SPOROZOITE CHALLENGE AND TRANSMISSION PATTERNS AS DETERMINANTS OF OCCURRENCE OF SEVERE MALARIA IN RESIDENTS OF KILIFI DISTRICT, KENYA.

BY

EPHANTUS WANJOHI KABIRU

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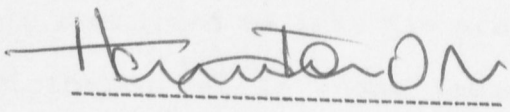
DECLARATION

I, EPHANTUS WANJOHI KABIRU, HEREBY DECLARE THAT THIS THESIS IS MY ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR A DEGREE IN ANY OTHER UNIVERSITY.

EPHANTUS WANJOHI KABIRU

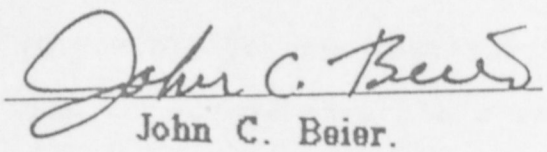
SUPERVISORS

Prof. Hannington O. Pamba
Department of Medical Microbiology
Faculty of Medicine, University of Nairobi.



Hannington O. Pamba

Prof. John C. Beier
School of Public Health
The Johns Hopkins University
Baltimore U. S. A.



John C. Beier.

DEDICATION

This thesis is dedicated to my wife, Pamela Pity Nyawira and my children, Jimm Wanjohi, Faith Gathoni, and Jane Wangeci.

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TABLE OF CONTENTS

CHAPTER 3:STUDY DESIGN AND METHODOLOGY

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

APPENDICES

GLOSSARY

ABTS - Azino-di[3-ethyl-benzethiazoline] sulphonate

Anopheles gambiae

- s.s - *An. gambiae sensu stricto.* One of the species in the *An. gambiae* complex.

- s.l - *An. gambiae sensu lato.* The An. gambiae complex.

ASAB - Asexual stage antibody

Compound - A group of houses within a confined area. In this thesis, the term compound has also been termed as homestead. Every compound has a head.

CS - Circumsporozoite

CSAB - Circumsporozoite antibody

DRI - Day resting (indoors)

DVBD - Division of Vector Borne Diseases

dl - decilitre

EIR - Entomological inoculation rate

ELISA - Enzyme linked immunosorbent assay

FITC - Fluorescein isothiocynate conjugate

HB - Haemoglobin

HBI - Human blood index

Herbalist - An expert on local trees and shrubs that are used to cure specific ailments.

Household - House or a group of houses belonging to one family, i.e. father, mother and the unmarried children.

IFAT - Indirect fluorescent antibody test

KEMRI - Kenya Medical Research Institute

MAB - Monoclonal antibodies

NBC(I) - Night biting collection (indoors)

NBC(O) - Night biting collection (outdoors)

OD - Optical density

PBS - Phosphate buffer saline

PCR - Polymerase chain reaction

PSC - Pyrethrum spray catches

sd - Standard deviation

TDR - Tropical Diseases Research and Training Programme.

µl - Microlitre

SUMMARY

A study was conducted in Kilifi district, Coast Province, Kenya, to examine the relationships between malaria parasite transmission and severe malaria. The goals were to determine the sporozoite loads for the major vector species in relation to severity of malaria, to determine the intensity of malaria transmission by examining malaria parasites and malaria antibodies in the human population and, to determine the heterogeneity of vector density in relation to prevalence of malaria morbidity, household, socio-demographic and environmental variables in relation to the occurrence of severe malaria. The study was carried out between February 1992 and June 1993.

In malaria endemic areas, many individuals normally suffer from bouts of malaria but the pressure due to the disease is mainly on children because of their low immunity to malaria. Many children in such endemic areas present with malaria symptoms, although the disease progresses into life-threatening condition only in a few of them. The relationships between man, parasite and vector are

responsible for the transmission and possibly the risk of developing severe malaria.

