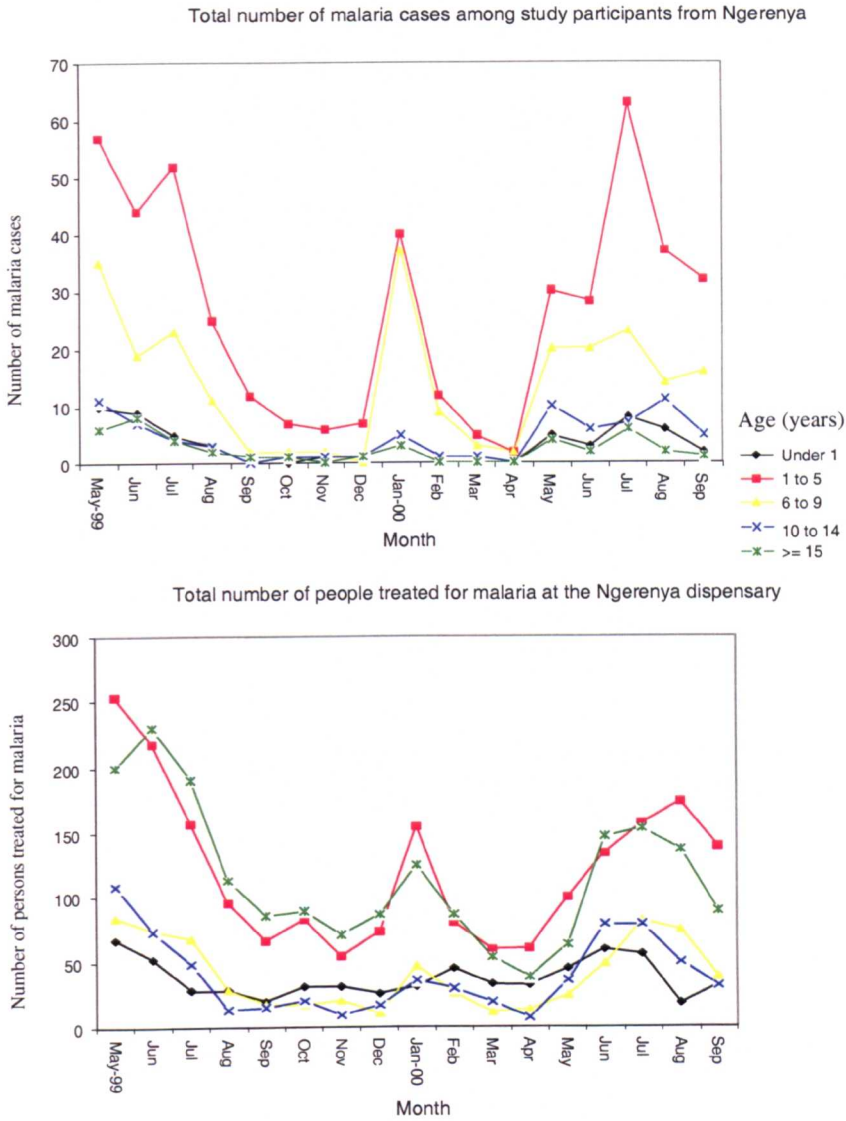
There are two government dispensaries that serve both Ngerenya and Chonyi areas (Ngerenya dispensary and Chasimba dispensary), and record sheets from the two health facilities were collected. The nurse in charge would record the names, sex, age, residence, diagnosis and treatments prescribed for each patient. At the Chasimba dispensary in Chonyi, which was the main government dispensary in the study area, the records did not have good age data. Age was recorded often as 'c' for child and 'a' for adult without specifying the exact or approximate age of the patients. Consequently, only records taken at the Ngerenya dispensary

were analysed. In these records, age estimates were recorded for those under 15 years of age. However, adult data was commonly aged as 'A' and rarely was an actual age provided for the adults. Complete dispensary data were available from March 1999 to September 2000 (19 months). Data for those diagnosed with 'clinical malaria' were tallied according to age for the different months of the years.

Numbers of patients that were diagnosed with clinical malaria in the dispensary were compared to those that were diagnosed with malaria at the same time period from the study participants from Ngerenya as illustrated in Figure 5.13. The seasonal pattern of presentation of people at both the dispensary and the study was similar. There were more people with malaria in the months of May to August and there was a small peak as well in the month of January after the short rains. More than half the cases of malaria from both the dispensary and the study were reported in the rainy season months of May to August (50.1% in the study and 57.4% in the dispensary). Presenting to the dispensary or the study clinic was less common in the dry season months of September to December and February to April.

We calculated the proportions of each age group diagnosed as malaria out of the total of all those attending the dispensary and compared these to the proportions among those in the study (i.e. if 'N' was all the people diagnosed with malaria at the dispensary and 'x' were those under one year, the proportion in the one year olds was 'x/N'). The proportions were compared to investigate if there were differences in the proportions diagnosed at the peripheral health centers compared to those diagnosed during an epidemiological survey.

Figure 5.13: Pattern of presentation of patients treated for malaria in the Ngerenya dispensary compared to that of confirmed malaria cases at the study clinic.



In children under one year of age, there was a slightly higher proportion of children treated at the dispensary (10.5%) compared to 6.1% diagnosed with malaria in the follow-up study ($\chi^2 = 22.9, p < 0.001$). Among those 1–5 years of age, a higher proportion were diagnosed with malaria from the study (53.2%) compared with 34.6% treated at the dispensary ($\chi^2 = 154.5, p < 0.001$). Similarly, there was higher proportion of children in the 6-9 year old age group

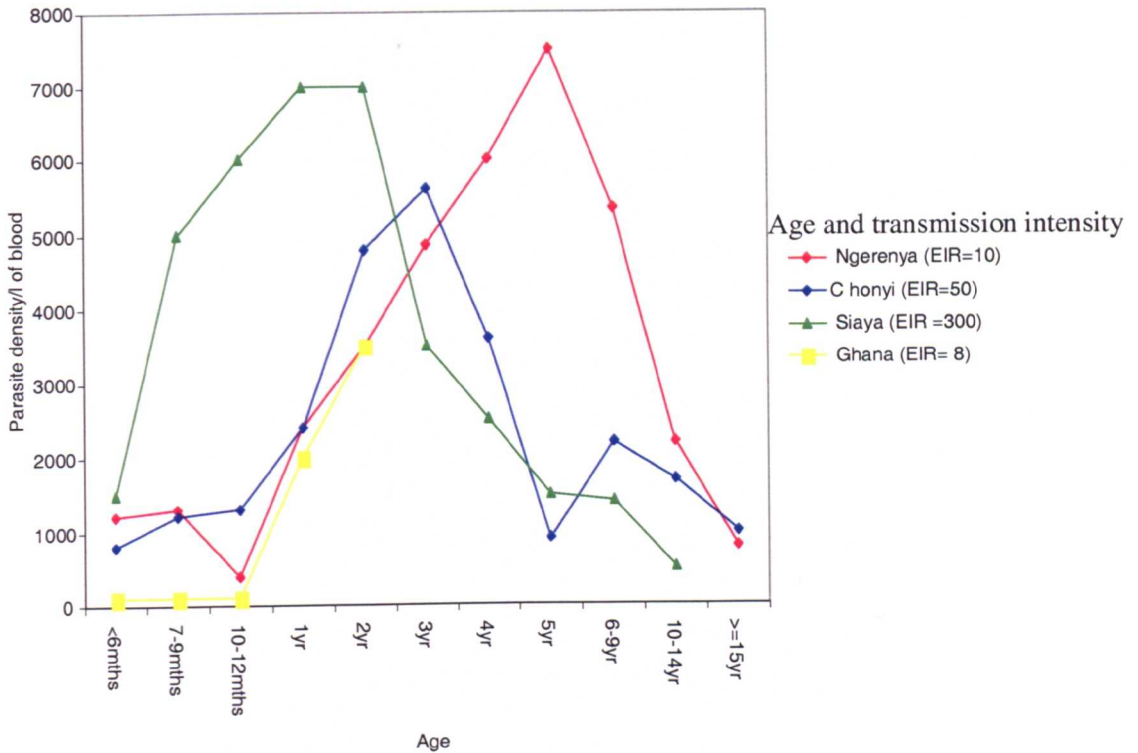
diagnosed with malaria in the study (27.3%) compared to 10.9% that were treated at the dispensary ($\chi^2 = 240.9$, $p < 0.001$) whereas a higher proportion of children in the 10-14 year old age group were treated for malaria at the dispensary (10.9%) compared to 8.6% diagnosed at the study clinic ($\chi^2 = 6.3$, $p < 0.01$). In those over 14 years of age, there was a much higher proportion of people treated for malaria at the dispensary (33.1%) compared to 4.9% that were diagnosed with malaria at the study clinic ($\chi^2 = 406.5$, $p < 0.001$)

5.4 Conclusions

The main aim of this analysis was to derive age specific malaria case definitions for the two Kilifi study sites. Differences in parasite density cut-offs for malaria case definitions to those observed in this study have been found in other studies in Africa. Figure 5.14 shows the pattern of parasite density cut-offs for malaria case definitions from this study compared to two other studies conducted in areas of differing malaria transmission in Africa. The Ghanaian study was conducted in an area with an EIR of 8 and data was available for children up to the age of 2 years only (McGuinness *et al.*, 1998). The Siaya study was conducted in an area with an EIR of 300 among people of all age groups (Bloland *et al.*, 1999).

This comparison brings out two main points, first that the age at which the peak parasite density cut-off for malaria case definition was achieved appeared to differ with transmission. This occurs at the youngest age in Siaya (EIR=300), and oldest in Ngerenya (EIR=10) with Chonyi (EIR=50) occupying the intermediate position.

Figure 5.14: A comparison of age-specific parasite cut-off used for malaria definitions for different areas in Africa. Data for Ghana available for under 2 yr olds only (McGuinness *et al*, 1998), Siaya data available up to 10 yrs (Bloland *et al.*, 1999)



Second, it suggests that it is probable that in most endemic areas, two malaria case definitions would be useful, one for very young children and adults and the other for older children. Transmission intensity may alter the age at which differences in malaria case definitions occur. In areas of high transmission like Siaya, children under 6 months and any person over 5 years may have one definition and those between 6 months and 5 years another. Within the two study areas in Kilifi, in those under a year old and ≥ 15 years of age, malaria would be defined as a fever accompanied by any level of parasitaemia whereas among those 1 to 14 years, malaria would be defined as a fever accompanied with a parasitaemia $\geq 2,500$

parasites/ μ l. These definitions lead to sensitivity and specificity estimates of >80% in both study areas.

Both age and transmission intensity affect the immune status of an individual and so does the HIV status. In a study conducted among HIV negative and positive adults in Uganda, there were differences in malaria case definitions among these two groups of adults (Whitworth *et al.*, 2000). The same definition (fever accompanied by a parasitaemia of >1,250 parasites/ μ l of blood) had a sensitivity of 51% and 84% of diagnosing malaria among HIV negative and positive adults respectively.

More detailed analysis needs to be conducted in areas with differing malaria transmission to study this phenomenon for firm conclusions to be made but it appears that there are differences in malaria case definitions that are age, transmission and HIV status dependant. These differences need to be considered when comparing malaria interventions in Africa, bearing in mind the probability that the interventions themselves are likely to alter some of these variables. This is especially so in the case of vaccine trials where it is probable that the immune status of the vaccinated group may be improved and this may alter the parasite density cut-off that would be appropriate for malaria case definitions. It is however worth noting that if studies are conducted differently i.e. some using only cross-sectional data and others both cross-sectional and longitudinal data, there are likely to be differences in the malaria case definitions derived as there are fewer numbers of fevers detected during cross-sectional surveys. Considering the data collected, cross-sectional data is not appropriate for case definitions unless very large numbers are used. If short periods of longitudinal follow-up are used, then data on CRP levels may be collected in order to increase the number of malaria

cases as the analysis shows an increase in cases of over 50% when additional CRP data was collected.

The use of arbitrary cut-offs with no estimates of sensitivity or specificity is likely to lead to misleading incidence estimates. Among children under five in Ngerenya for example, whereas 699 have fevers attributable to parasitaemia, 809 (16% more cases) would be defined as malaria cases were fever accompanied with any parasitaemia used as a case definition and 662 (5% less cases) would be defined as cases were fever accompanied with a parasitaemia $\geq 5,000$ parasites/ μ l of blood used as a malaria case definition. Among children under five years of age in Chonyi, there were 574 fevers attributable to parasitaemia, there would be 652 (14% more cases) were fever accompanied with any parasitaemia used as a case definition and 521 (9% less cases) would be defined as cases were fever accompanied with a parasitaemia $\geq 5,000$ parasites/ μ l of blood used as a malaria case definition. The definition used would therefore alter greatly the incidence estimate and also the sample size calculation.

The second aim of this analysis was to quantify malaria in these two Kilifi populations. At the population level, the fraction of fevers attributable to parasitaemia is a good measure of malaria morbidity. There were however differences in the estimate of malaria attributable fractions when using the classical compared to the logistic regression approaches. The differences were more pronounced in Chonyi where malaria attributable fevers were underestimated using the classical approach. This was associated with the presence of a higher number of asymptomatic infections in Chonyi compared to Ngerenya and has been found to be so in areas where malaria endemicity was high (Smith *et al.*, 1994). However, overall 50% of the fevers in Ngerenya and 48% of the fevers in Chonyi were attributable to parasitaemia, therefore if malaria was eliminated in these study areas, reported fevers would be halved in

both areas. However, there were differences in the fractions of fever due to malaria in the different age groups in the two areas. A higher number of under 1 year old children had parasitaemia attributable fevers in Chonyi compared to Ngerenya although a higher fraction of older children with malaria attributable fevers in Ngerenya compared to Chonyi.

Overall, there was a higher incidence of malaria in Ngerenya, the area of low transmission compared to Chonyi, which was the area of high transmission (IRR=0.66, (95% CI: 0.61 – 0.72), $p<0.001$). The life-time experience of clinical malaria episodes by the age of 60 years was 17 for an individual from Ngerenya compared to 11 for an individual from Chonyi. This was despite the finding that there was higher bednet cover in Ngerenya compared to Chonyi (section 3.1.4). The age incidence patterns of malaria in areas with differing transmission will be discussed in more detail in Appendix XI, which is a systematic review of studies where the rate of malaria was measured in various sites across Africa with differing transmission.

Finally, there appears to be more adults treated at dispensaries for malaria that would be diagnosed using the epidemiological definitions. This may be as a result of misdiagnosis of malaria at the health facility. Using clinical symptoms and signs to improve on malaria diagnosis for treatment is the focus of the next chapter.

CHAPTER SIX

USE OF SIGNS AND SYMPTOMS IN DEFINING CLINICAL MALARIA.

6.1: Introduction

Malaria is the commonest cause of fever in most malaria endemic areas. It is however difficult to differentiate from other fever-causing conditions yet since malaria is potentially fatal, it ought to be correctly diagnosed and treated at initial presentation to a health facility. Through the use of the Integrated Management of Childhood Illness (IMCI) guidelines, the WHO has made recommendations on the diagnosis and treatment of malaria among children living in malaria endemic areas (Gove, 1997). The guidelines recommend that children living in areas of high malaria endemicity be treated for malaria if they present to a health facility with fever (which includes a history of fever, feels hot, or a temperature $\geq 37.5^{\circ}\text{C}$) whereas children living in areas of low malaria endemicity be treated for malaria if they present with fever (which includes a history of fever, feels hot, or a temperature $\geq 37.5^{\circ}\text{C}$) in the absence measles, running nose and any other cause of fever.

Among the studies conducted in health facilities across Africa, between 8 and 70% of children treated for malaria have been reported to have a positive slide or met the studies definition for malaria (Hendrickse *et al.*, 1971; Okeahialam *et al.*, 1972; Olivar *et al.*, 1991; Redd *et al.*, 1996; Weber *et al.*, 1997; Olaleye *et al.*, 1998; Muhe *et al.*, 1999; Tarimo *et al.*, 2001). Among adults, between 14 and 48% of those treated for malaria at various health centres across Africa had a positive slide (Mkawagile and Kihamia, 1986; Jonkman *et al.*, 1995; Oster *et al.*, 2000). This suggests a high level of malaria misdiagnosis in health facilities across Africa.

There are two main problems associated with malaria misdiagnosis. First, this may result in an increased risk of morbidity and mortality from other conditions whose diagnosis is deferred in favour of a malaria diagnosis (O'Dempsey *et al.*, 1993). Second, over treatment is expensive. There is a lack of a cheap and safe anti-malaria drugs since the loss of chloroquine due to resistance in much of Africa. The drugs that are currently available for malaria treatment are more expensive and potentially more dangerous. Malaria treatment of all children presenting to health facilities with a history of fever also leads to unnecessary excessive use of the available anti-malaria drugs, which may precipitate rapid development of drug resistance.

It has been suggested that malaria misdiagnosis may be reduced by the introduction of microscopes to all health facilities across Africa. Jonkman *et al.* (1995), reported that the introduction of microscopes to a rural health center in Malawi led to a reduction in the prescription of anti-malarials for adults from 21.1 to 6.6%. However, under non-trial conditions in Zambia, the introduction of microscopes did not alter anti-malarial use (Barat *et al.*, 1999). Introduction of microscopes in rural health facilities would also require adequate technical support and the reading of slides may be inaccurate due to large work loads (Barat *et al.*, 1999). However, even if microscopes were introduced into all health facilities with good technical support, diagnosis may not be improved especially in highly endemic areas where about 80% of the healthy children will be parasitised (Greenwood *et al.*, 1987).

In addition, or as an alternative to microscopy, malaria diagnosis could potentially be improved by using algorithms incorporating various symptoms and clinical signs. A number of studies have been conducted in Africa to try and identify clinical syndromes with reliable predictive values for malaria diagnosis. In rural Tanzania, the use of intermittent fever of 2-3 days as a predictor of malaria had a sensitivity of 73% and a

specificity of 98% in diagnosing malaria (Rooth and Bjorkman, 1992). In a study conducted in rural Malawi, Redd *et al.* (1996), found that the use of a rectal temperature $\geq 37.7^{\circ}\text{C}$, together with nailbed pallor or splenomegaly resulted in an algorithm with a sensitivity of 85% and a specificity of 41% for diagnosing malaria compared to the use of a history of fever alone, which had a sensitivity of 93% and a specificity of 21%. In rural Ethiopia, an algorithm consisting of a history of fever accompanied by either a previous attack of malaria, pallor or absence of cough was found to have a sensitivity of 83% and a specificity of 51% for diagnosing malaria (Muhe *et al.*, 1999). Two studies from peri-urban Gambia have been conducted to investigate the value of algorithms in malaria diagnosis. Weber *et al.* (1997), found that the presence of chills, sweating or shaking had a sensitivity of 93% and specificity of 19% for diagnosing malaria whereas Olaleye *et al.* (1998), using a malaria score of ≥ 8 predictors reported a sensitivity of 88% and a specificity of 64% for diagnosing malaria. However not all studies have reported a potential in the improvement in malaria diagnosis with the use of malaria algorithms as in a study conducted in Zimbabwe by Bassett *et al.* (1991).

A review of studies conducted in various parts of the world on the use of clinical algorithms for malaria diagnosis was conducted by Chandramohan *et al.* (2002). Algorithms derived from these studies varied due to cultural perceptions, malaria epidemiology and gold standard definitions. It was also difficult to compare algorithms across various sites as not only were the algorithms site specific but also different studies reported different symptomatology. However, using algorithms with the best predictive values (ability of a test to predict disease status) derived from six African studies, Chandramohan and colleagues (2002), compared drug wastage (proportion of febrile children falsely classified as malaria) and failure to treat (proportion of febrile children falsely classified as non-malaria). They concluded that since there was a high risk of failure to treat malaria in areas of high malaria endemicity, then it was important that all

children that present to a health facility with fever or a history of fever be treated with an anti-malarial drug. However, in areas of low malaria endemicity where there is a higher rate of drug wastage and less risk of failure to treat, use of algorithms for malaria diagnosis may be of benefit.

In this chapter, the use of malaria diagnostic algorithms was investigated in a longitudinal study population under moderate malaria transmission. Various algorithms were derived for the various age groups and compared to those already derived in other studies conducted in Africa and suggested in the IMCI guidelines.

6.2: Materials and methods

6.2.1: Data collection

Data were collected from the study clinic among subjects who reported with a history of fever as described in chapter four (section 4.4). All patients with a history of fever had a smear taken for malaria parasites. Once the results from the slide readings were available, the study clinician attended to the patient and a questionnaire was filled out. These questionnaires had 27 symptoms and 27 clinical signs (Appendix VII). The clinician initially recorded all the symptoms that the patient volunteered as spontaneous symptoms and then prompted for the symptoms that the patient did not volunteer. All records were double entered into a database (FoxPro[®] version 2.5) and both entries cross-checked for errors before cleaning.

6.2.2 Data analysis

Data analysis was conducted using STATA[®] software, version 7.0. Data from Chonyi and Ngerenya study areas was combined as there were few differences in the symptomatology from people of the same age groups in the two areas (section 4.4). Data collected in the first year from May 1999-May 2000 were used to derive the malaria algorithms and data

collected in May 2000 to May 2001 were used to assess the value of these algorithms.

Since the data were collected over a long period of time, there was concern that repeat patient appearances at the clinic would result in an increase in the reporting of certain symptoms due to repeated prompting for them by the clinician. An analysis was carried out to investigate changes in the proportions of various clinical signs and symptoms over time. This analysis showed that there was negligible variation of symptoms (prompted or volunteered) reported over the period of the study that would alter the association between symptoms and malaria. To investigate whether repeat episodes in an individual would have altered symptom-malaria associations, we analysed data using the first malaria episode per individual only and compared this with the use of the complete data set. There were minimal differences in the proportion with varying symptoms and their association with malaria. The full data set was therefore used in the analysis of the symptom/sign – malaria associations.

Symptoms were grouped into those volunteered by the patient (spontaneous symptoms) and a combination of those that were volunteered and those that were prompted (spontaneous and prompted symptoms). To construct the algorithm, variables with a negative association with malaria were reworded so that they became positively associated, for example, 'presence of diarrhoea' was negatively associated with malaria but the variable 'absence of diarrhoea' was positively associated with malaria.

Data were analysed according to three age groups: 0-5 years of age, between 6 – 14 years and adults (≥ 15 years). A distinction was made between children under the age of six years and those 6-14 years as the older children were thought to be better able to articulate their symptoms than younger children who rely on the guardian to interpret their condition. Two definitions of clinical malaria were used:

1) Epidemiological definition: The presence of fever (axillary temperature of $\geq 37.5^{\circ}\text{C}$) accompanied by any level of parasitaemia in those under a year old and adults. For those 1-14 years old, fever accompanied by a parasitaemia of $\geq 2,500$ parasites/ μl was used. These were the clinical definitions derived for malaria diagnosis from chapter five and will be referred to as 'Emalaria' for the rest of the chapter.

2) Treatment definition: A history of fever accompanied by the presence of parasitaemia in all the age groups. This is the group who would be considered for treatment if presenting to a clinic with microscopy facilities and will in the rest of the chapter be referred to as 'Tmalaria'.

Both malaria definitions were used in order to investigate how clinical algorithms performed in diagnosing 'malaria for treatment' or 'malaria defined epidemiologically'. A set of four algorithms was derived for each of the age groups separately as described on Table 6.1.

Table 6.1: Four sets of algorithms derived for malaria case diagnosis.

Malaria definition	*Symptom description	Term for algorithm
Emalaria	Volunteered only	Spon-e
	Volunteered and prompted	Prompt-e
Tmalaria	Volunteered only	Spon-t
	Volunteered and prompted	Prompt-t

Key: *- All algorithms included clinical signs as well as symptoms.

The set of four was arrived at to investigate if there are any differences in the algorithms derived in each of the age groups if a different malaria definition was used and whether it makes any difference to derive the algorithms from only volunteered symptoms or using prompted symptoms as well.

Associations between various clinical symptoms and signs and malaria (using both definitions separately) were estimated using Maentel-Haenzel unadjusted odds ratios. Using statistical significance (unadjusted odds ratios with a $p < 0.05$), the clinical symptoms and signs associated with malaria were identified and termed 'predictors' of malaria. Logistic regression models were used to estimate the association between each predictor and malaria after adjusting for all the other predictors. Using both backward and forward logistic regression modelling, predictors independently associated with malaria (adjusted odds ratios with a $p\text{-value} < 0.05$) were identified. Sensitivities, specificities, positive predictive values (PPV) and negative predictive values (NPV) of these independent predictors were estimated.

Finally, a malaria score was calculated for each individual using a simple count of these independent predictors of malaria. For each patient, a score of '1' was given if the predictor was present and '0' if absent. If a predictor had an odds ratio > 10 , then the score of '2' was assigned if the predictor was present and '0' if absent. For each patient, a total score was calculated for each outcome in each age group. The sensitivity and specificity of each total score was estimated and the score with the highest sensitivity and specificity selected as the algorithm of choice.

The performance of these algorithms was then tested on the second year of data that was collected from May 2000 to May 2001. The sensitivity and specificity of each of the derived algorithms was estimated along with the positive and negative predictive values, where algorithms derived using one malaria definition were tested against the same definition unless stated. The score with the highest sensitivity and specificity was selected as the best score for each algorithm. For children, these algorithms with the highest scores were compared with those from the IMCI guidelines and those derived from other studies in Africa where similar data were collected. Scatter plots of the sensitivity and '1-

specificity' of the various selected algorithms were generated. This is a modification of the Receiver Operator Curves (ROC), which is used to compare diagnosis cut-offs with the highest sensitivity and specificity (Altman, 1997). This normally involves a curve with sensitivity VS '1-specificity' with the best cut-off being the one which maximises these two estimates, which is the point nearest the left-hand corner. In the case of this study, the algorithm nearest to the left-hand upper corner is selected as the best for diagnosing malaria.

6.3: Results

In the period May 1999 to May 2000, a total of 4,379 people reported to the study clinic with a history of fever and had smears made. Of these, 2,355 (54%) were children under six years of age of whom 947 (40%) had 'Tmalaria' and 369 (16%) had 'Emalaria'. A total of 1,188 (27%) were children between 6-14 years of age of whom, 593 (50%) had 'Tmalaria' and 123 (10%) had 'Emalaria'. A total of 836 (19%) were adults, of whom 163 (20%) had 'Tmalaria' while 19 (2%) had 'Emalaria'.

6.3.1 Deriving algorithms for malaria diagnosis in children 0-5 years old

The commonest clinical symptoms and signs reported to the study clinic among children with 'Tmalaria' were: axillary temperature $\geq 37.5^{\circ}\text{C}$ (44%), 'hot on palpation' (40%), pallor (17%) and palpable spleen (18%). The commonest clinical symptoms included: cough (46%), vomiting (31%), shivering (27%), runny nose (13%) and poor appetite (22%).

Proportions of those with the 27 clinical signs and 27 symptoms using 'spon-t', spon-e', 'prompt-t' and 'prompt-e' were calculated. Estimates of the association of each clinical symptom or sign with malaria (using either definition) were made using univariate analysis and those found to be associated with malaria ($p < 0.05$) identified. Estimates of adjusted

odds ratios, sensitivities and specificities of these independent predictors are listed on

Table 6.2.

6.3.1.1: Clinical malaria predictors using various malaria definitions among children ≤ 5 years old

6.3.1.1.1 Clinical malaria predictors for 'Emalaria'

Five broad sets of clinical symptoms and signs were found to be independent predictors of 'Emalaria' among children under six years of age. In the univariate analysis, being 'hot on palpation' was the best predictor of 'Emalaria'. After controlling for other predictors, those children that were 'hot on palpation' were 23 times more likely to have 'Emalaria' than those that were not 'hot on palpation' whether the outcome was 'spon-e' [Adjusted OR= 23.2 (95%CI: 17.3-31.3, $p < 0.001$)] or 'promp-e' [Adjusted OR= 22.6 (95%CI: 16.2-30, $p < 0.001$)]. After controlling for other predictors, those children that reported vomiting or had no diarrhoea were found to be two and three times more likely to have 'Emalaria' than those that did not report vomiting or those that reported diarrhoea ($p < 0.001$). After controlling for other predictors, those children that did not report respiratory symptoms and signs were also found to be two to six times more likely to have 'Emalaria' than those children that presented with any of these respiratory symptoms or signs ($p < 0.04$).

6.3.1.1.2: Clinical malaria predictors for 'Tmalaria'

All the symptoms and signs that were associated with 'Emalaria' were also predictive of 'Tmalaria', however, additional respiratory predictors and the absence of skin infections were added independent predictors of 'Tmalaria'. Children without the listed skin conditions were three to seven times more likely to have 'Tmalaria' than children that had these skin conditions ($p < 0.03$).

Table 6.2: Estimates of adjusted odds ratios, sensitivities and specificities of malaria predictors in children 0-5 years old from both

Chonyi and Ngerenya in the period May 1999-May 2000.

Clinical sign or symptom (* = clinical signs)	Epidemiological definition (Emalaria)						Treatment definition (Tmalaria)					
	Volunteered symptoms (Spon - e)			Prompted symptoms (Promp-e)			Volunteered symptoms (Spon - t)			Volunteered and prompted symptoms (Promp-t)		
	Odds ratio	Sen. (%)	Spec. (%)	Odds ratio	Sen. (%)	Spec. (%)	Odds ratio	Sen. (%)	Spec. (%)	Odds ratio	Sen. (%)	Spec. (%)
Fever												
* Hot on palpation	22.6	81.3	81.3	23	81.3	81.3	2.2	39.7	79	2.1	39.7	79
* Axillary temperature $\geq 37.5^{\circ}\text{C}$	NA	NA	NA	NA	NA	NA	1.4	44	73.2	1.4	44	73.2
Shivering	-	-	-	1.7	25.8	87.6	2.3	7.9	97.9	3.7	26.6	93.7
Digestive problems												
Vomiting	2.1	26.1	86.6	2	34.5	81.3	2.1	22.4	89.3	2.2	30.9	85.3
Absence of diarrhoea	3.0	94	11.4	2.6	92.7	12.3	3.7	93.8	13.4	3.1	92.5	14.3
Respiratory problems												
* Normal chest sounds	5.5	97.6	8.9	6.4	97.6	8.9	4.3	97.5	11.5	4.5	97.5	11.5
* Absence of rhinitis	3.3	98.4	5.8	-	-	-	2.9	97.9	7.2	3.0	97.9	7.2
* Cough not heard	-	-	-	-	-	-	1.4	89.4	19	1.4	89.4	19
* Normal respiratory rate	-	-	-	-	-	-	1.9	95.8	8.1	1.8	95.8	8.1
Absence of cough	1.8	68	51.7	1.8	60.4	58.4	1.6	62	55.7	1.5	53.8	61.7
Absence of running nose	-	-	-	1.9	90.5	19.9	1.7	95.5	9.9	1.5	87.2	22
Skin problems												
* Wounds not seen	-	-	-	-	-	-	-	-	-	3.0	98.6	4.6
Absence of rashes	-	-	-	-	-	-	7.7	99.3	4.6	4.0	98.6	4.6
Absence of wounds	-	-	-	-	-	-	2.9	98.9	2.8	-	-	-
Absence of ear ache and discharge	-	-	-	-	-	-	5.1	99.7	1.7	-	-	-
Others												
* Pallor	2.6	17.3	92.4	2.8	17.3	92.4	3.1	16.6	95.8	3.4	16.6	95.8
* Palpable spleen	1.9	16.8	91.9	1.8	16.8	91.4	4.8	18.2	96.5	4.2	18.2	96.5
Absence of red discharging eyes	-	-	-	-	-	-	-	-	-	4.7	99.7	1.4
Sleepiness	-	-	-	-	-	-	-	-	-	2.6	7.9	97.9
Best score	≥ 5 (hot on palpation=2)						≥ 5 (hot on palpation=2)					
Sensitivity and Specificity	Sens=63%, Spec. = 87%						Sens=81%, Spec = 58%					
NPV and PPV	NPV=95%, PPV=39%						NPV=82%, PPV=56%					

Key: Sens.- Sensitivity Spec. - Specificity NPV- Negative predictive value PPV- Positive predictive value

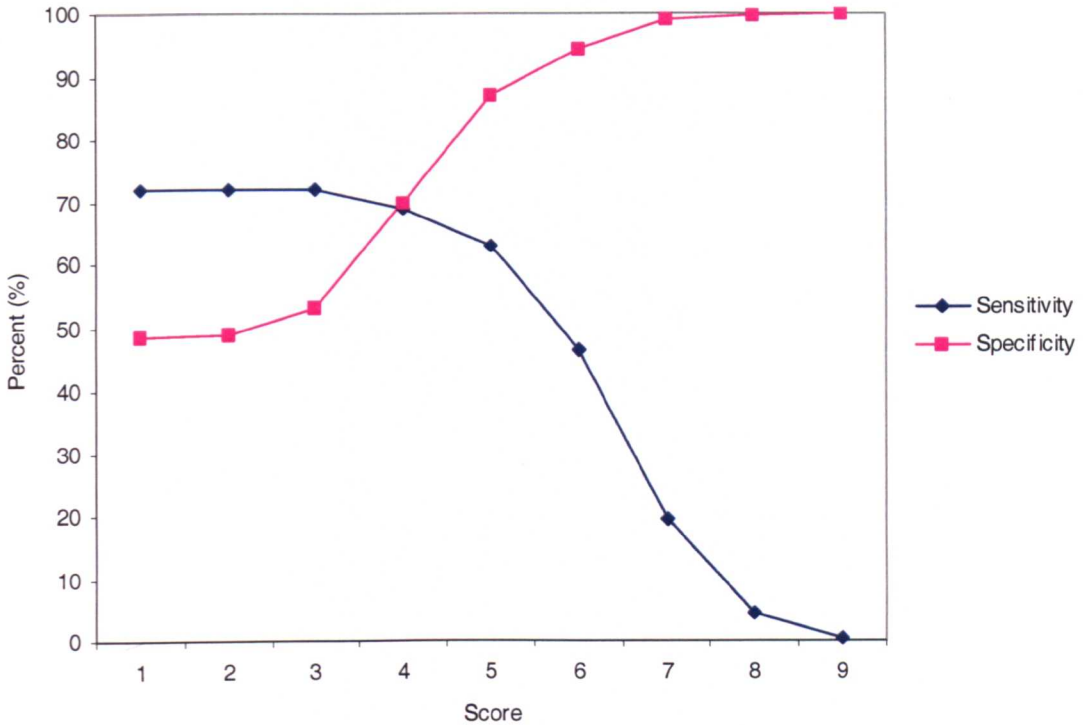
Although being ‘hot on palpation’ was associated with an increased risk of ‘Tmalaria’, the odds ratio was a tenth of that associated with ‘Emalaria’ whether using volunteered symptoms alone or both prompted and volunteered symptoms [Adjusted OR= 2 (95%CI: 1.5 - 2.7, $p < 0.001$) & Adjusted OR= 2.2 (95%CI: 1.6 – 2.9, $p < 0.001$)].

6.3.1.2: Process of deriving malaria diagnosis algorithms among children ≤ 5 years old

To derive algorithms for diagnosing malaria, independent malaria predictors were scored ‘1’ if present and ‘0’ if absent. However, in the algorithms where the outcome was ‘Emalaria’, the predictor ‘hot on palpation’ was scored as ‘2’ if present and ‘0’ if absent as it had an odds ratio >10 (as discussed in section 6.2.2). For each individual, a sum of the score for the presence of these predictors was made. Sensitivity and specificity estimates were calculated for the various total scores. Figure 6.1 shows sensitivity and specificity estimates for various scores that would be used in ‘Emalaria’ diagnosis using volunteered symptoms among children under six years of age.

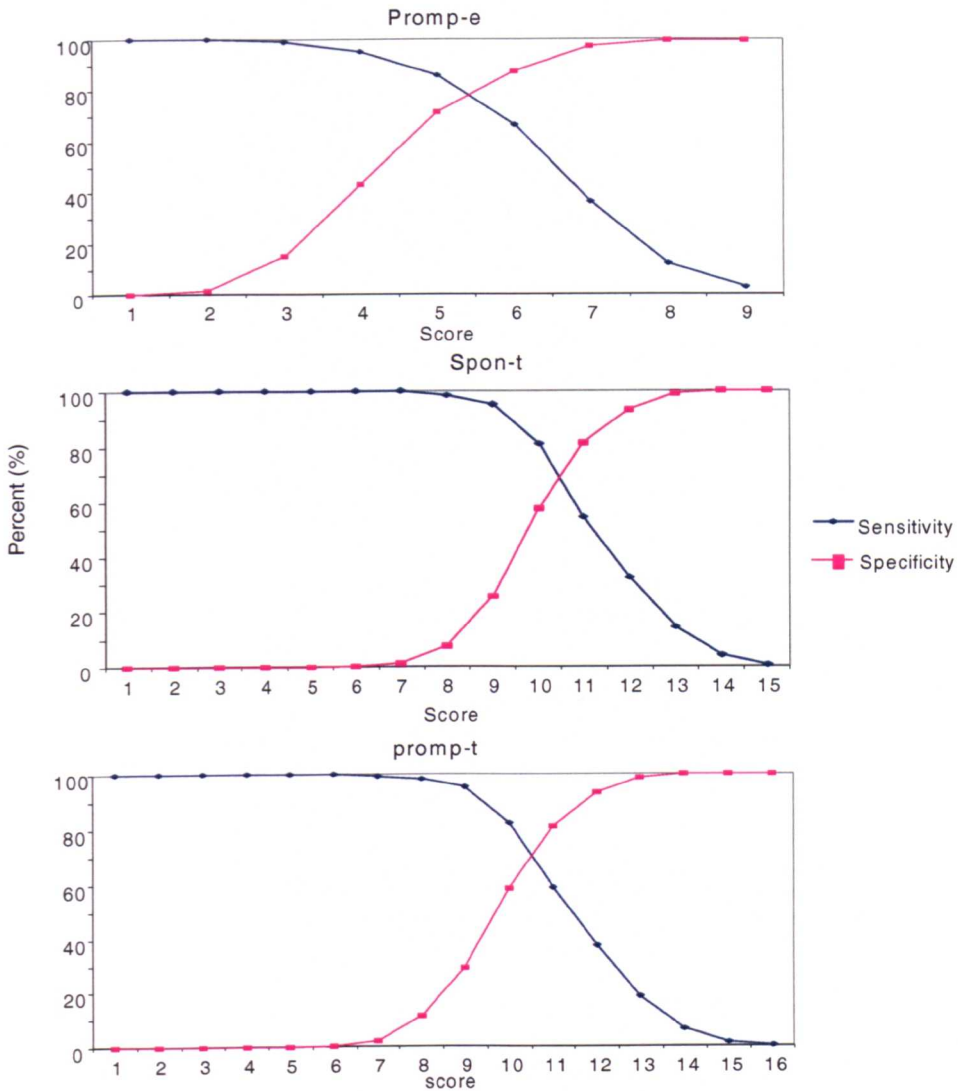
The use of an algorithm with a score of one (the presence of a malaria predictor with a score of ‘1’ if present) had a sensitivity estimate of 72% and a specificity estimate of 49% for diagnosing ‘Emalaria’. These estimates were similar to those derived using an algorithm with a score of ‘2’ (the presence of two clinical signs or symptoms or only ‘hot on palpation’). Although the sensitivity and specificity curves cross at the score of 4 with sensitivity and specificity estimates of 69%, the best score was selected as ≥ 5 , which had a sensitivity of 63% but a higher specificity of 87%. Whereas a score of ≥ 4 had a PPV of 23% and a NPV of 95%, a score of ≥ 5 had PPV of 39% and a NPV of 95%.