The study was conducted in 9 sites with varying levels of severe malaria. Each site was divided into 4 quadrants. One house for night biting collection (NBC) was randomly chosen from each quadrant. This was the index house around which 5 other houses were chosen for day resting (indoor) collection (DRI) and pyrethrum spray collection (PSC). A total of 216 houses were selected.

Four techniques were used to sample the mosquitoes: 1. night biting collections (indoors), and 2. night biting collections (outdoors) using human volunteers, 3. day resting (indoors) and 4. pyrethrum spray collection techniques. The salivary glands of the mosquitoes were dissected for sporozoites which were enumerated using a haemocytometer. The mosquito samples were also tested for circumsporozoite protein by ELISA. The type of houses where mosquitoes were sampled were observed and recorded.

A cross-sectional survey was done in which a total of 2,366 individuals from the study sites were bled for

parasitological and serological examinations. The population was also screened for enlarged spleens. Haemoglobin (Hb) was estimated for children ≤ 10 years of age.

Blood-fed mosquitoes were also collected. The aim of testing these mosquitoes was to find out whether CSAB detected in mosquito bloodmeals could be used instead of finger pricking human beings to map out malaria transmission in a particular area.

Out of 4,961 *Anopheles* vectors collected, 4,926 (99.3%) were *An. gambiae s.l.* Other species identified were *An. funestus, An. nili, An. squamosas* and *An. coustani.* In 8 of the sites vector density was generally low and only a total of 946 (19.1%) of the 4,961 mosquitoes were collected from these sites. Densities of *Anopheles* mosquitoes were not influenced by the type of house construction, environment of the house or the limited control measures practised by the resident population. Although there was a significant difference in *Anopheles* density between the sites studied, no significant association was observed between the density of mosquitoes and severe malaria.

Out of 2,055 mosquitoes dissected to determine sporozoite loads, 48 (2.3%) were positive. The number of sporozoites ranged from 125 to 79,875 with a geometric mean of 1,743 sporozoites. Twenty two (45.8%) of positive mosquitoes had sporozoite loads of <1,000.

The overall *Plasmodium falciparum* sporozoite rate by ELISA was 2.5% during the study period. The annual entomological inoculation rates (EIR) averaged 6.7 infective bites per person and varied from site to site in the range of 0 to 31.4. No significant association was observed between sporozoite loads of mosquitoes or EIR and severe disease.

Out of 2,366 individuals screened during the cross-sectional survey, 1,129 (52.0%) had malaria parasites. *P. falciparum* was responsible for 97.2% of all malaria infections. The malaria parasite positive rate rose from 22.5% in children up to 1 year of age to 70.2% in 5 to 9 years age group. The rate then gradually dropped to 25.1% in individuals over 40 years old. Three hundred and twenty four (26.4%) of the population had *P. falciparum* gametocytes in their blood. Gametocyte rate was 66.7% in children up to 1 year of age and

decreased to 7.5% in individuals over 40 years old. Overall, the spleen rate was 18.6% in all the sites studied. The spleen rates varied from site to site. Of 1,229 individuals who had malaria parasites, 427 (35%) had enlarged spleens. A significant association was observed between the parasite rate and spleen rate. Children with Hb <5.1gm/dl were considered severely anaemic. Fourteen (71.0%) severely anaemic children were also parasitaemic. No significant association occurred between Hb and parasite intensities. The CSAB was detected in 46.7% whereas asexual stage antibodies were detected in 92.9% of the population tested. In spite of the fact that almost every individual had an infection with *P. falciparum* by their 1st birthday, the parasite rates varied significantly between sites ranging from 49.5% to 67.2%. No significant association was observed between the parasite intensities or CSAB positive rates according to site, and severe disease.