Figure 6.1: Sensitivity and specificity of various malaria scores for the algorithm ‘spon-e’ among children 0-5years of age from data collected in the period May 1999- May 2000.



Derivation of the other three algorithms are not discussed in detail to avoid repetition but Figure 6.2 shows the sensitivity and specificity curves using different scores for the three algorithms. The algorithm derived using ‘Emalaria’ whether ‘spon-e’ or ‘promp-e’ was a combination of predictors with a score of ≥ 5 . For algorithms derived with ‘Tmalaria’ as an outcome, whether ‘spon-t’ or ‘promp-t’, a score of ≥ 10 was found to be the best predictor of malaria. The algorithms derived using ‘Tmalaria’ as an outcome had a higher PPV than those algorithms derived using ‘Emalaria’ as an outcome however, the later had lower specificities.

Figure 6.2: Sensitivity and specificity of various malaria scores for the three algorithms ‘pomp-e’, spon-t’ and ‘pomp-t’ among children aged 0-5 years of age.



6.3.1.3 Comparisons between different algorithms

The four algorithms generated from the data collected from May 1999-May 2000 were compared to the IMCI guidelines and two algorithms that were derived from similar studies in Africa, one using ‘Tmalaria’ as an outcome (Muhe *et al.*, 1997) and the other using ‘Emalaria’ as an outcome (Olaleye *et al.* 1998). The two sets of IMCI guidelines were used. The first one (IMCI-1), was a modification of the algorithm derived for areas of low endemicity, which was presenting to the clinic with a history of fever or the presence of a history of fever in the absence of a running nose or measles. The second was an

algorithm for the low transmission area (IMCI-2), which was presenting to the clinic with a history of fever in the absence of measles, running nose and any other cause of fever. The data used to compare algorithms was that collected from the same study population in the subsequent year (May 2000 to May 2001). The data were used to calculate estimates of the sensitivity, specificity, positive and negative predictive values of each of the algorithms (Table 6.3).

Table 6.3: Comparison of estimates of sensitivity, specificity, positive and negative predictive values for various algorithms in children ≤ 5 years old.

Algorithm	Sens (%)	Spec (%)	PPV (%)	NPV (%)
∇ Epidemiological definition (volunteered symptoms only) = Spon-e	78.2	81.3	42.4	95.5
∇ Epidemiological definition (volunteered + prompted symptoms) = Prompt-e	79.8	79.9	41.2	95.7
*Treatment definition (volunteered symptoms only) = Spon-t	80.2	53.5	62.9	73.3
*Treatment definition (volunteered and prompted symptoms) = Prompt-t	80	55.1	63.6	73.7
* IMCI-1 : History of fever, no running nose nor measles	95.1	11.5	51.2	70.6
* IMCI-2 : History of fever, no running nose, no measles, no cough or diarrhoea	58.4	67.6	63.8	62.5
∇ Gmb-e : 5 out of 9 predictors (Olaleye <i>et al.</i> , 1998)	83.5	84.6	37.8	97.9
* Ethiopia-t : Absence of cough or presence of pallor (Muhe <i>et al.</i> , 1997).	67.9	53.8	58.9	63.1

Key:

* - Malaria treatment definition (Tmalaria)

∇ - Malaria epidemiological definition (Emalaria)

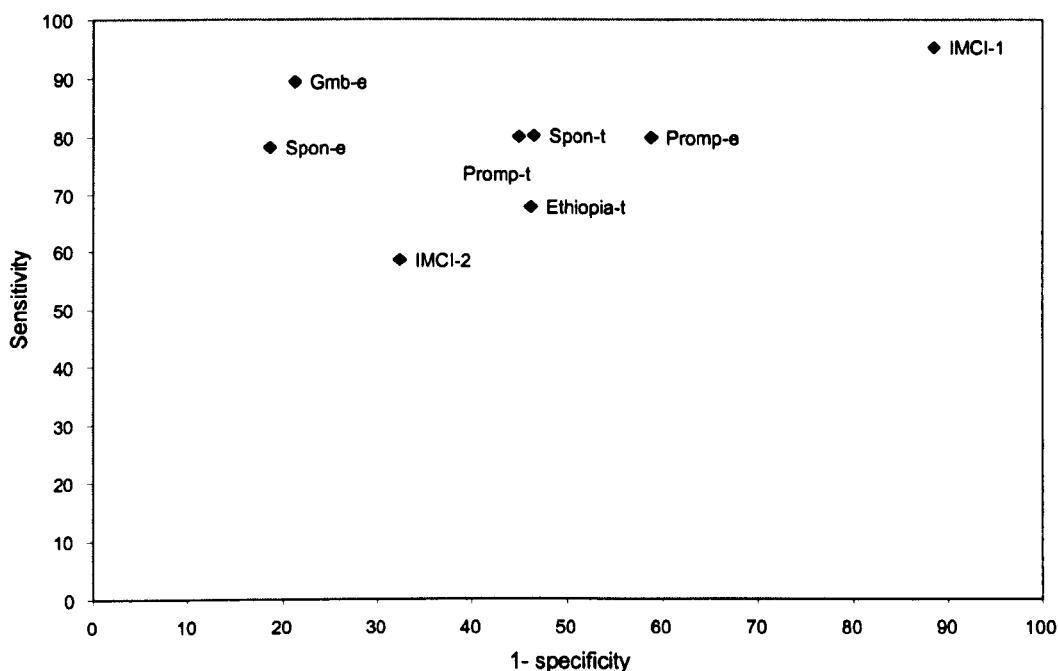
Sens: Sensitivity Spec: Specificity

PPV- Positive predictive value NPV- Negative predictive value

Except IMCI-2 and the Ethiopian algorithm (Muhe *et al.*, 1997), all the other algorithms were able to diagnose malaria with a sensitivity of approximately 80%. The sensitivities and specificities of diagnosing malaria appeared to be about 80% when the outcome was 'Emalaria'. However, when the algorithms were used to diagnose 'Tmalaria', the specificity was low. All the algorithms had a negative predictive value of >60% but those

derived with 'Emalaria' as an outcome had negative predictive values >95%. However, the positive predictive values for the algorithms derived using 'Emalaria' as an outcome were <43% compared to those using 'Tmalaria' as an outcome where algorithms had positive predictive values >60%. Figure 6.3 shows the sensitivity and '1-specificity' of the algorithms described on table 6.3. The 'best' definition was the one closest to the left hand upper corner.

Figure 6.3: Plot of sensitivity and '1-specificity' of various algorithms from data collected in the period May 2000 to May 2001 from children 0-5years of age from both Chonyi and Ngerenya.



Key: 'e' - Epidemiological malaria definition
't' and IMCI- Malaria defined for treatment

For the algorithms derived from this analysis, use of volunteered symptoms performed better than the use of both prompted and volunteered symptoms. The best algorithms using 'Emalaria' as an outcome were that derived in the Gambia study (Olaleye et al., 1998) and

the 'spon-e' algorithm. The best algorithms using 'Tmalaria' as an outcome was 'promp-t'. The IMCI-1 algorithm would have resulted in the treatment of all children with a history of fever for malaria but about 90% of those without malaria would have been treated as well were this algorithm used for 'Tmalaria' diagnosis. Although IMCI-2 had an improved specificity, the sensitivity was less than 40%.

This next section is a hypothetical scenario investigating how many children would be correctly treated if these algorithms were to be used among the children presenting to the study clinic with a history of fever in the period May 2000-May 2001. There were 2,688 children under the age of six years that presented to the study clinic with a history of fever within that period. Out of these, 1,328 (49.4%) had parasitaemia and should have received anti-malarials, while 1,360 should not have received anti-malarials. When a child with a history of fever and parasitaemia was treated with an anti-malarial drug or a child with no parasitaemia not treated with an anti-malarial, then this was considered 'correct treatment'. However, if a child with a history of fever and parasitaemia was not treated with an anti-malaria or a child with no parasitaemia treated with an anti-malarial, then this was considered 'wrong treatment'. Table 6.4 shows the numbers and proportions that would have been treated correctly or wrongly treated if any of the selected algorithms were used on data from patients presenting to the study clinic between May 2000 to May 2001 with a history of fever.

If the algorithm IMCI-1 were to be used for treatment, about 95% of the children with parasitaemia would have be treated. However, a further 89% of the children with no parasitaemia at all would have been treated as well. If absence of cough and absence of diarrhoea were added to the IMCI guidelines (IMCI-2), this would have resulted in a reduction in the number of children with parasitaemia correctly treated and would have also resulted in a reduction in the number of children that had no parasitaemia treated for

malaria. If the best algorithm (derived from the Kilifi analysis) for ‘Tmalaria’ were to be used (‘prompt’), then 80% of the children with parasitaemia would have been correctly treated while 45% of children with no parasitaemia would have been wrongly treated for malaria.

Table 6.4: The number of patients that would have been treated or not treated according to parasitaemia status using five algorithms among children aged 0-5 years from Chonyi and Ngerenya presenting to the clinic with a history of fever in the period May 2000-May 2001.

Definition	*Number treated that had parasitaemia, n (%)	VNumbers treated that had no parasitaemia, n (%)	*Number not treated that had parasitaemia, n (%)
Spon-e	689 (52%)	326 (24%)	633 (48%)
Gmb-e	545 (41%)	290 (21%)	783 (59%)
IMCI-1	1,263 (95%)	1,204 (89%)	65 (5%)
IMCI-2	776 (58%)	441 (32%)	552 (42%)
Prompt	1,057 (80%)	605 (45%)	264 (20%)

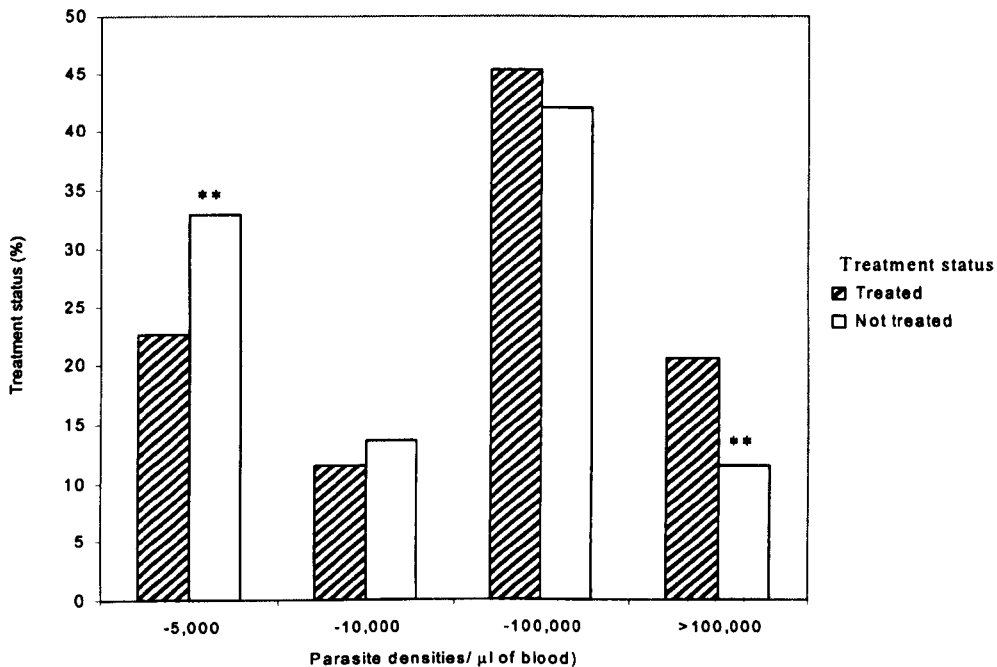
*-As a fraction of the 1,3068 children with a history of fever and parasitaemia

∇- As a fraction of the 1,360 children with a history of fever without parasitaemia

Using the best algorithm for defining ‘Emalaria’ (‘spon-e’), only 52% of the children under five years of age with parasitaemia would have been correctly treated while a quarter of those without parasitaemia would have been wrongly treated. Using the Gambian algorithm, about 60% of the children with parasitaemia would not have been treated. Although the algorithms ‘spon-e’ and ‘Gmb-e’ performed well in diagnosing ‘Emalaria’, this hypothetical investigation shows them to be poor at diagnosing ‘Tmalaria’. It is however possible that though they would not capture all the children with parasitaemia, they may be able to capture all the cases with a high parasitaemia and leave out those with low parasite densities. The next section describes the proportions that would be treated or not treated among those with various parasite densities using the best algorithms for diagnosing ‘Emalaria’ (Spon-e) and the best algorithm for diagnosing ‘Tmalaria’ (prompt) in children under six years of age.

Figure 6.4 seeks to investigate this by showing proportions of children with different parasite densities who would have been treated and those that would not have been treated had the 'spon-e' algorithm been used for diagnosing 'Tmalaria'.

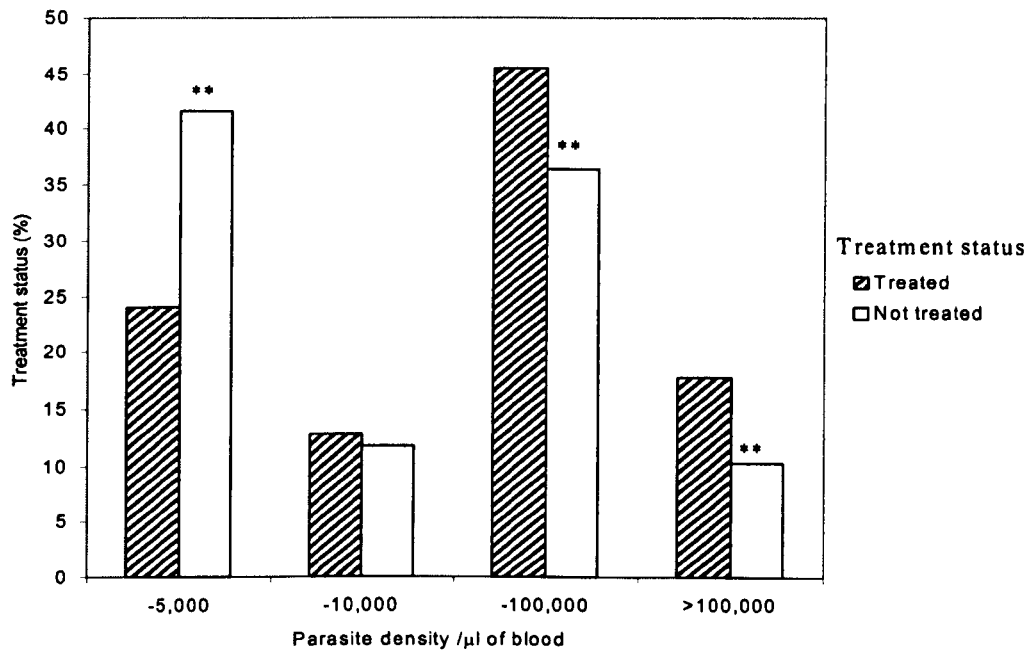
Figure 6.4: Percentage of children 0-5years of age that would and would not be treated if the 'spon-e' algorithm were to be used for 'Tmalaria' diagnosis (data from May 2000-May 2001 from Chonyi and Ngerenya).



The levels of parasite density among those children that would not have been treated were very similar to those among children that would have been treated. The majority (>40%) in either group have a parasitaemia between 10,000-100,000 parasites/ μ l of blood. There was evidence of more children not treated with parasitaemia <math>< 5,000</math> parasites/ μ l of blood than those treated (32.9% Vs 22.6%, $\chi^2=17.3$, $p<0.01$) and more children with a parasitaemia >100,000 parasites/ μ l of blood among those treated compared to those not treated (20.5% Vs 11.5%, $\chi^2=19.4$, $p<0.01$).

Figure 6.5 seeks to investigate proportions of children with different parasite densities among children who would have been treated and those that would not have been treated had the 'promp-p' algorithm been used for diagnosing 'Tmalaria'.

Figure 6.5: Percentage of children 0-5years of age that would and would not be treated if the 'promp-t' algorithm were to be used for 'Tmalaria' diagnosis (data from May 2000-May 2001 from Chonyi and Ngerenya).



There was evidence that more children with a parasitaemia <5,000 parasites/ μ l of blood were not treated compared to those that were treated (41.7% Vs 24%, $\chi^2=32.9$, $p<0.01$). There was also evidence that more children were treated among those with a parasitaemia between 10,000-100,000 parasites/ μ l of blood than not treated (45.6% Vs 36.4%, $\chi^2=7.2$, $p<0.01$) and this was found to be the same among children with a parasitaemia >100,000 parasites/ μ l of blood (17.7% Vs 10.2%, $\chi^2=8.7$, $p<0.001$).

6.3.2 Deriving algorithms for malaria in children 6-14 years old

The same methodology used to derive algorithms for children under six years of age was used in those 6-14 years of age. The commonest clinical signs among children six to fourteen years of age that reported to the study clinic with 'Tmalaria' were: axillary temperatures $\geq 37.5^{\circ}\text{C}$ (26%), hot on palpation (22%), and palpable spleen (17%). The commonest clinical symptoms were: headache (41%), cough (28%), vomiting (17%), shivering (17%) and abdominal pain (16%).

6.3.2.1. Clinical malaria predictors for both 'Emalaria' and 'Tmalaria' among children 6-14 years old

Table 6.5 is a tabulation of predictors independently associated with malaria and the performance of selected algorithms. Four independent predictors of 'Emalaria' were identified. They were the clinical sign 'hot on palpation' and the symptoms vomiting, shivering and absence of cough. Children 6-14 years that were 'hot on palpation' were more than 25 times more likely to have 'Emalaria' compared to children that were not 'hot on palpation' [Adjusted OR= 27.6 (95%CI: 16.9 – 45.1, $p < 0.001$) & Adjusted OR= 25.1 (95%CI: 15.4 – 40.7, $p < 0.001$)]. However, there was no evidence of an association between being 'hot on palpation' and having 'Tmalaria' using volunteered symptoms alone [Adjusted OR= 1.4 (95%CI: 0.9 – 2.3, $p = 0.1$)] although there was some association when both volunteered and prompted symptoms were used [Adjusted OR= 1.9 (95%CI: 1.3 – 2.6, $p < 0.001$)]. Children that reported vomiting were four times more likely to have 'Emalaria' than those children that did not report vomiting [Adjusted OR= 4.2 (95%CI: 2.5 – 7, $p < 0.001$)].