Between June 1992 and May 1993, 37 cases of severe malaria were recorded in the study sites, ranging from 8.58 to 38.10 per 1000 children. The 37 severe cases came from 34 households within the study sites. Thirteen

severe cases were recorded from 12 of 216 houses in which mosquitoes were sampled.

One thousand three hundred and three blood-fed *Anopheles*, *Culex* and *Aedes* (with visible blood in their guts) with a human blood index of 0.93 were collected for CSAB screening. The overall CSAB positive rate from the blood-fed mosquitoes was 42.0%. Mosquito bloodmeal CSAB positive rate (42.0%) was comparable to CSAB from human sera (46.7%) but had no significant association at site level.

The results from this study demonstrated that sporozoite loads were comparable to sporozoite loads from *Anopheles* mosquitoes in other areas where transmission is more intense. Although *Anopheles* vectors occurred in low numbers throughout the study period they maintained continuous malaria transmission which varied significantly between sites.

Overall, no significant association was found between the sporozoite loads, transmission challenge and severe malaria in the study sites.

CHAPTER 1

INTRODUCTION

1.1 Malaria

1.1.1 Definition and global situation

Malaria is a disease caused by a protozoan blood parasite of the genus *Plasmodium*. It is acquired when an infective female *Anopheles* mosquito bites a vertebrate host. Over 2,000 million of world's population, living in 100 countries, are at risk of contracting malaria while 270 million are currently infected with the parasite (WHO, 1991). The disease is more prevalent in tropical Africa where it kills one out of every 20 children before the age of 5 years (WHO, 1993).

1.1.2 Life cycle of malaria parasites

There are about 20 mammalian species of *Plasmodia* but only four of them infect human beings, namely *Plasmodium falciparum*, *P. malariae*, *P. ovale* and *P. vivax* (Garnham,

1966). During blood feeding an infective female *Anopheles* mosquito injects sporozoites into a human being. The sporozoites disappear from the blood system into the parenchyma cells (figure 1.1). The parasites undergo a somatic cycle with subsequent release of liver merozoites. The liver merozoites are liberated into the blood where they invade red blood cells (Garnham, 1966).

The parasites undergo schizogonic phase in the blood from trophozoites through schizonts and when the infected red blood cell ruptures the merozoites are released. It is during this schizogonic phase that the clinical symptoms of malaria are experienced. After some generations in the blood, some of the merozoites differentiate into male and female gametocytes that initiate the sexual cycle in the mosquito vector (Miller and Carter, 1976). When an appropriate female *Anopheles* mosquito feeds on a human being with circulating gametocytes it ingests some gametocytes. In the midgut of the mosquito, the gametocytes develop to male and female gametes. Fertilization takes place and a zygote is formed. This develops into a motile ookinete which traverses the peritrophic membrane and lodges on the outer side of the midgut. An oocyst is formed. Inside the oocyst nuclear subdivision takes place with formation of sporozoites