Table 6. 5: Adjusted odds ratios, sensitivities and specificities of malaria predictors in children 6- 14 years old from both Chonyi and Ngerenya in the period May 1999-May 2000.

Clinical signs or symptom (* = clinical signs)	Epidemiological definition (Emalaria)						Treatment definition (Tmalaria)					
	Volunteered symptoms (Spon - e)			Volunteered and Prompted symptoms (Promp-e)			Volunteered symptoms (Spon - t)			Volunteered and prompted symptoms (Promp-t)		
	Odds ratio	Sen. (%)	Spec. (%)	Odds ratio	Sen. (%)	Spec. (%)	Odds ratio	Sen. (%)	Spec. (%)	Odds ratio	Sen. (%)	Spec. (%)
Fever												
* Hot on palpation	28	74.8	88.5	25	74.8	88.5	-	-	-	1.9	21.9	85.9
* Axillary temperature $\geq 37.5^{\circ}\text{C}$	-	-	-	-	-	-	1.9	26	83.7	-	-	-
Shivering	-	-	-	1.8	39.8	77.3	1.6	17.2	89.6	1.5	32	83
Sweating	-	-	-	-	-	-	-	-	-	2.8	6.2	98.5
Digestive problems												
Vomiting	4.4	27.6	89.5	4.3	40.7	84.2	2	16.9	92.3	2.1	25.5	88.7
Respiratory problems												
* Normal chest sounds	-	-	-	-	-	-	3.4	99	3	4.4	99	3
* Absence of rhinitis	-	-	-	-	-	-	3.4	99	3.4	-	-	-
Absence of cough	2.1	74.8	36.7	-	-	-	1.8	72	43	1.5	65.8	47.9
Absence of running nose	-	-	-	-	-	-	-	-	-	2.8	95.1	12.6
Skin problems												
* Wounds not seen	-	-	-	-	-	-	6.6	99.5	3.4	5.2	99.5	3.4
* Rash not seen	-	-	-	-	-	-	-	-	-	5.2	99	3.2
Absence of rashes	-	-	-	-	-	-	7	99.7	2.7	-	-	-
Others												
* Palpable spleen	-	-	-	-	-	-	3.5	16.7	95	4.1	16.7	95
Headache	-	-	-	-	-	-	-	-	-	1.3	57.7	55.5
Weakness	-	-	-	-	-	-	-	-	-	3.1	3.5	99
Best score	≥ 2 (hot on palpation=2)			≥ 2 (hot on palpation=2)				≥ 5			> 6	
Sensitivity and Specificity	Sens & Spec. = 81%			Sens=78%, Spec. = 84%				Sens=86%, Spec. = 72%			Sens=71%, Spec. = 61%	
NPV and PPV	NPV=97%, PPV=35%			NPV=97%, PPV=36%				NPV=36%, PPV=86%			NPV=68%, PPV=61%	

Key: Sens. – Sensitivity

Spec. – Specificity

NPV -Negative predictive value

PPV- Positive predictive value

Children who did not present to the clinic with a number of respiratory and skin related signs and symptoms were two to seven times more likely to have 'Tmalaria' than those children who presented with listed respiratory and skin related conditions ($p < 0.03$). None of these skin or respiratory symptoms were associated with 'Emalaria'. Children with a palpable spleen were four times more likely to have 'Tmalaria' than those without a palpable spleen ($p < 0.001$).

The presence of headache and weakness were associated with 'Tmalaria' but only if both prompted and volunteered symptoms were both included in the algorithm. There appeared to be differences in the inclusion of symptoms when either volunteered symptoms were used alone or both volunteered and prompted symptoms were used especially when the outcome was 'Tmalaria'.

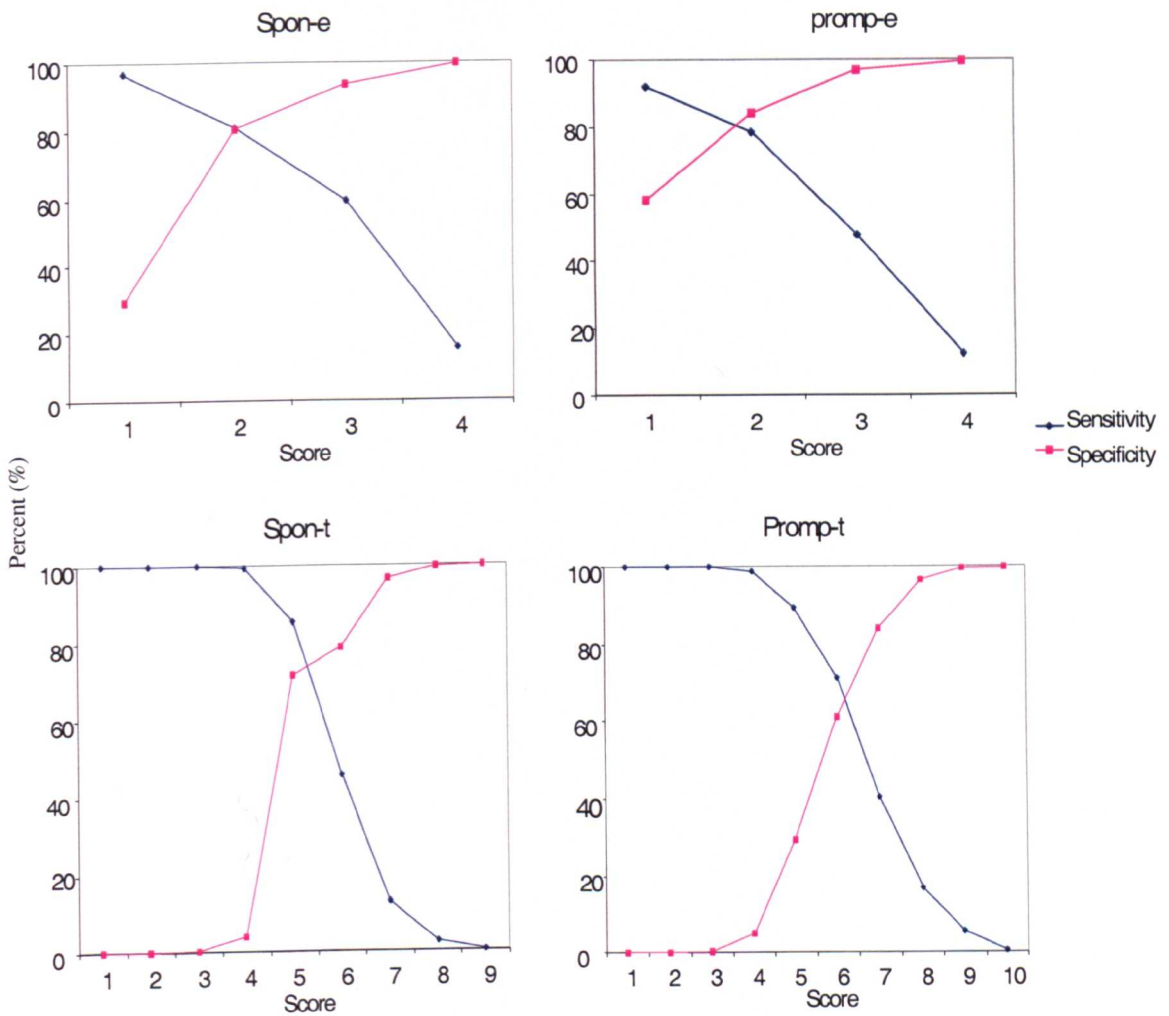
6.3.2.2. Process of deriving malaria diagnosis algorithms

As was the case with children under six years of age, to derive algorithms, independent malaria predictors were scored '1' if present and '0' if absent. However, as in children under six years of age, the predictor 'hot on palpation' was scored as '2' if present and '0' if absent when the outcome used was 'Emalaria', as it had an odds ratio > 10 . For each individual, the sum of the score was made and sensitivity and specificity estimates made for each score. Figure 6.6 shows the sensitivity and specificity estimates for various scores for all four algorithms among children 6-14 years of age.

For algorithms derived using 'Emalaria' as an outcome, a score of ≥ 2 was found to have a high sensitivity and specificity of diagnosing 'Emalaria'. A score of '2' would include either two symptoms or being 'hot on palpation'. Whether volunteered symptoms or both prompted and volunteered symptoms were used did not make a difference to the algorithm score or the PPV and NPV. The algorithms for diagnosing 'Tmalaria', included a higher

number of predictors. The use of volunteered symptoms performed well in terms of sensitivity and specificity but the use of the prompted alongside the volunteered symptoms contributed to an improvement on both the PPV and the NPV of the algorithm.

Figure 6.6. The sensitivity and specificity scores for diagnosing malaria using various algorithms among children 6-14 years of age



6.3.2.3 Comparisons between different algorithms in children 6-14 years old

Table 6.6 shows the estimates of sensitivity and specificity, PPV and NPV of various algorithms when using the data collected from May 2000- May 2001. The algorithms derived from this analysis were compared to those of the IMCI guidelines as well as two studies from The Gambia and Ethiopia that collected similar data.

Table 6.6: Comparison of sensitivity, specificity, positive and negative predictive values of various algorithms in children 6-14 years old.

Algorithm	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Epidemiological definition (volunteered symptoms only) = Spon -e	83.5	81.4	36.9	97.4
Epidemiological definition (volunteered + prompted symptoms) = Prompt-e	81.3	85.7	42.4	97.3
*Treatment definition (volunteered symptoms only) = Spon-t	46.8	77.8	75.4	50.2
*Treatment definition (volunteered and prompted symptoms) = Prompt-t	32.6	88.1	79.9	47.3
* IMCI-1 : History of fever, no running nose nor measles	97.4	5.4	59.8	58.8
* IMCI-2 : History of fever, no running nose, no measles, no cough or diarrhoea	72.2	45.4	65.6	53.1
[∇] Gmb-e : 5 out of 9 predictors (Olaleye <i>et al.</i> , 1998)	91.8	79.1	27.5	99.1
* Ethiopia-t : Absence of cough or presence of pallor (Derived by Muhe <i>et al.</i> , 1997).	75	40.8	64.7	53.1

Key:

* - Malaria treatment definition (Tmalaria)

[∇] - Malaria epidemiological definition (Emalaria)

Sens: Sensitivity Spec: Specificity

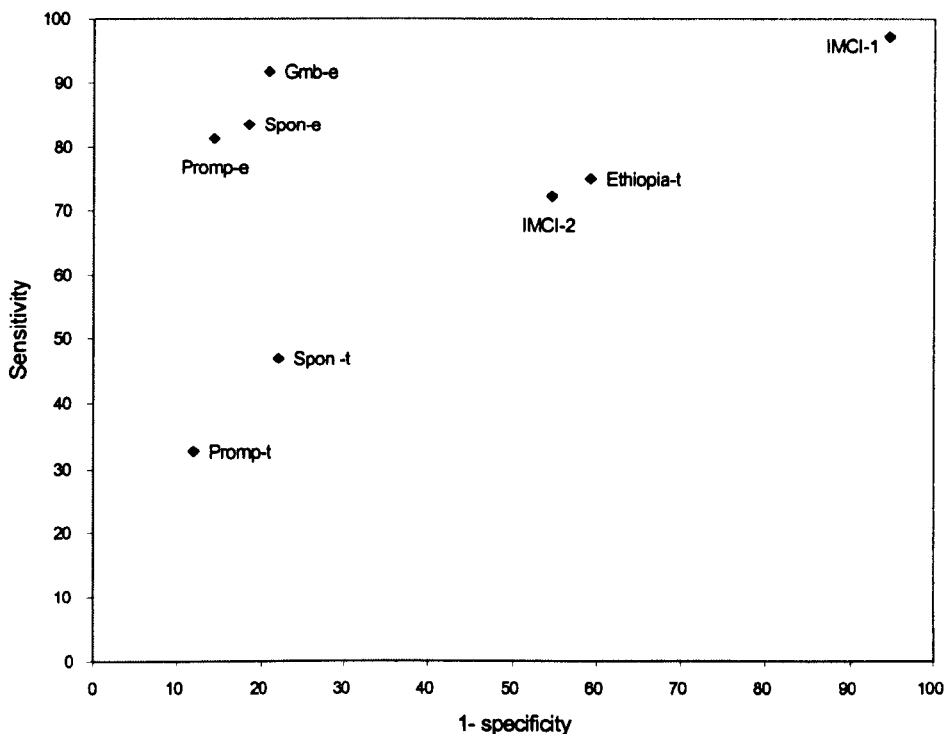
PPV- Positive predictive value NPV – Negative predictive value

As with children under the age of six years, the algorithms using 'Emalaria' as an outcome performed better with sensitivities and specificities >80%. However, they had very low PPV but high NPV and are therefore better at diagnosing non-malaria than malaria cases. The IMCI guidelines when used to define malaria for treatment had a high sensitivity as expected but a low specificity and moderate predictive values. The algorithms derived for 'Tmalaria' had poor sensitivities but high specificities; these also had moderate predictive values. The algorithms for diagnosing 'Tmalaria' were therefore as good at identifying a malaria case, as they were good in picking a non-malaria case.

Figure 6.7 below, shows the sensitivity vs. 1-specificity of the various algorithms for children 6-14 year of age. The best algorithms for diagnosing 'Emalaria' was the Gambian algorithm and 'spon-e' algorithm. The algorithm using both volunteered symptoms alone

of both prompted and volunteered symptoms did not differ very much. None of the algorithms derived for diagnosing 'Tmalaria' appeared to be superior over the other as they either had good sensitivity and poor specificity or poor sensitivities with good specificities.

Figure 6.7: Plot of sensitivity and '1-specificity' of various algorithms from data collected in the period May 2000 to May 2001 from children 6-14 years of age from both Chonyi and Ngerenya.



Key:

'e' - Epidemiological malaria definition 't' and IMCI- Malaria defined for treatment

The section that follows is a hypothetical investigation of the numbers and proportion of children 6-14 years of age that presented to the clinic with a history of fever from May 2000-May 2001 that would be treated correctly if some of these algorithms were used for treatment. Correct treatment in this case is assumed to be malaria treatment for a person who presents to the clinic with a history of fever and an accompanying parasitaemia. There were 1,347 children 6-14 years old who presented to the study clinic with a history of fever

in the period May 2000-May 2001 of whom 796 (59.1%) had parasitaemia and should have been treated for malaria. Table 6.7 shows the numbers and proportions of those with parasitaemia who would have been treated correctly or not treated (wrongly) and those without parasitaemia that would have been treated (wrongly) were these algorithms used on this study population.

Table 6.7: The number of patients that would have been treated or not treated according to parasitaemia status using various algorithms in children 6 – 14 years old from Chonyi and Ngerenya who presented to the study clinic with a history of fever in the period May 2000-May 2001

Definition	*Number with parasitaemia treated n (%)	∇ Numbers treated that had no parasitaemia n (%)	*Number not treated that had parasitaemia n (%)
Spon-e	355 (45)	149 (27)	441 (55)
Prompt-e	307 (39)	121 (22)	489 (61)
Gmb-e	276 (35)	99 (18)	520 (65)
IMCI-1	775 (97)	521 (95)	21 (3)
IMCI-2	575 (72)	301 (55)	221 (67)

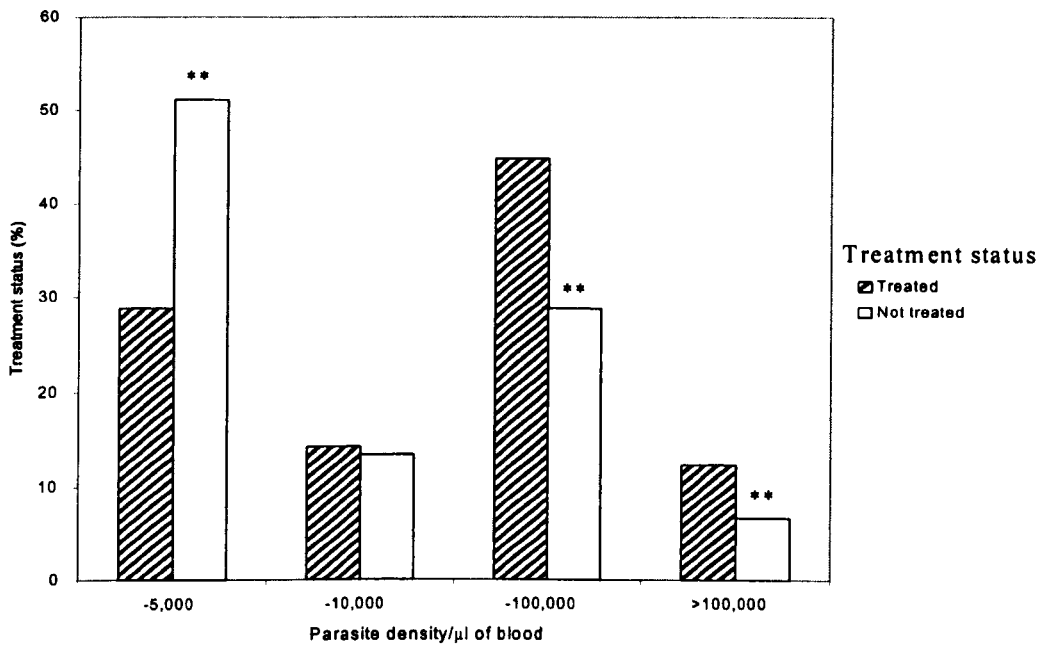
*-As a fraction of the 796 people that had parasitaemia

∇- as a fraction of the 551 people that had no parasitaemia

Using the three best algorithms derived for 'Emalaria', 45% of the children with parasitaemia would be correctly treated and 55% of the children who had parasitaemia would not have been treated. About 20% of children that had no parasitaemia would have been treated. Using a history of fever in the absence of a running nose and absence of measles (IMCI-1), about 97% of the children would have been correctly treated but about 95% of children who had no parasitaemia at all would have been treated for malaria. If the absence of a cough and the absence of diarrhoea were included (IMCI-2) in the analysis, then there would have been less children with parasitaemia that would have been correctly treated (72%) but less children without parasitaemia would have been treated for malaria (55%).

Figure 6.8 describes the distribution of parasite densities among those that had any parasitaemia that would have been treated compared to those that would not have been treated if 'best' algorithm ('spon-e') were used as a diagnostic tool.