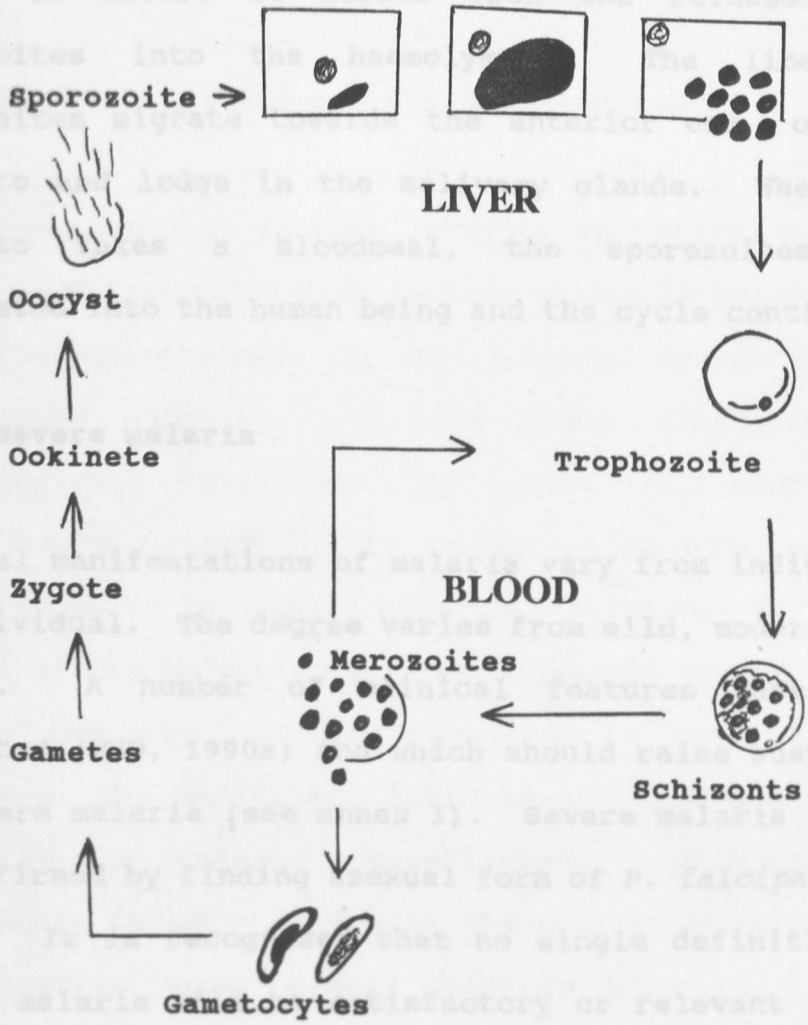


Figure 1.1 Diagram showing the life cycle of malaria parasites

which are the infective stage in the mosquito. When the oocyst is mature it bursts open and releases the sporozoites into the haemolymph. The liberated sporozoites migrate towards the anterior end of the mosquito and lodge in the salivary glands. When the mosquito takes a bloodmeal, the sporozoites are inoculated into the human being and the cycle continues.

1.1.3 Severe malaria

Clinical manifestations of malaria vary from individual to individual. The degree varies from mild, moderate to severe. A number of clinical features have been suggested (WHO, 1990a) and which should raise suspicion of severe malaria (see annex 1). Severe malaria should be confirmed by finding asexual form of *P. falciparum* in blood. It is recognized that no single definition of severe malaria will be satisfactory or relevant in all clinical situations. The purpose of defining and describing severe malaria is to make the health workers aware of the symptoms and signs associated with progression of disease to life-threatening conditions. In parts of the world where malaria is holo- or hyperendemic most severe malaria occurs among children over the age of 6 months. It is less common in older

children because of progressive acquisition of partial immunity (WHO, 1990a).

1.1.4 Risk factors for malaria transmission

There are many risk factors involved in transmission of malaria. The possible risk factors relate to human host, infecting parasite species, environmental parameters and the vector mosquito. In the human host genetic, physiological, behavioural and economic factors all affect man's susceptibility to infection with malaria parasites while in natural malaria infections, the final outcome of the infection will be dependent on the degree of host immunity and the virulence of the infecting species of the parasite. Environmental factors play a key role in malaria transmission. Climatic and topographic features determine the ecology of both human and arthropod hosts as well as their contacts. Human-vector contact will affect the frequency of vector feeding on man and thus increase the probability of transmitting the infection.

1.1.5 Malaria situation in Kenya

1.1.5.1 Distribution

Malaria in Kenya is widespread and varies from region to region. The endemicity pattern is illustrated in figure 1.2. Stable malaria occurs in most parts of coast and Lake Victoria basin where transmission is high. In these areas where malaria is stable many children become infected and experience clinical episodes of malaria but it is only in a few that the disease progresses to life-threatening conditions.

Unstable malaria occurs in many other parts of the country but manifestations appear seasonally during periods of transmission. Epidemic malaria usually occurs in highland areas, sometimes above 2,500m above sea level and bordering endemic areas. There has been a series of such epidemics in the highland region and considerable adult and child mortality due to malaria was reported in 1988, 1989 and 1990 (Ministry of Health annual reports).

Legend:

Holoendemic (very high prevalence of malaria)

Hyperendemic (high prevalence of malaria)

Mesoendemic (moderate prevalence of malaria)

Hypoendemic (low prevalence of malaria)

Malaria-epidemic prone areas

Malaria - free

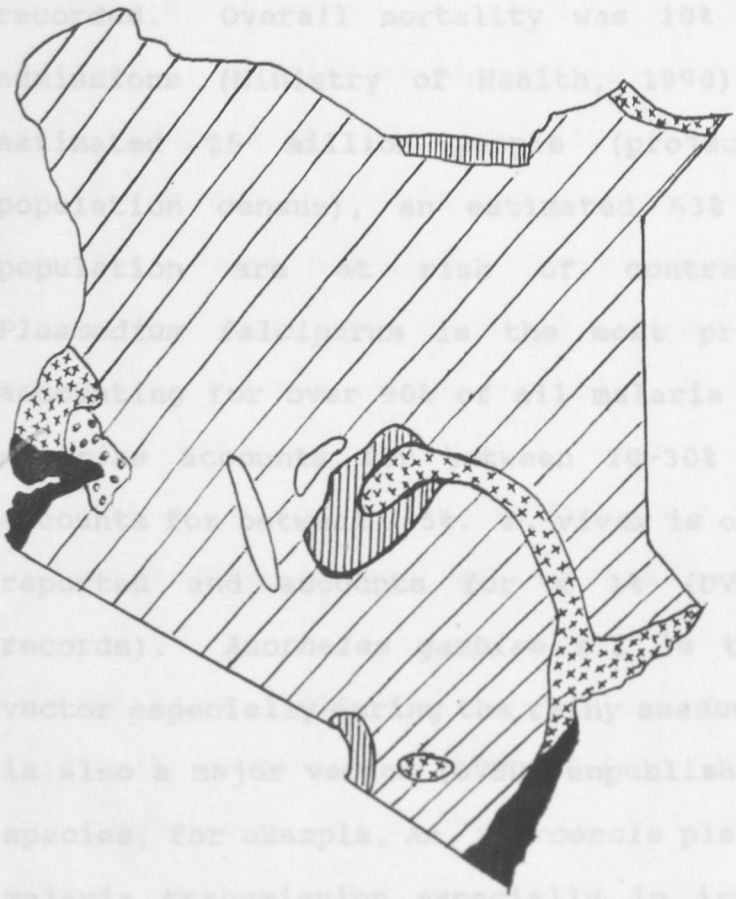


Figure 1.2:Sketch map of Kenya showing endemicity of malaria. Source:Ministry of Health.

1.1.5.2 Epidemiology

In Kenya malaria is a leading cause of morbidity and mortality. In 1989 malaria was the leading cause of out-patient morbidity, contributing to 26% of total morbidity recorded. Overall mortality was 10% of the malaria admissions (Ministry of Health, 1990). Out of the estimated 25 million people (projected from 1989 population census), an estimated 63% of the Kenyan population are at risk of contracting malaria. *Plasmodium falciparum* is the most prevalent species accounting for over 90% of all malaria infections. *P. malariae* accounts for between 10-30% while *P. ovale* accounts for between 3-5%. *P. vivax* is only occasionally reported and accounts for ≤ 1% (DVBD, unpublished records). *Anopheles gambiae s.l* is the main malaria vector especially during the rainy seasons. *An. funestus* is also a major vector (DVBD, unpublished data). Other species, for example, *An. pharoensis* play a minor role in malaria transmission especially in irrigation schemes (Mukiama and Mwangi, 1989).