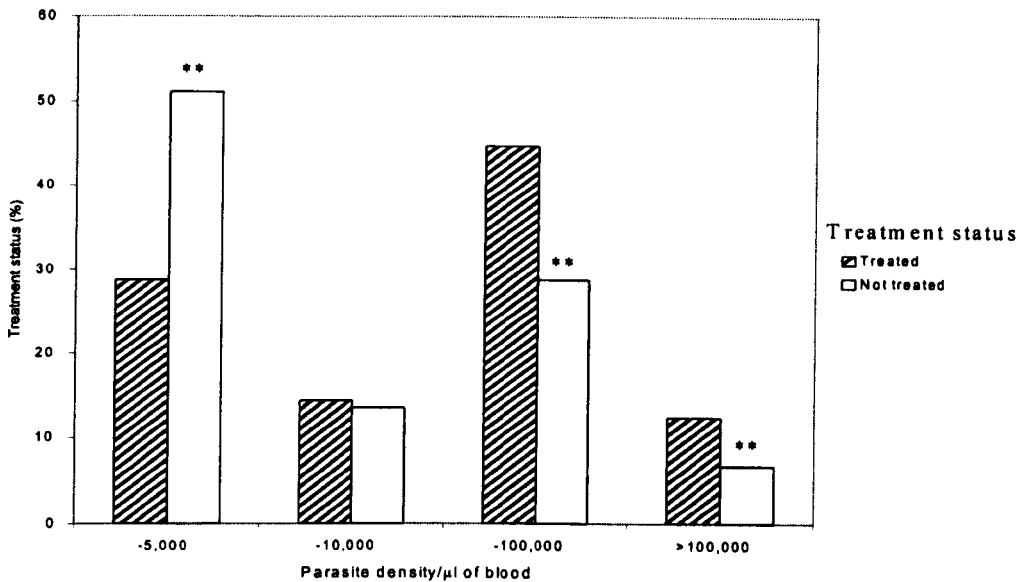
Figure 6.8: Fractions of children 6-14 years of age that would and would not be treated if the 'spon-e' algorithm were to be used for malaria diagnosis (data from May 2000-May 2001 from Chonyi and Ngerenya used).



There was evidence that more children with parasitaemia <5,000parasites/μl of blood would not be treated than would have been treated had 'spon-e' used to diagnose 'Tmalaria' (50.1% Vs 29%, $\chi^2=36.27$, $p<0.001$). However, there was evidence that more children with parasitaemia between 10,000 and 100,000 parasites/μl of blood would be treated compared to those not treated (41.7% Vs 31.5%, $\chi^2=8.2$, $p=0.003$) and the same would be the case among those with a parasitaemia >100,000 parasites/μl of blood (14.4% Vs 5.2%, $\chi^2=19.5$, $p<0.001$).

Figure 6.9 shows the proportions of children 6-14 years of age that would be treated for 'Tmalaria' were the algorithm 'spon-t' used for malaria diagnosis.

Figure 6.9: Fractions of children 6-14 years of age that would and would not be treated if the 'spon-t' algorithm were to be used for malaria diagnosis (data from May 2000-May 2001 from Chonyi and Ngerenya used).



Surprisingly, there was little difference in the proportions between those that would be treated using this algorithm and 'spon-e'. There was evidence that more children with parasitaemia <5,000parasites/μl of blood would not be treated than would have been treated had 'spon-t' used to diagnose 'Tmalaria' (51.1% Vs 28.6%, $\chi^2=41.4$, $p<0.001$). However, there was evidence that more children with parasitaemia between 10,000 and 100,000 parasites/μl of blood would be treated compared to those not treated (44.7% Vs 28.7%, $\chi^2=21.8$, $p<0.001$) and the same would be the case among those with a parasitaemia >100,000 parasites/μl of blood (12.4% Vs 6.7%, $\chi^2=7.7$, $p=0.006$).

6.3.3 Deriving algorithms for malaria in adults

The same methodology used in children 0-5 years age was used to derive algorithms in adults. The commonest clinical signs among adults that reported to the study clinics with 'Tmalaria' were: axillary temperatures $\geq 37.5^{\circ}\text{C}$ (11.7%), hot on palpation (9.2%), and pallor (10%). The commonest clinical symptoms were: headache (67.5%), shivering (27%), cough (25%), joint pains (26%), Abdominal pain (15%) and 'aching all over' (13%). All clinical symptoms and signs were evaluated as described in the younger age groups. Table 6.8 is a tabulation of predictors independently associated with malaria using various definitions and either symptoms volunteered only or both volunteered and prompted symptoms.

6.3.3.1 Clinical malaria predictors for both 'Emalaria' and 'Tmalaria' among adults

There was only one clinical sign ('hot on palpation') and two clinical symptoms (vomiting and joint pains) that were found to be independently associated with 'Emalaria'. Adults that were 'hot on palpation' were more than 60 times more likely to have 'Emalaria' than those adults that were not 'hot on palpation'. ($p < 0.001$). If only volunteered symptoms were used, adults that reported having joint pains were four times more likely to have 'Emalaria' than adults that did not report joint pains [Adjusted OR= 4.1 (95%CI: 1.4 – 12.5, $p = 0.01$)]. When prompted and volunteered symptoms were used, those that reported vomiting were seven times more likely to have 'Emalaria' than those that did not [Adjusted OR= 7.4 (95%CI: 2.1 - 26, $p = 0.002$)] while those that had joint pains were about 10 times more likely to have 'Emalaria' than those adults that did not report having joint pains [Adjusted OR=9.5 (95%CI: 2.5 – 35.4, $p = 0.001$)]. It is worth noting however that only 19 adults fitted the epidemiological malaria definition in the period May 1999-May 2000.

There was one clinical sign and two symptoms when using ‘spon-e’ and four when using ‘promp-e’ were significantly associated with ‘Tmalaria’. Individuals who were ‘hot on palpation’ were about three times more likely to have ‘Tmalaria’ than those that were not ‘hot on palpation’ ($p < 0.001$). When using either ‘spon-t’ or ‘promp-t’, individuals with joint pains were about twice as likely to have ‘Tmalaria’ than those without joint pains [Adjusted OR= 1.8 (95%CI: 1.2 – 2.8, $p = 0.005$ & Adjusted OR= 1.7 (95%CI: 1.2 – 2.5, $p = 0.008$)]. Using clinical signs and volunteered symptoms, individuals without chest pains were 2.4 times more likely to have ‘Tmalaria’ than those with chest pains [Adjusted OR= 2.4 (95%CI: 1.3 – 4.2, $p = 0.004$)]. Using clinical signs and volunteered and prompted symptoms, individuals without a running nose were 6 times more likely to have ‘Tmalaria’ than those with a running nose [Adjusted OR= 5.9 (95%CI: 1.8 – 19.4, $p = 0.003$)], while individuals who reported vomiting were twice as likely to have ‘Tmalaria’ as those that did not report vomiting [Adjusted OR= 1.8 (95%CI: 1 – 3.5, $p = 0.04$)]. This later association was similar to the association between history of nausea and ‘Tmalaria’ [Adjusted OR= 1.7 (95%CI: 1 – 2.8, $p = 0.04$)].

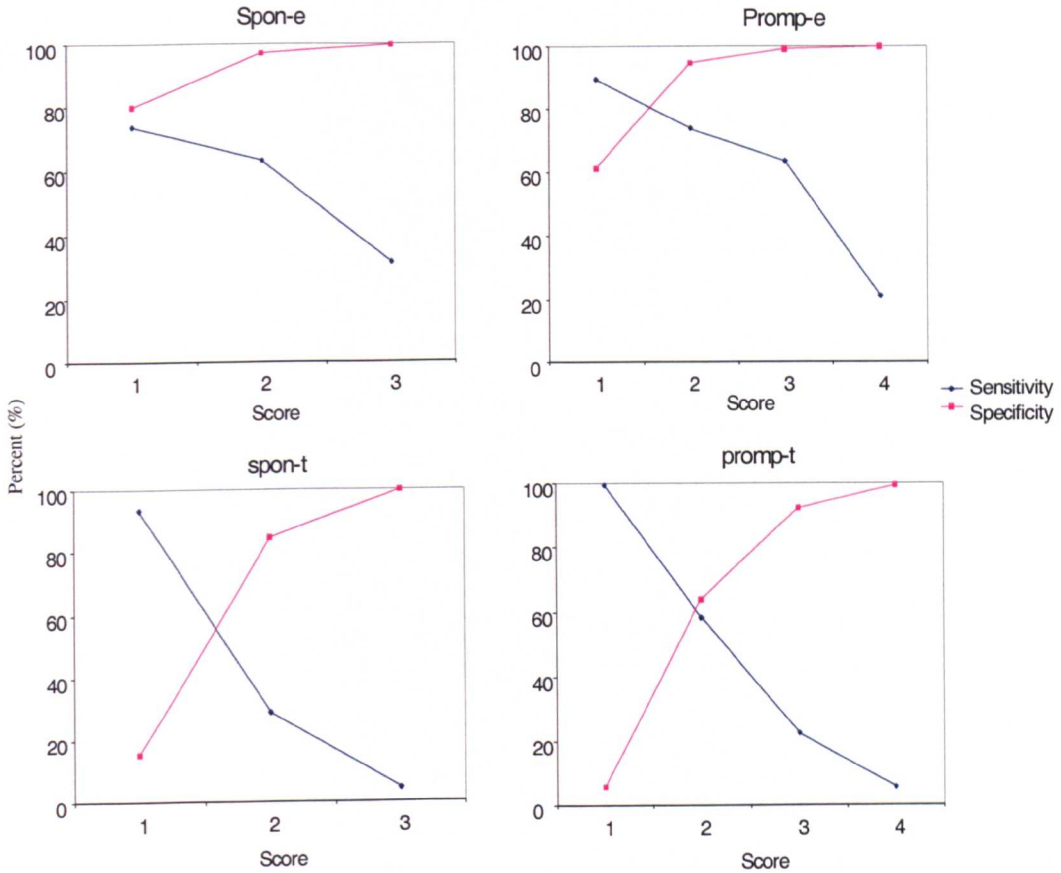
6.3.3.2 Process of deriving malaria algorithms in adults

The same process was used as that in children, for each individual, each predictor was scored as ‘1’ if present and ‘0’ if absent except for those that had an odds ratio of association with malaria that was larger than 10. The sum of score was calculated at an individual level and specificity and sensitivity estimates for each score provided for all four algorithms.

Figure 6.10 shows the sensitivity and specificity of using various scores for malaria diagnosis. The best algorithms for ‘Emalaria’ diagnosis was a score of ‘2’ which meant having two of the clinical symptoms present or just being ‘hot on palpation’. The

sensitivity and specificity of a score of '2' was very high but the PPV or the ability of the test to diagnose an episode of 'Emalaria' was very low.

Figure 6.10: Sensitivity and specificity for malaria diagnosis using the four algorithms among adults.

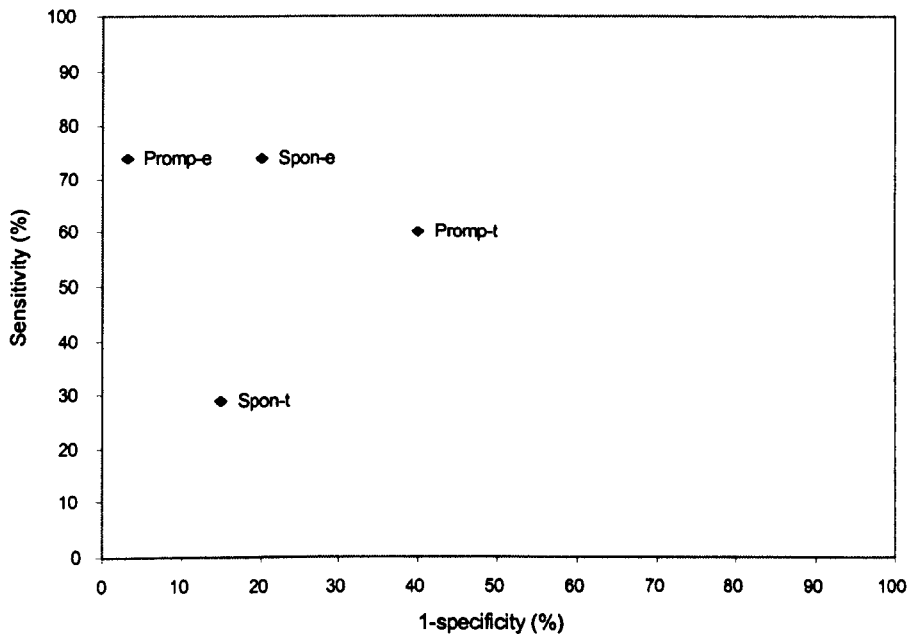


For 'Tmalaria' and volunteered symptoms, the presence of any two 'Tmalaria' predictors had a sensitivity of 29% and a specificity of 85% for. When using both prompted and volunteered symptoms, the presence of any two predictors had a sensitivity of 58% and a specificity of 64% for diagnosing 'Tmalaria'. The positive predictive value of any of the selected algorithms was very low (7.9% to 31%), while the negative predictive values were high (83 to 99.2%). These algorithms were therefore more useful in predicting non-malaria than in predicting malaria.

6.3.3.3 Comparison of different algorithms among adults

There are no specific guidelines for the treatment of malaria in adults in endemic areas and there are few studies that have looked at algorithms of symptoms and signs that could be used for the treatment of malaria in adults. Figure 6.11 however compared the performance of the algorithms that have been derived from this analysis on data collected from May 2000 to May 2001 in adults reporting to the study clinic with a history of fever.

Figure 6.11: Plot of sensitivity and ‘1-specificity’ of various algorithms from data collected in the period May 2000 to May 2001 from adults from both Chonyi and Ngerenya.



The best algorithm for ‘Emalaria’ was ‘promp-e’. There were 641 adults that presented with a history of fever in the study clinic during the time period May 2000-May 2001 of whom 158 (25%) had parasitaemia (compared to 20% for the period May 1999-May 2000). If the algorithm ‘promp-e’ was used, only 24 (15%) of those with a parasitaemia would be treated correctly, 31 would be treated that did not have a parasitaemia and 134

(85%) of those with parasitaemia would not be treated. If this algorithm were used, there would be about 100 people not treated with a parasitaemia of $<5,000$ parasites/ μ l of blood and 35 persons with a parasitaemia higher than this. About 77% of those not treated would have no parasitaemia.

6.4 Conclusions

There are no unique features that set clinical malaria apart from other febrile conditions. One way to differentiate those presenting to a health facility with a fever from other conditions and a fever due to malaria is to use microscopy. This however assumes that anyone presenting to the clinic with a history of fever and parasitaemia (Tmalaria) ought to be treated for malaria. Algorithms that were derived based on 'Tmalaria' were capable of detecting 80% of children under six that had a history of fever and an accompanying parasitaemia, however, the use of this algorithm would lead to the treatment of 45% of the children that did not have parasitaemia. Among children 6-14 years of age, algorithms derived for 'Tmalaria' diagnosis would lead to the treatment of 72% of the children with a history of fever and parasitaemia but also 55% of the children without parasitaemia. The use of algorithms for diagnosing 'Tmalaria' were moderately good at picking out most of the children that had parasitaemia but would also lead to the treatment of many children with a history of fever but with no accompanying parasitaemia as malaria cases.

As described in Chapter five, the presence of fever and any level of parasitaemia may not be a good indicator of malaria illness. Therefore among those with 'Tmalaria', only a fraction of these fevers would have been truly attributable to parasitaemia. If algorithms derived for 'Emalaria' in children under six years of age were used in the treatment of 'Tmalaria', this would lead to the correct treatment of 45% of those with a history of fever and accompanying parasitaemia, but also about 20% of those with no parasitaemia and about 50% of those with parasitaemia would not be treated. In this age group however,

there would be more children with a parasitaemia $<5,000$ parasites/ μl of blood not treated and more children with parasitaemia $>100,000$ parasites/ μl of blood that were treated. Among those 6-14 years of age, a similar pattern was repeated.

Chandramohan *et al.*, (2002) reviewed studies that have attempted to derive malaria algorithms for the diagnosis of malaria in children. Their review used a mixture of algorithms derived to define either 'Emalaria' or 'Tmalaria'. Algorithms that were derived from areas of high malaria endemicity would lead to more failure to treat and less drug wastage whereas algorithms derived in areas of low malaria endemicity would lead to less failure to treat but more drug wastage. However, due to the high risk of failure to treat whatever the endemicity, the paper concluded by encouraging treatment of all febrile children presenting to health facilities in endemic countries with an anti-malarial.

The same conclusion would be made in this analysis of data from Kilifi as more than 50% of children under 15 years of age that were not treated had a parasitaemia $>10,000$ parasites/ μl of blood. If such children are sent home, there is likely to be an increased risk of severe malaria and deaths and the risk of treating more children without parasitaemia for malaria may be considered a small price to pay.

Adults present a different scenario however. Studies conducted in Africa have concentrated on childhood malaria and few studies have derived clinical algorithms for diagnosing malaria in adults. In a study conducted in a holo-endemic area in Tanzania, it was found that the symptoms traditionally associated with malaria such as fever, pallor and splenomegally were not associated with clinical malaria in adults (Oster *et al.*, 2000). However, a study conducted in an area of low malaria endemicity in India, derived an algorithm with a sensitivity of 71% and a specificity of 40% that performed in the same

way as a clinician without microscopy (Chandramohan *et al.*, 2001). A study conducted in Papua New Guinea among adults found that abnormal stool (constipation or diarrhoea), associated with a normal spleen were good predictors of malaria (Genton *et al.*, 1994a).

It has been difficult to derive an algorithm for adults in areas of either low or high endemicity and common predictors for malaria in children are not necessarily good predictors of malaria in adults. The algorithms also have very poor specificity and predictive values are often low due to the low prevalence of adult malaria in areas with endemic transmission. Only a quarter of the adults that presented to the study clinic in Kilifi had a positive slide, whereas, in Tanzania, positive slides were found only during the malaria season (Oster *et al.*, 2000).

From this study it would be recommended that in the absence of specific diagnosis (microscopy or dipstick), all children that present to the clinic with a history of fever be treated for malaria. For adults however, a slide ought to be taken so that malaria can be eliminated from the diagnosis. A delay in the treatment of adults for malaria is unlikely to have deleterious effects as compared to children. Avoidance of failure to treat should be a priority in children whereas in adults the priority should be a reduction in drug wastage.

CHAPTER SEVEN

MALARIA ADMISSIONS IN KILIFI

7.1 Introduction

It has been estimated that about 750,000 deaths occur each year among African children as a result of malaria (Snow *et al.*, 1999a). In endemic areas, almost all cases of severe malaria and malarial deaths occur in children as adults have developed solid immunity to severe malaria. Severe malaria has traditionally been regarded as falling into two main syndromes: severe malaria anaemia and cerebral malaria. Recently however, an additional syndrome of respiratory distress has been incorporated into the definition for severe malaria (WHO, 1986, Marsh *et al.*, 1995).

Descriptions of the clinical pattern of severe malaria have been published in the last ten years from several countries within Africa (Snow *et al.*, 1994; Slutsker *et al.*, 1994; Snow *et al.*, 1997; Modiano *et al.*, 1998; Schellenberg *et al.*, 1999; Marsh and Snow, 1999). Several patterns have emerged. Firstly, within any given area, severe malaria anaemia is more common in younger children whereas cerebral malaria has been observed to be more common in older children. Secondly, children from areas with high malaria transmission also tend to get severe malaria when they are younger than children from areas with moderate to low malaria transmission. As a result, severe malaria in children from areas of high transmission presents more commonly as severe malaria anaemia, however in areas of moderate to low transmission, cerebral malaria is relatively more common than in areas of high malaria transmission (Snow *et al.*, 1997). These conclusions about the relationship between transmission and clinical pattern of disease have been drawn from comparisons of widely separated areas in which it is possible that other factors also affect the clinical picture.

Severe malaria anaemia is associated with lower mortality compared to cerebral malaria (Marsh *et al.*, 1995; Brewster *et al.*, 1990). However, due to the HIV-AIDS epidemic in Africa, there is an increased health risk among children with severe malaria anaemia that need blood transfusions (Snow *et al.*, 1999a). Cerebral malaria is not only associated with a higher mortality but also with the occurrence of neurological sequelae (Molyneux *et al.*, 1989; Walker *et al.*, 1992; Crawley *et al.*, 1996). Therefore, the burden of severe malaria is not restricted to disease and death but also a reduction in the quality of life in children that survive.

In this chapter, data is presented comparing the patterns of severe malaria in two areas (Chonyi and Ngerenya) under differing malaria transmission that are 30 kilometres apart but contain populations from the same ethnic group living in very similar circumstances. Two sets of analysis are performed. The first set of data is from paediatric admissions in children that had been under active surveillance in selected households as described in earlier chapters. As there were few admissions from these study participants in that time period, a second set of data was analysed. This data was derived from paediatric admissions from all households in the wider Chonyi and Ngerenya study area in a one year period between January to December 2000.

7.2 Materials and methods

All the data used in this analysis were collected at the paediatric wards at the Kilifi District Hospital (KDH). This section describes the data collection process and analysis.

7.2.1 Clinical surveillance

The Kilifi District Hospital (KDH) is located on the Kenyan coast in a mainly rural malaria-endemic area. There are approximately 5,000 paediatric admissions annually, the majority of whom are treated in the general paediatric ward (36-bedded ward one).

However, there is a high dependency Kenya Medical Research Institute (KEMRI) unit with 6 beds which is situated within KDH to which children requiring more intensive care are admitted. All paediatric admissions to the KDH were assessed by a KEMRI clinician and a standard set of laboratory parameters were collected routinely depending. Baseline investigations included determination of venous blood gas urea and creatinine (CIBA corning diagnostics Ltd., London, UK), sodium and potassium (CIBA corning, ion-selective electrode, London, UK), blood glucose (Analox™ GM6, microstat analyser, London, UK) haemoglobin concentration and white cell count (Coulter counter, Coulter electronics, UK). Thick and thin blood films were stained with Giemsa and counted for asexual stages of *P. falciparum*. Blood cultures were taken as part of the standardised admission procedure.

A primary diagnosis of malaria was made if the child had a positive blood film for *P. falciparum* at admission with no other detectable cause for the clinical presentation. A child was considered to have severe malaria if they had a positive slide and were either severely anaemic, prostrated, had impaired consciousness or respiratory distress. Severe anaemia was defined as a haemoglobin level <5g/dL. Prostration was defined as the inability to sit up unaided for children >1 year old or the inability to breastfeed in those ≤ 1 year of age (Marsh *et al.*, 1995). Impaired consciousness was defined as a Blantyre Coma Score (BCS) of less than 5 in those children aged > 8 months or less than 4 in children aged ≤ 8 months (Molyneux *et al.*, 1989).

7.2.2 Data analysis

Data analysis was conducted using STATA® software, version 7.0 and is reported in two parts. The first is the analysis of the longitudinal data collected from the study participants from selected households that were followed up for at least two years (as described in

section 3.2.4) and who were admitted to hospital. During the period from September 1998 to June 2001, there were a total of 182 children admitted to the paediatric ward at the Kilifi District hospital from the Ngerenya cohort whereas there were 86 admissions from the Chonyi cohort from May 1999 to June 2001.

Due to the small number of admissions among the study participants, comparisons of proportions between the two study areas were difficult to make. A second set of data was reviewed which included all admissions in the wider Ngerenya and Chonyi study areas. This 'wider' area included all the children admitted from all the households that had not been selected into the longitudinal studies alongside those that had been. Admissions from the wider study area between January and December 2000 were included in this analysis. This included 431 paediatric admissions from the wider Ngerenya and 337 paediatric admissions from wider Chonyi.

Proportions with various clinical criteria were estimated and comparisons made between the two study areas using Chi-squared tests. Odds ratios were used to estimate the strength of the associations between various clinical criteria and admissions with malaria compared to non-malaria admissions. The Kruskal-Wallis test was also used to compare median age, parasite density and haemoglobin levels from paediatric admissions from these two study areas to test the hypothesis that the different sets of data come from a population with the same median.

7.3 Results

Results of paediatric admissions shall be described separately for data collected from study participants involved in the longitudinal study (section 7.3.1) and those from paediatric admissions from the wider study areas (section 7.3.2).

7.3.1. Admissions from the longitudinal morbidity study

Out of a total of 268 admissions, 92% and 86% from Chonyi and Ngerenya respectively were children under five years of age. Only 6(2.2%) of these admissions were transferred to the high intensive KEMRI ward, five of whom had a peripheral parasitaemia (two from Chonyi and three from Ngerenya). There were 4 (1.7%) deaths in total, two from each of the study areas, none of whom were admitted to KEMRI ward. None of the children that died had parasitaemia on admission and it could be assumed that the deaths were not attributable to malaria.

A malaria admission was defined as a child that had a peripheral parasitaemia at admission and a final diagnosis of malaria. In Chonyi, 51 (59.3%) of the admissions were due to malaria compared to 35 due to other causes whereas in Ngerenya, 110(60.4%) of the admissions were due to malaria compared to 72 due to other causes. Two children fitted this criteria but were included in the non malaria admissions as they were also diagnosed with tuberculosis and pyomyositis.

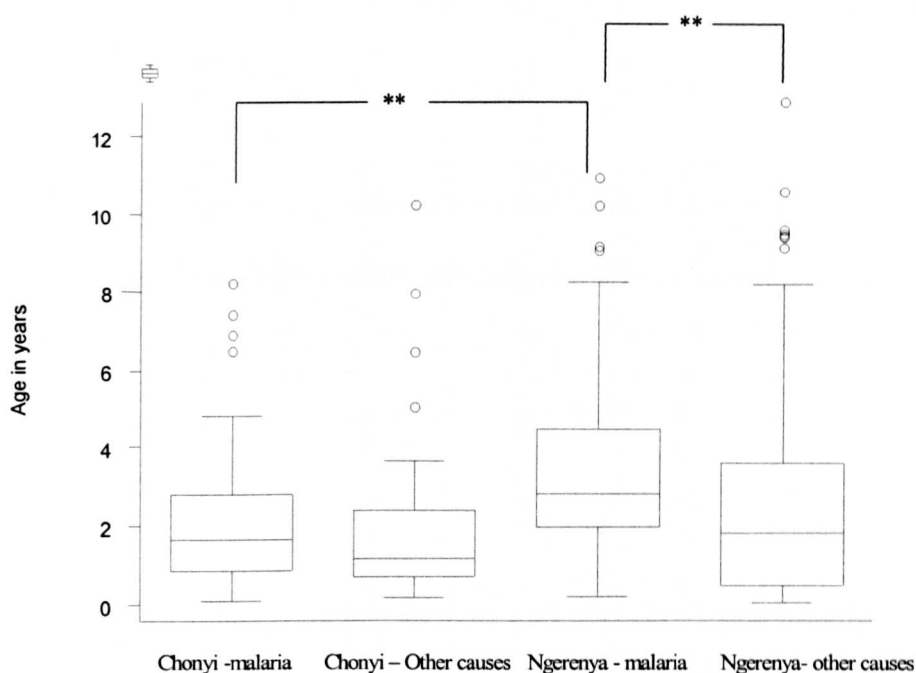
It is possible to calculate the risk of malaria admissions using the population of children under 12 years of age under surveillance in June 2000, which was 474 and 496 for Chonyi and Ngerenya respectively. Therefore the risk of being a malaria admission for children under 12 years old in Ngerenya was one in every 22 whereas in Chonyi, it was one every 10 children.

7.3.1.1 Age of malaria and non-malaria admissions

Figure 7.1 shows the age distribution of paediatric admissions with or without malaria in the two study areas. The median age among paediatric malaria admissions from Chonyi was 1.6 years (IQR 0.9 – 2.8) compared to 1.1 years (IQR: 0.7 – 2.4) among non-malaria paediatric admissions from the same area. The median age among paediatric malaria

admissions from Ngerenya was 2.8 years (IQR: 1.9 – 4.5) compared to 1.8 years (IQR:0.5-3.4) among non-malaria paediatric admissions from the same area.

Figure 7.1: Box and whisker plots for age of malaria and non-malaria paediatric admissions among study participants from Chonyi and Ngerenya.



Key : Chonyi-malaria – Paediatric malaria admissions from Chonyi
 Chonyi-other causes- Non- malaria paediatric admissions from Chonyi
 Ngerenya-malaria – Paediatric malaria admissions from Ngerenya
 Ngerenya-other causes- Non- malaria paediatric admissions from Ngerenya

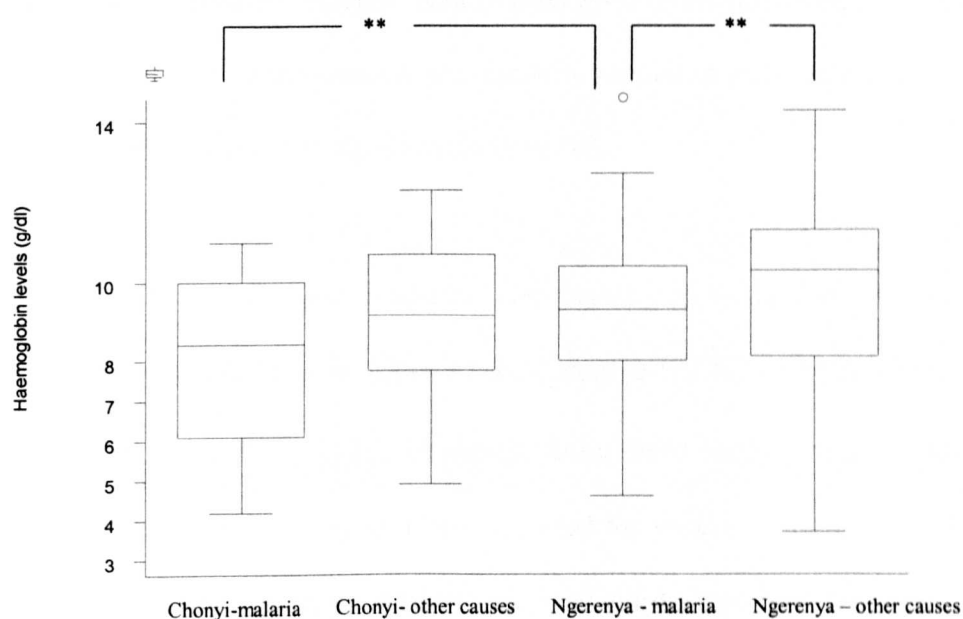
The Kruskal-Wallis test was used to test the hypothesis that these four groups of children were from a population with the same median age. Malaria paediatric admissions from Ngerenya had a higher median age than non-malaria admissions from the same area ($p < 0.001$) whereas there was no evidence of a difference in the median age between malaria and non-malaria paediatric admissions from Chonyi ($p = 0.2$). There was no evidence of a difference in the median age among paediatric admissions that were due to

other causes other than malaria from Chonyi and Ngerenya ($p=0.7$). However, there was evidence that paediatric malaria admissions from Ngerenya had a higher median age compared to those from Chonyi ($p<0.001$)

7.3.1.2 Haemoglobin levels among malaria and non-malaria admissions

Figure 7.2 shows the haemoglobin (Hb) levels in paediatric admissions with or without malaria in the two study areas.

Figure 7.2: Box and whisker plot of haemoglobin levels of malaria and non-malaria paediatric admissions among study participants from Chonyi and Ngerenya.



Key: Chonyi-malaria – Paediatric malaria admissions from Chonyi
 Chonyi-other causes- Non- malaria paediatric admissions from Chonyi
 Ngerenya-malaria – Paediatric malaria admissions from Ngerenya
 Ngerenya-other causes- Non- malaria paediatric admissions from Ngerenya

The median Hb levels among paediatric malaria admissions from Chonyi was 8.4g/dL (IQR: 6.1 – 10) compared to 9.2g/dL (IQR: 7.8 – 10.7) among non-malaria paediatric

admissions from the same area. The median Hb level among malaria paediatric admissions from Ngerenya was 9.3g/dL (IQR: 8 – 10.4) compared to 10.3g/dL (IQR: 8.1 – 11.3) among non-malaria paediatric admissions from the same area.

The Kruskal-Wallis test was used to compare whether the four groups were from a population with the same median. There was a significantly higher median Hb levels among children admitted for other causes compared to those admitted for malaria among paediatric admissions from Ngerenya ($p=0.007$) however this difference was of borderline significance among paediatric admissions from Chonyi ($p=0.05$). There was evidence of a higher median haemoglobin level among children admitted for malaria in Ngerenya compared to those admitted for malaria from Chonyi ($p=0.008$). However, the difference in the median haemoglobin levels among non-malaria paediatric admissions from the two areas was of borderline statistical significance ($p=0.08$).

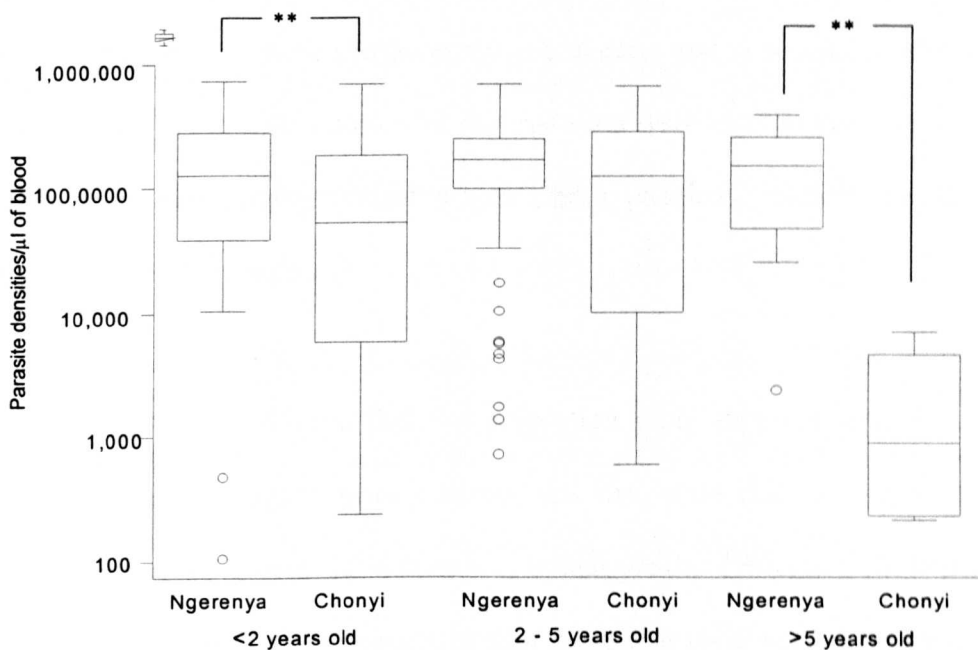
Levels of mild ($Hb < 11g/dL$) and moderate ($Hb < 8g/dL$) anaemia were similar among malaria paediatric admissions from Chonyi and Ngerenya [(57% Vs 64%, $\chi^2=0.5$, $p=0.5$) and (33% Vs 24%, $\chi^2=2.03$, $p=0.2$)]. However, there were slightly more children with severe anaemia ($Hb < 5g/dL$) among children admitted for malaria from Chonyi compared to Ngerenya though the statistical significance was borderline (6% Vs 0.9%, $\chi^2=3.6$, $p=0.06$). There was however a higher proportion of children with $Hb \geq 11g/dL$ from Ngerenya than Chonyi (12.8% Vs 2%, $\chi^2=4.7$, $p=0.03$).

7.3.1.3 Comparison of admission parasite densities

The median parasite density among paediatric malaria admissions from Ngerenya was 152,582 parasites/ μ l of blood (IQR: 72,208 – 271, 772) compared to 60,635 parasites/ μ l of blood (IQR: 3,072 – 220,094) from Chonyi. There was evidence of a higher parasite

density among malaria paediatric admissions from Ngerenya compared to Chonyi ($p=0.001$). Figure 7.3 shows the parasite densities among children in the different age groups from Chonyi and Ngerenya. The median parasite density among paediatric malaria admissions aged <2 years of age was 125,790 parasites/ μl of blood (IQR: 38,870 – 275,104) in Ngerenya compared to 53,259 parasites/ μl of blood (IQR: 5,856 – 180,956) from Chonyi.

Figure 7.3: Box and whisker plot of parasite density (log scale) by age of paediatric malaria admissions among study participants from Chonyi and Ngerenya.



The median parasite density among malaria paediatric admissions aged 2-5 years of age was 167,838 parasites/ μl of blood (IQR: 98,591 – 246,478) in Ngerenya compared to 122,999 parasites/ μl of blood (IQR: 18,518 – 280,349) in Chonyi. The median parasite density among paediatric malaria admissions 6-11 years of age from Ngerenya was 148,002 parasites/ μl of blood (IQR: 46,334 – 251,743) compared to 1,659 parasites/ μl of blood (IQR: 229 – 5,032) in Chonyi.

There was evidence of a higher median parasite density among paediatric malaria admissions <2 years old from Ngerenya compared to those from Chonyi ($p=0.04$). There was however no evidence of a difference in the median parasite density among paediatric malaria admissions from the two areas among children aged 2-5 years old ($p=0.3$). There was evidence of a higher median parasite density among malaria paediatric admissions aged 6-11 years of age from Ngerenya compared to Chonyi ($p=0.005$).

7.3.1.4 Clinical criteria for malaria admissions

Table 7.1 shows proportions with varying clinical criteria for severe malaria among children admitted to the Kilifi district hospital from the longitudinal study. Proportions of those with varying criteria were compared among malaria and non-malaria admissions by univariate analysis using odds ratios. Chi-squared tests were used to compare differences in the proportions with varying clinical criteria among paediatric malaria admissions only from both Chonyi and Ngerenya.

In Ngerenya, there was evidence that the proportion with impaired consciousness and respiratory distress was higher among admissions that were due to causes other than malaria. The only malaria specific marker was hyperpyrexia. Children with hyperpyrexia were 5.5 times more likely to have malaria than those that were not hyperpyrexia among paediatric admissions from Ngerenya [Unadjusted odds ratios= 5.5 (CI: 1.2 – 25.7), $p=0.01$]. No such relationship was observed in Chonyi, in fact the data showed a reduction in the odds of hyperpyrexia among malaria admissions compared to non-malaria admissions which though not statistically significant was unexpected. Hyperparasitaemia was the only one of the selected clinical criteria for which there was evidence of an association characterising a malaria admissions from Chonyi.

Table 7.1: Proportions and risk of being a malaria admission using various clinical criteria among paediatric admissions from study participants from Ngerenya and Chonyi.