1.1.5.3 Malaria control in Kenya

A number of government ministries and divisions, private and public sectors, non-governmental organizations as well as international agencies participate in various limited ways in malaria control in Kenya. Case management and personal protection are the main control strategies. Strategies for personal protection, however, largely depend on individuals and these activities are usually chemoprophylaxis and reduction of human-vector contact. Some agencies support community programmes aimed at promoting personal protection especially reduction of human-vector contact. Limited and sporadic vector control, especially source reduction, is undertaken by some municipalities but the exercise is always hampered by lack of financial support.

In realising that malaria is becoming increasingly difficult to manage, and following WHO recommendations for each country affected by malaria to formulate its own control strategy, the government of Kenya launched a 5 year national plan of action for malaria control in April 1994. The main goal of the plan is to develop an infrastructure and system that will ensure access to

malaria prevention and curative services to those at risk of malaria with the aim of substantially reducing illness and death. The objectives of the plan are among others 1) to improve and sustain services within the community for reducing malaria morbidity and mortality and 2) to coordinate and focus the malaria control efforts of ministries, divisions, international agencies and private sector on malaria as a national health problem.

1.2 Objectives of the present study

The overall goal of the study was to examine the relationship between the sporozoite loads of the major anopheline vectors, the transmission intensity, and the severity of malaria in different sites in Kilifi District, Kenya.

Specific objectives were:

1. To determine sporozoite loads for the major vector species of malaria in the study area in relation to the severity of disease.

2. To determine the intensity of transmission in different sites of the study area by examining the

malaria parasite (including gametocyte) rate, circumsporozoite (CS) and antibodies to asexual stages in the population residing in the study area.

3. To determine the heterogeneity of vector density in time and space and to correlate it with parasite rate, prevalence of malaria morbidity, incidence of severe malaria and, house types, socio-demographic, and environmental variables.

4. To determine whether the presence of malaria-specific human antibodies in mosquito blood meals can be used as a method to measure malaria prevalence and the degree of malaria parasite transmission.

HYPOTHESIS

It was hypothesised that in specific sites within the study area, the sporozoite loads of the major vector species and the transmission patterns determine sporozoite challenge, and influence the occurrence of severe malaria.

Specific Hypotheses were:

1. The frequency distribution of sporozoite loads varies from site to site but does not parallel the heterogeneity of vector density, and high loads are localised in micro-environments associated with severe malaria.

2. Although age differences may occur, asexual parasitaemia and asexual stage antibody prevalence and density, is uniform in the study sites because transmission is continuous throughout the year in the study area. Age-specific levels of circumsporozoite antibodies, and gametocyte rates vary from site to site and this indicate the pattern of sporozoite challenge and occurrence of severe malaria. The heterogeneity of gametocyte rates affects the distribution of sporozoite loads.

3. Heterogeneity of vector density occurs in relation to household, socio-demographic and environmental variables, and it is associated with the frequency of severe malaria.

4. The presence of malaria-specific human antibodies detected in mosquito bloodmeals demonstrates

the sporozoite challenge pattern in the host population. It provides a good alternative way to establish the malaria transmission in an area, without necessarily bleeding the local residents.

JUSTIFICATION

In endemic areas most cases of clinically severe malaria occur in children. It is not evident as to why only a minority of these children present with these symptoms. Many factors involved in transmission of malaria have relationships between man, parasite and environment. These factors are all possibly linked to the risk of occurrence of severe malaria. Entomological indices could also play a role in the development of severe malaria. Vector-related elements also affect the micro-distribution pattern of transmission, incidence and degree of severity of malaria infections and that is why these parameters should be considered as potential determinants of severe malaria. The risk of malaria in individuals living in endemic areas depends upon exposure to infective vectors and the number of clinical episodes experienced by an individual in a household could be related to the exposure levels of the infective vector (Schmidt, 1982).