Clinical criteria	Ngerenya			Chonyi		
	Prevalence - n (%)		Odds ratio (P-value)	Prevalence - n (%)		Odds ratio (P-value)
	Malaria	Other causes		Malaria	Other causes	
^a Severe anaemia	1 (0.9)	2 (2.8)	0.32 (p=0.3)	3 (5.9)	1 (2.9)	2.1 (p=0.5)
^b Hypoglycaemia	3 (16.7)	2 (8.3)	2.2 (p=0.4)	1 (12.5)	1 (10)	1.3 (p=0.9)
^c Acidosis	7 (25.9)	6 (37.5)	0.6 (p=0.4)	10 (40)	6 (54.6)	0.6 (p=0.4)
^d Impaired consciousness	2 (1.9)	6 (11.8)	0.15 (p=0.01)*	4 (9.8)	3 (10.7)	0.9 (p=0.9)
^e Repeated convulsions	4 (3.6)	3 (4.2)	0.9 (p=0.9)	1 (2)	1 (2.9)	0.7 (p=0.8)
^f Hyperparasitaemia	75 (68.2)	0	>>>	20 (39.2)	1 (2.9)	21.9 (p<0.001*)
^g Hyperpyrexia	15 (13.6)	2 (2.8)	5.5 (p=0.01)*	1 (2)	1 (2.9)	0.7 (p=0.8)
^h Prostration	3 (2.8)	3 (4.3)	0.6 (p=0.6)	1 (2.3)	1 (2.9)	0.8 (p=0.9)
ⁱ Respiratory distress	3 (5.9)	25 (45.5)	0.08 (p<0.001)*	8 (24.2)	10 (34.5)	0.6 (p=0.4)
Jaundice	2 (1.8)	3 (4.2)	0.4 (p=0.3)	3 (5.9)	0	-

key

^aAnaemia - Hb <5g/dl

^bHypoglycaemia - glucose <2.2 mmol/l – data available from 60 children

^cAcidosis - plasma bicarbonate <15mmol/l – data available for 79 children

^dImpaired consciousness – Inability to localise pain for those >9 months and lack of directed eye movements for those under 9 months of age

^eRepeated convulsions - 3 or more fits in 24 hours

^fHyperparasitaemia - >100,000 parasites/μl of blood

^gHyperpyrexia - Axillary temperatures >40°C

^hProstration – Patient cannot sit unaided or breastfed in those <1 year old

ⁱRespiratory distress – Those with either nasal flaring, chest indrawing or deep breathing

*- Those with statistically significant associations between the clinical criteria and the risk of admission with malaria

>>>- odds ratio was extremely high

Denominator for proportions admissions from the morbidity study areas.

Next, instead of comparing malaria and non-malaria admissions in the same area, proportions with various clinical criteria among malaria admissions from Chonyi and Ngerenya are compared. There was evidence of a higher proportion of malaria paediatric admissions with respiratory distress from Chonyi compared to Ngerenya (24.2% Vs 5.9%, $\chi^2 = 5.31$, p=0.02). However, there was evidence of a higher proportion of malaria paediatric admissions with hyperparasitaemia in Ngerenya compared to Chonyi (68.2% Vs 39.2%, $\chi^2 = 12.1$, p=0.001). There was also evidence of a higher proportion of malaria admissions with hyperpyrexia in Ngerenya compared to Chonyi (13.6% Vs 2%, $\chi^2 = 5.31$,

p=0.02). However, there was evidence that a higher proportion of malaria paediatric admissions from Chonyi that had severe anaemia compared to malaria admissions from Ngerenya however the difference was of borderline statistical significance (5.9% Vs 0.9%, $\chi^2 = 3.56$, p=0.06).

As there were only 51 malaria admissions from Chonyi that were compared to 110 malaria admissions from Ngerenya, admission data from the wider Ngerenya and Chonyi study area was incorporated into the analysis.

7.3.2. Analysis of data from wider study area admissions

As with the analysis using the longitudinal data, a malaria definition was based on the presence of peripheral *P. falciparum* parasitaemia at admission accompanied by the clinicians final diagnosis of malaria. There were 431 paediatric admissions from the wider Ngerenya study area of which 184 (43%) were attributed to malaria. There were 337 paediatric admissions from the wider Chonyi area of which 134 (40%) were due to malaria. Forty-nine (49) children had peripheral parasitaemia but the final diagnosis was not malaria and therefore these children were excluded from the malaria group.

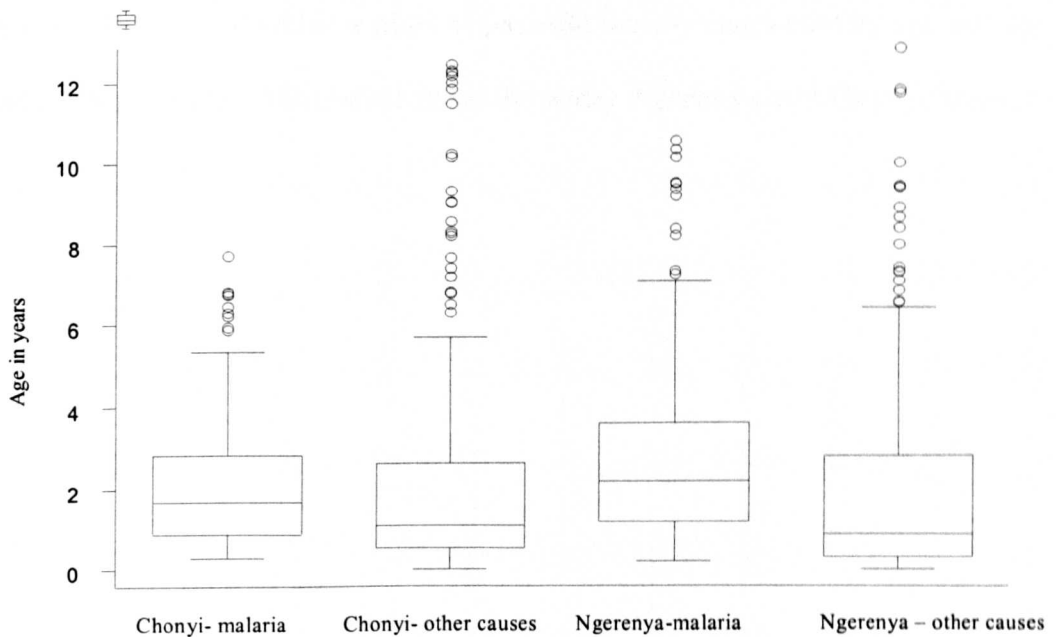
A total of 38 deaths (4.9%) occurred among these paediatric admissions, 15 (3.5%) from the wider Ngerenya area and 23 (6.8%) from the wider Chonyi area. Only five of the deaths were due to malaria two from Ngerenya and three from Chonyi. Only 2 of the children with malaria (one from each area) were discharged with neurological sequelae.

7.3.2.1 Age patterns among the malaria and non-malaria admissions

The age pattern of malaria and non-malaria admissions from both study areas are shown on Figure 7.4. The median age among paediatric malaria admissions from Ngerenya was 2.2 years (IQR: 1.2-3.6) compared to 11 months (IQR: 0.3 – 2.8) among non-malaria

admissions from the same area. The median age for paediatric malaria admissions from Chonyi was 1.7 years (IQR: 0.9 – 2.8) compared to 1.1 years (IQR: 0.5 – 2.6) among non-malaria admissions from the same area.

Figure 7.4: Box and whisker plots for age of malaria and non-malaria paediatric admissions from the wider Ngerenya and Chonyi areas.



Key : Chonyi-malaria – Paediatric malaria admissions from Chonyi

Chonyi-other causes- Non- malaria paediatric admissions from Chonyi

Ngerenya-malaria – Paediatric malaria admissions from Ngerenya

Ngerenya-other causes- Non- malaria paediatric admissions from Ngerenya

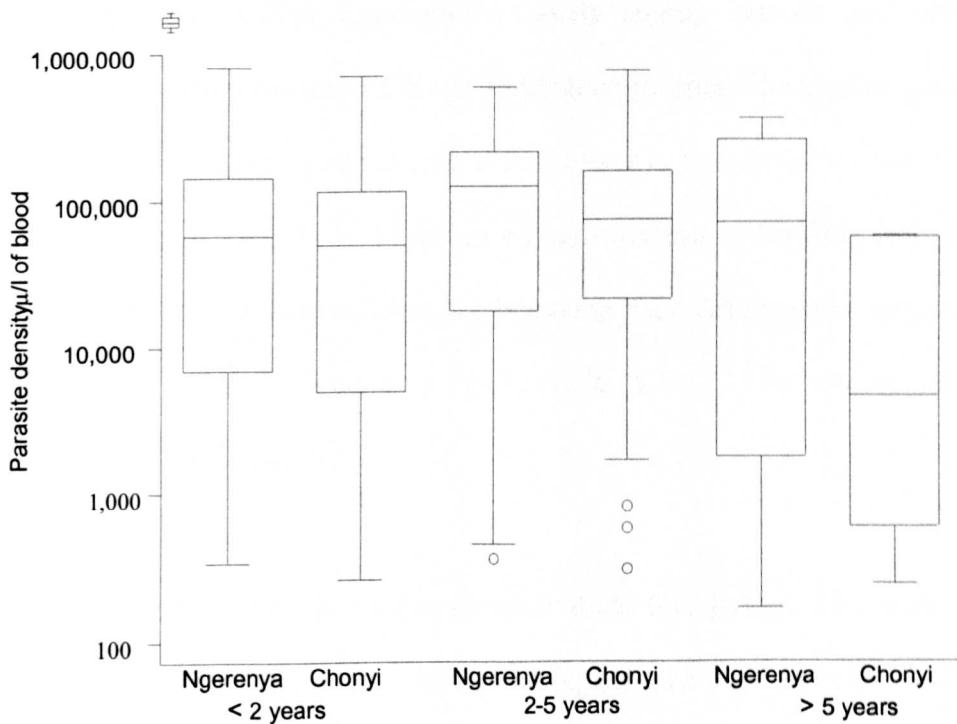
The Kruskal-Wallis test was used to compare the medians among the four groups from Ngerenya and Chonyi. The median age of malaria paediatric admissions was higher than that of paediatric admissions due to other causes in both Ngerenya ($p < 0.001$) and Chonyi ($p = 0.009$). There was evidence of a higher median age of paediatric malaria admissions for Ngerenya compared to Chonyi ($p = 0.004$) but there was evidence that the median age was

higher among children admitted for other causes in Chonyi compared to Ngerenya ($p=0.02$).

7.3.2.2 Parasitaemia among the malaria and non-malaria admissions

The levels of parasitaemia in paediatric malaria admissions from the two study areas by age were demonstrated on Figure 7.5.

Figure 7.5: Box and whisker plots of parasite density (log scale) by age among malaria paediatric admissions from the wider Ngerenya and Chonyi areas.



The median parasite density among paediatric malaria admissions aged <2 years of age was 49,617 parasites/ μ l of blood (IQR: 5,688 – 134,185) in Ngerenya compared to 42,618 parasites/ μ l of blood (IQR: 4,646 – 112,392) from Chonyi. The median parasite density among malaria paediatric admissions aged 2-5 years of age was 115,459 parasites/ μ l of

blood (IQR: 7,872 – 212,006) in Ngerenya compared to 72,698 parasites/ μ l of blood (IQR: 21,276 – 156,956) in Chonyi. The median parasite density among paediatric malaria admissions 6-11 years of age from Ngerenya was 31,620 parasites/ μ l of blood (IQR: 1,320 – 256,805) compared to 1,314 parasites/ μ l of blood (IQR: 246 – 35,680) in Chonyi.

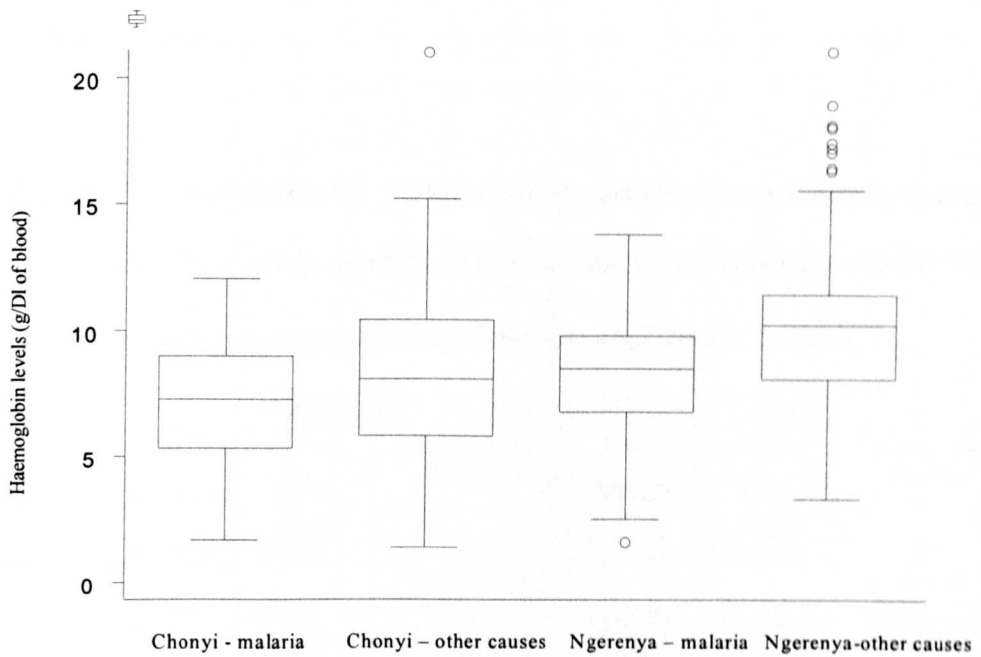
When the Kruskal –Wallis test was used to compare the median parasite densities in all the groups, there appeared to be no evidence of a difference in the median parasite density between any of these groups ($p>0.3$).

7.3.2.3 Haemoglobin levels among the malaria and non-malaria admissions

Figure 7.6 shows the median haemoglobin levels among malaria and non-malaria paediatric admissions from the wider Chonyi and Ngerenya area. The median haemoglobin levels among malaria paediatric admissions from Ngerenya was 8.5g/dL (IQR: 6.8 – 9.8) compared to 10.2g/dL (IQR: 8.1 – 11.4) among non-malaria admissions from the same area. In Chonyi, the median haemoglobin levels among paediatric malaria admissions was 7.3g/dL (IQR: 5.3 - 9) compared to 8.1g/dL (IQR: 5.8 – 10.4) among non-malaria admissions from the same area.

When the Kruskal-Wallis test was used to compare these four groups, they were all found to differ from each other. There was evidence of higher haemoglobin levels among non-malaria paediatric admissions compared to malaria admissions from both Ngerenya ($p<0.001$) and Chonyi ($p<0.001$). There was evidence of a higher median haemoglobin level among paediatric admissions from Ngerenya compared to those from Chonyi, whether malaria admissions ($p<0.001$) or non-malaria admissions ($p<0.001$) were compared.

Figure 7.6: Box and whisker plot of haemoglobin levels among malaria and non-malaria paediatric admissions from the wider Chonyi and Ngerenya areas



Key : Chonyi-malaria – Paediatric malaria admissions from Chonyi
 Chonyi-other causes- Non- malaria paediatric admissions from Chonyi
 Ngerenya-malaria – Paediatric malaria admissions from Ngerenya
 Ngerenya-other causes- Non- malaria paediatric admissions from Ngerenya

Among children admitted for malaria, there were differences in the levels of mild (hb<11g/dL) moderate (hb<8g/dL) and severe (hb<5g/dL) anaemia in the two areas. There was a higher level of mild anaemia in Ngerenya compared to Chonyi (58.3% Vs 36.6%, $\chi^2=3$, p=0.08) though the statistical significance was borderline. There was a higher proportion with moderate as well as severe anaemia in Chonyi compared to Ngerenya [(38.1% Vs 27.7%, $\chi^2=3.8$, p=0.05) and (20.9% Vs 11.4%, $\chi^2=5.3$, p=0.02)]

7.3.2 4 Clinical criteria of severe malaria

Table 7.2 demonstrates the prevalence of the various clinical criteria in the two study areas and their associations with a malaria diagnosis investigated using univariate analysis. There was evidence that those paediatric admissions with hyperparasitaemia, hyperpyrexia

and severe anaemia were more likely to be malaria admissions than those without any of these clinical criteria ($p<0.03$). There was also evidence that those without respiratory distress from both study areas were more likely to be malaria cases than those that had respiratory distress ($p<0.01$).

Table 7.2: Proportions and risk for malaria using various clinical criteria among paediatric admissions from study participants from the wider Ngerenya and Chonyi areas. Green lettering represents associations also found in Table 7.1.

Clinical criteria	Ngerenya			Chonyi		
	Prevalence - n (%)		Odds ratio (P-value)	Prevalence - n (%)		Odds ratio (P-value)
	Malaria	Other causes		Malaria	Other causes	
^a Severe anaemia	21 (11.4)	7 (2.8)	4.4 ($p<0.001$)*	28 (20.9)	20 (9.9)	2.4 ($p=0.005$)*
^b Hypoglycaemia	3 (6.8)	6(6.8)	1 ($p=1$)	4 (11.8)	13 (19.1)	0.56 ($p=0.9$)
^c Acidosis	21 (26.3)	23(23.7)	1.1 ($p=0.7$)	31 (40.3)	39 (36.5)	1.2 ($p=0.6$)
^d Impaired consciousness	16 (8.2)	36 (15.3)	0.56 ($p=0.06$)	17 (12.1)	24 (12.2)	0.96 ($p=0.9$)
^e Repeated convulsions	10 (5.6)	11 (4.5)	1.3 ($p=0.6$)	6 (4.7)	5 (2.5)	1.9 ($p=0.3$)
^f Hyperparasitaemia	85 (46.2)	2 (0.8)	105.2 ($p<0.001$)*	44 (32.8)	1 (0.5)	98.8 ($p<0.001$)*
^g Hyperpyrexia	28 (15.2)	12 (4.9)	3.5 ($p<0.001$)*	10 (7.5)	5 (2.5)	3.2 ($p=0.03$)*
^h Prostration	21 (11.4)	43 (17.4)	0.8 ($p=0.5$)	24 (17.9)	39 (19.2)	0.8 (0.5)
ⁱ Respiratory distress	17 (9.2)	89 (36)	0.18 ($p<0.001$)*	26 (19.4)	64 (31.5)	0.52 ($p=0.001$)*
Jaundice	7 (3.8)	9 (3.6)	1.1 ($p=0.9$)	5 (3.7)	9 (4.4)	0.84 ($p=0.8$)

key

^aAnaemia - Hb $<5g/dl$

^bHypoglycaemia - glucose <2.2 mmol/l – data available from 234 persons

^cAcidosis - plasma bicarbonate <15 mmol/l – data available for 361

^dImpaired consciousness – Inability to localise pain for those >9 months and lack of directed eye movements for those under 9 months of age

^eRepeated convulsions - 3 or more fits in 24 hours

^fHyperparasitaemia - $>100,000$ parasites/ μ l of blood

^gHyperpyrexia - Axillary temperatures $>40^{\circ}C$

^hProstration – Patient cannot sit unaided or breastfed for those <1 year old

ⁱRespiratory distress – Those with either nasal flaring, chest indrawing or deep breathing

*- Statistically significant association between clinical criteria and being a malaria admission

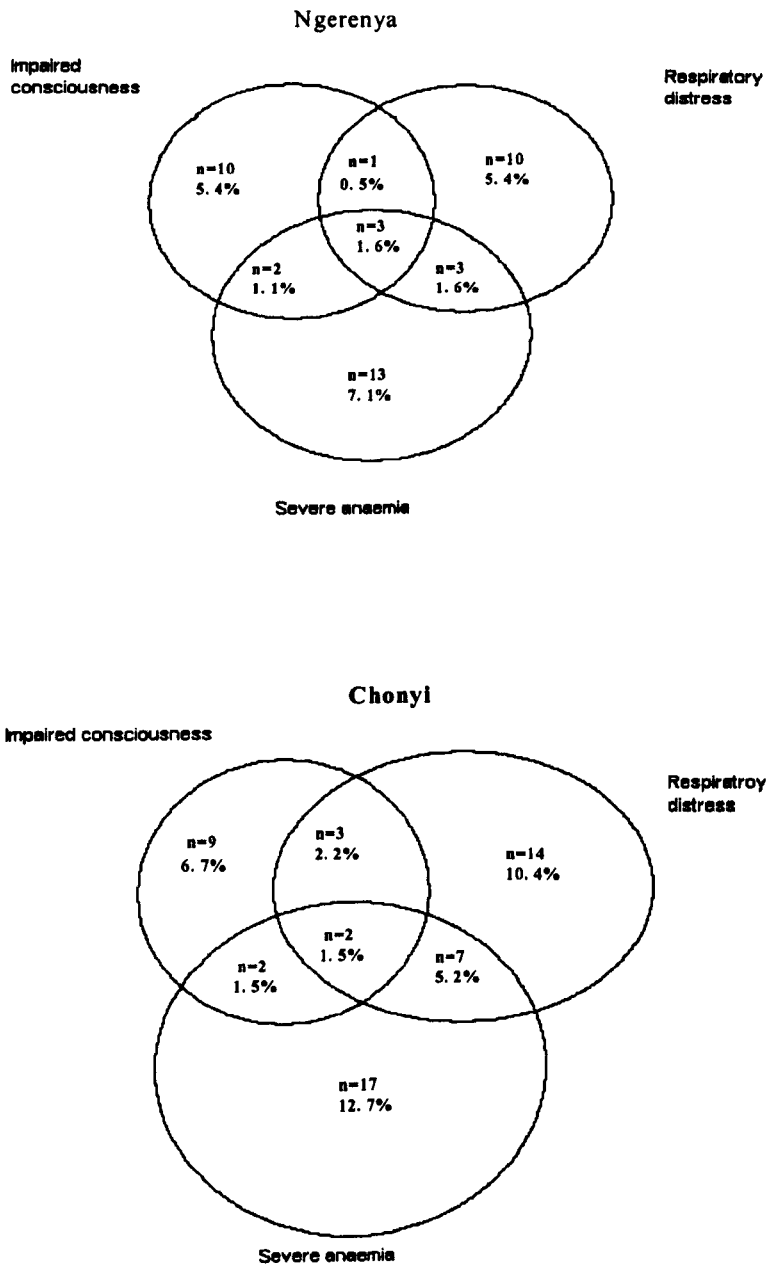
There were some similarities between the findings found using this larger database and that found with the smaller database as described on Table 7.1. These included associations between being a malaria admission and hyperparasitaemia in both study areas. Hyperpyrexia, no respiratory distress and no impaired consciousness were also associated

with being a malaria admission in the smaller data base though in this wider study area data base, the statistical significance between no impaired consciousness and malaria has a borderline statistical significance.

Comparisons were also made between proportions diagnosed with malaria in the two study areas that had these clinical criteria to find out if there were differences in malaria clinical presentation in the two study areas. There was evidence of a higher proportion of malaria admissions with hyperparasitaemia from Ngerenya than from Chonyi (46.2% Vs 32.8%, $\chi^2 = 5.74$, $p=0.02$) and also a higher rate of hyperpyrexia in Ngerenya compared to Chonyi (15.2% Vs 7.5%, $\chi^2 = 4.43$, $p=0.04$). These two associations were similar to those found in the smaller database (Table 7.1). There was also evidence of a higher proportion of malaria paediatric admissions with acidosis in Chonyi compared to Ngerenya although the differences were of borderline statistical significance (40.3% Vs 26.3%, $\chi^2 = 3.48$, $p=0.06$), this association that was not found earlier in Table 7.1.

However, of great interest were differences in the proportion with severe anaemia, impaired consciousness and respiratory distress in the two areas, these are described graphically on Figure 7.7. When severe malaria was classified as either severe malaria anaemia, respiratory distress and impaired consciousness, then there were 54 (40.3% of malaria admissions) cases of severe malaria from Chonyi and 42 (22.8% of malaria admissions) cases from Ngerenya. Therefore despite the high numbers of paediatric malaria admissions in Ngerenya, more of those from Chonyi had severe malaria than those from Ngerenya.

Figure 7.7: Proportions of severe malaria admissions using the three definitions of severe malarial anaemia, respiratory distress and impaired consciousness from the wider Ngerenya and Chonyi study areas (As a percentage of total malaria admissions).



There were differences in the proportion with severe anaemia in Chonyi compared to Ngerenya (20.9% Vs 11.4%, $\chi^2 = 5.35$, $p=0.02$) but there was no difference in the proportion with impaired consciousness among the malaria admissions from Ngerenya and

Chonyi (8.2% Vs 12.1%, $\chi^2 = 1.4$, $p=0.2$). There was however evidence of a higher proportion of malaria paediatric admissions with respiratory distress from Chonyi compared with Ngerenya (19.4% Vs 9.2%, $\chi^2 = 6.84$, $p=0.009$). These relationships were similar to those observed in the smaller database (Table 7.1.). Figure 7.8 compares the age pattern of severe anaemia and impaired consciousness among malaria paediatric admissions from Ngerenya and Chonyi.

Figure 7.8: Age patterns of severe anaemia and impaired consciousness among study participants from the wider Ngerenya and Chonyi study areas

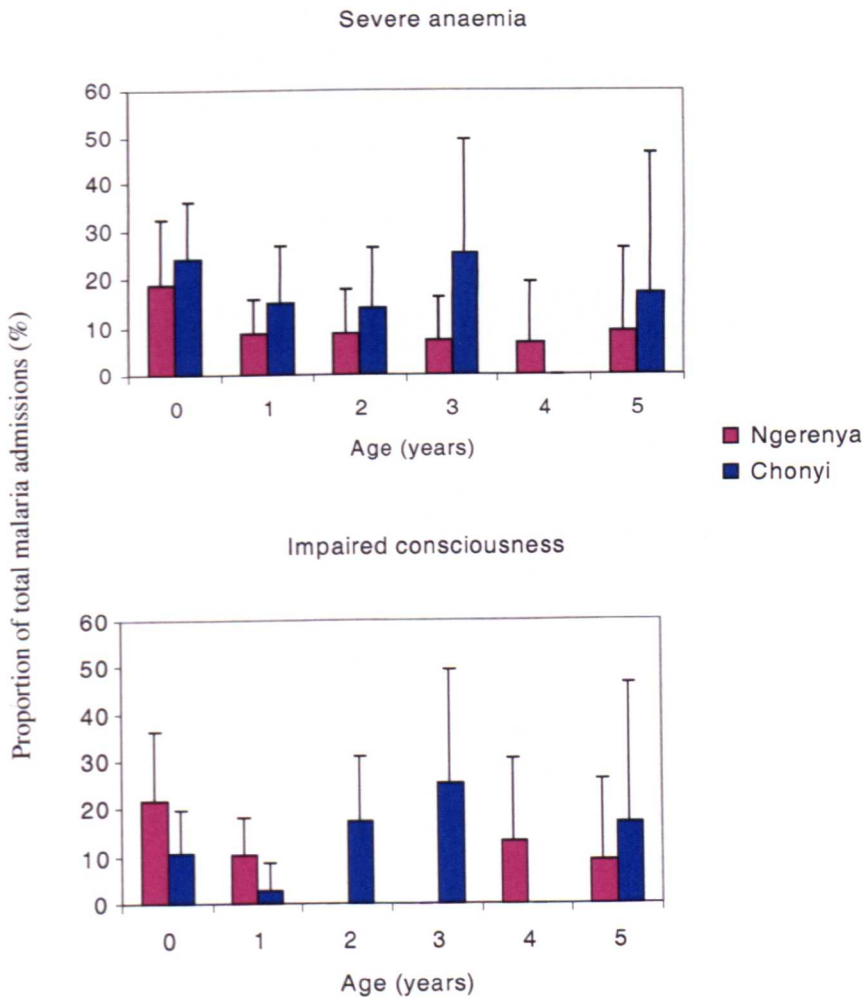


Figure 7.8a describes the pattern of severe malarial anaemia with age in the two study areas. When proportions within the different age groups in the two study areas were compared, there was no evidence of a difference in the proportions with severe anaemia in

the two areas. There were however differences in the rates of admissions with impaired consciousness among children from Ngerenya and Chonyi. There were 8 children with impaired consciousness among those aged two and three years of age in Chonyi, whereas there were no children admitted with malaria with impaired consciousness among children of this age from Ngerenya. There was also no consistent pattern in the rates of these two syndromes by age in the two study areas, however there were few cases and the confidence intervals were wide.

7.3.2.5 Measures of malarial admissions

We have described both malaria admissions and severe malaria admissions in the two study areas. This section is an attempt at quantifying malaria admissions in the two areas. The rates of malaria admission were derived using admissions from the wider Ngerenya and Chonyi and the denominator was the population living in this wider area in the year 2000 as provided from the demographic surveillance system in place within the study area (Bauni, personal communication).

Table 7.3: Paediatric admission rates among children from the wider Ngerenya and Chonyi area in the year 2000.

Area	Age	Population	Admission rates (per 1,000 children)	
			Malaria admissions	Non-malaria admissions
Chonyi	0 - 5 years	6,233	20.4	28.7
	6 - 12 years	6,684	1.0	3.6
Ngerenya	0 - 5 years	3,793	45.1	58.3
	6 - 12 years	3,896	3.3	6.7

The rate of malaria admission in children under six year of age in Ngerenya was 45.1 admissions/1,000 children compared to 20.4 admissions/1,000 children from Chonyi. However, the rate of malaria admissions in children 6-12 years of age was 1 admission/1,000 children in Chonyi compared to 3.3 admissions/1,000 children in Ngerenya. The rate of malaria admissions in Ngerenya was higher than that of Chonyi,

however, the rate of non-malaria admissions in Ngerenya were similarly higher than those from Chonyi.

There were only five deaths attributed to malaria in both areas. Using the WHO definition of severe malaria admissions (Prostration and respiratory distress), 39 children in Chonyi fitted this description, three of whom were over 5 years old. The severe malaria admission rate among children under five years of age in Chonyi was there 5.8/1,000 children with a mortality rate of 0.48/1,000 children.

In Ngerenya, 30 children were admitted with severe malaria according to this definition, one of whom was over five years of age. The severe malaria admission rate in this area was therefore 7.6/1,000 children with a mortality rate of 0.53/1,000 children.

7.4 Conclusion

From both the admissions that included study participants only and those from the wider study area, the median age for malaria admissions was higher among paediatric malaria admissions from Ngerenya (EIR=10) compared to those from Chonyi (EIR=50). This is consistent with data from other studies that have shown the average age for malaria admissions to be higher among children from areas with lower malaria transmission than that of children from areas with higher malaria transmission (Snow *et al.*, 1997). Snow and colleagues found this to be the case when they compared data from five study areas, three in The Gambia and two from Kenya with varying malaria transmission. However, one interesting finding in this study was that the median age of malaria admissions was higher compared to that of admission with other causes both in the low and high transmission setting. This may suggest that most causes of childhood morbidity and mortality exert their effect in very young children whereas malaria continues to be a problem for longer.

Haemoglobin levels were higher among non-malaria admissions compared to malaria admissions. This was consistent for both data sets comparing study participants only and the wider study area data and between the two study sites. This is not surprising and in most malaria endemic areas, malaria is one of the leading contributors to childhood anaemia (Newton *et al.*, 1997b; Kahigwa *et al.*, 2002). This may result from both chronic and acute malaria infections in these areas. Not surprisingly either median haemoglobin levels were consistently higher among the study participants from Ngerenya compared to those from Chonyi whether they were admitted for malaria or for other causes. This is consistent with the higher parasite prevalences among children from Chonyi compared to children from Ngerenya.

There were no differences in the median parasite densities among paediatric malaria admissions in the different age groups from the two study areas in the wider study area analysis. There were however differences in the median parasite densities among malaria admissions in children ≤ 1 year and over 5 years of age with Ngerenya study participants having a higher median parasite density. It is difficult to interpret these differences as they were not consistent in the two data bases and may have arisen in the smaller data set purely by chance but would be consistent with the idea that immunity regulating parasitaemia is distinct from immunity controlling malaria disease.

Overall there was a higher rate of severe malarial anaemia in Chonyi compared to Ngerenya which was consistent with earlier studies (Snow *et al.*, 1997). In both Chonyi and Ngerenya there appeared to be a gradual reduction in the rate of severe malaria anaemia being highest in those a year old and declining gradually upto the age of two years, however, the prevalence of anaemia remains high into the fourth year, this however may be as artefact of the small sample size. There was a higher proportion of children with

impaired consciousness among paediatric admissions from low transmission area compared to a high transmission area as observed in other studies (Snow *et al.*, 1997).

There was a higher rate of paediatric malaria admissions in Ngerenya (45.1 episodes/1,000 children) compared to Chonyi (20.4 episodes/1,000 children), there was similarly a higher severe malaria admission rate in Ngerenya (7.6 episodes/1,000 children) compared to Chonyi (5.6 episodes/1,000 children). This is despite a higher bednet use in Ngerenya compared to Chonyi. From the bednet survey conducted among study participants in July 2000, there was a higher coverage of bednets in households in Ngerenya (81.2%) compared to Chonyi (21.6%).

Despite the higher rate of severe malaria admissions from Ngerenya compared to Chonyi, the malaria mortality rate was the same in both areas. This may mean that though the admissions from Chonyi are fewer, they are more severe than those from Ngerenya, hence a similar mortality rate. However, if malaria cases were more severe in Chonyi than Ngerenya, then the non-malaria admissions would have the same pattern. Though the non-malaria admissions in the under five year olds was higher in Ngerenya compared to Chonyi, the non-malaria mortality rate in Ngerenya (2.63 deaths /1,000 children) was similar to that from Chonyi (2.72 deaths/1,000 children). It is therefore possible that more severely ill children from Chonyi come to the KDH compared to children from Ngerenya. During the longitudinal study, there were also more clinic attendants among study participants from Ngerenya compared to Chonyi (section 4.4). This was thought to be as a result of ease of getting vehicles to KDH.

It is therefore possible that there are more malaria cases that should be admitted from Chonyi that do not present to the hospital. It is therefore difficult to conclude that there is a higher severe malaria admission rate in Ngerenya, the area of low transmission compared

to Chonyi, the area of high transmission due to these differences in admission rates. It is possible that the true rate of severe malaria in these two areas is the same.

Conclusions

- 1- This study was conducted with the main aim of deriving case definitions for clinical malaria in two areas of moderate malaria transmission at the coast of Kenya. It was found that case definition varied with age and transmission and that probably for all malaria endemic areas in Africa, malaria case definitions will differ in the same way. This is a useful factor to bear in mind in interpretation of intervention studies where individual cases of malaria are used as an outcome. Younger children and adults are likely to have lower parasite density cut-offs in case definitions compared to older children. Interventions that alter immune status are also likely to alter case definition and this should be borne in mind when interpreting the data. For example if a malaria vaccine is introduced, the immune status changes and therefore the case definition may have to be changed. However, that would require a different case definition for the vaccinated and non-vaccinated groups, which will give the impression that the results have been manipulated in favour or against the vaccine. Using the same case definition for people of the same age group with varying immune status due to HIV was found to result in large differences in sensitivity of case detection (Whitworth *et al.*, 2000). Different case definitions that take into account the difference in immune status (vaccine or HIV status) ought to be considered in order to assess the effect of differences in case definition to the magnitude of intervention efficacy.

2. There were more episodes of non-severe malaria among those living under lower malaria transmission than those living under a higher transmission. This data seems to suggest therefore that a reduction of transmission from the level found in Chonyi to that in Ngerenya would lead to an increase in the rate of non-severe malaria and also to a shift in the age pattern of disease presentation. This is an undesirable trait as it suggests a disadvantage in lowering transmission. However, a review conducted as part of the thesis of studies across Africa under differing transmission (Appendix X1) has suggested that there was an increase in the number of non-severe malaria episodes in areas of low to moderate-high transmission after which the number of malaria episodes start to fall with increasing transmission. It maybe that Ngerenya and Chonyi are at the transition point where an increase in transmission leads to a reduction in disease.
3. An interesting finding emerged in this study that has also been reported in Senegal where long-term longitudinal studies have been conducted in the same population for a number of years (Trape et al., 2003). They reported that there are children with a higher susceptibility to clinical malaria and have more episodes than are expected for the age group. Using the occurrence of an episode of clinical malaria as an outcome in intervention of immuno-epidemiological studies may be replaced with the occurrence of multiple malaria episodes as this suggests increased susceptibility. Investigations into what makes these children more susceptible than others may help in the understanding of how malaria immunity is acquired and may lead to identification of effective vaccine targets.

4. Deriving clinical algorithms for malaria diagnosis for children (≤ 14 years) results in high predictive values but would result in a large number of children being sent home untreated but with high parasite densities which may lead to severe malaria and death. However, the pursuit of clinical algorithms in this age group is tempting due to their otherwise good performance but are however impractical. However, only a quarter of the adults with a history of fever had parasitaemia in this study. Algorithms among adults performed poorly. However, adults account for much of the malaria treatments in health facilities in Africa as observed in section 5.3.3. and therefore to much of the drug wastage. If rapid tests or microscopy was made available in health facilities in rural Africa, they could be used to differentiate adults that present to the health facilities with parasitaemia and those without and only those with parasitaemia treated. Very few studies have been conducted on adult malaria in Africa especially when it comes to the use of health facilities and drug wastage. A review by Snow *et al.* (2003), has however shown that adults account for almost half of the malaria treatments in malaria endemic areas yet in such areas, immunity to non-severe malaria is high and very few of the adults treated would actually have malaria if investigations were conducted. It is therefore probable that if more resources were spent on investigating malaria treatment in adults within rural health facilities in Africa, it would emerge that most of those treatments are unnecessary. This field of investigations would lead to more investment into diagnosing the presence of parasitaemia in adults presenting to health facilities with a history of fever while treating all children presenting to the clinic with a history of fever as

malaria cases. However, for this to be efficient, diagnostic kits have to be cheaper than the malaria treatments.

5. Our analysis of the effect of untreated bednets on the occurrence of clinical malaria has confirmed recent studies that untreated bednets offer a significant protection from clinical disease as compared to untreated bednets (Appendix XII). This suggests that in the absence of poor uptake of net re-treatments (as occurs in most studies in Africa), there is a great advantage of using untreated bednets than not using them at all while we await the permanently treated bednets.

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