This study was coordinated with a WHO/TDR sponsored case-control study to identify factors affecting the severity of clinical malaria. These clinical studies were conducted by a joint Kenya Medical Research Institute (KEMRI)/Oxford University team. The purpose of the study was to determine whether the distribution patterns of severe malaria in the study area were associated with transmission patterns.

CHAPTER 2

LITERATURE REVIEW

2.1 General

The transmission dynamics and distribution of malaria are complex issues. The elements involved in this matrix include the vector mosquitoes, the human host and the infecting malarial parasites. Within each of these elements are a series of underlying factors that interact and effect the transmission dynamics.

2.2 Vectors

2.2.1 The vector mosquitoes

Out of more than 400 described species of *Anopheles* (White *et al.*, 1977) some 45 of them are implicated in the transmission of malaria. Different species of *Anopheles* are responsible for transmission of malaria in specific geographical areas. In tropical Africa the An.

gambiae complex and *An. funestus* are the main vectors. The *An. gambiae* complex is now known to contain six species. The salt water breeding *An. melas* Theobald and *An. merus* Donitz are found in West and East Africa respectively. The fresh water breeding *An. gambiae s.s* Giles, *An. arabiensis* Patton and *An. quadriannulatus* Theobald are widespread in many parts of Africa (White, 1975) while the sixth species (*An. bwambae*), has been found only in the Bwambwa hot springs of Western Uganda (Davidson and White, 1972; White, 1973). On the East African coast, 3 species of *Anopheles* have been recorded. They are *An. merus* Donitz, *An. gambiae s.s* Giles and *An. arabiensis* Patton (Muirhead-Thompson, 1951; White *et al.*, 1972; Highton *et al.* 1979; Mosha and Petrarca, 1983; Mutero and Mosha, 1984). Of the 6 species of the *An. gambiae* complex, *An. gambiae s.s* and *An. arabiensis* are most closely associated with man and represent the major vectors of malaria. The distribution of these two species overlaps and they occur sympatrically in large areas of tropical Africa. Investigations in northern Nigeria by Colluzi *et al.* (1979) showed that the two species occurred at different frequencies along an ecological gradient. Highton *et al.* (1979) studying *An. gambiae s.s* and *An. arabiensis* in Western Kenya, found

An. arabiensis on the plains and *An. gambiae s.s* on the foothills. *An. merus* and *An. melas* are restricted to a narrow coastal range (Muirhead-Thomson, 1951) due to the fact that they prefer to breed in salty water (Muirhead-Thomson, 1951; Ribrands, 1941; Mutero *et al.* 1984). In the Kenya coastal belt village of Jimbo, in Kwale district, 96% of *An. gambiae s.l.* caught were *An. merus* (Mosha and Petrarca, 1983).

The density of mosquito population is dependent on larval ecology. Irrigation schemes (particularly used for growing rice), for example, are preferred breeding sites for open country species of *An. gambiae s.l* and *An. funestus* while *An. balabacensis* and *An. dirus* are abundant in the forested areas where they transmit forest malaria as in South Eastern Asia (Scanlon and Sandhinand, 1965). *An. melas* and *An. merus* may not be as efficient malaria vectors as *An. gambiae* (Gelfand, 1955) but nevertheless they are important vectors because of high densities as a result of having extensive breeding sites within the tidal limits of the coastal line (Bryan, 1983). Information on absolute densities may not be routinely valuable for malaria transmission but would be important in vector population studies (Gillies, 1955; Reisen and Asalamkhan, 1979).

Under natural conditions where man, vector, and local parasite strains co-exist, transmission is usually intense provided that the vector is abundant and feeds on man. These vectors must also live long enough to transmit the infection. However, changes in parasite strain introduced by migrating population, climate or mosquito fauna may limit the role of a potent vector. A good example of this type of situation was failure of *An. maculipennis* in Europe to become infected by Indian or African strains of *P. falciparum* (James *et al.*, 1932). The fact that some mosquitoes within a population are more susceptible to malaria infection compared to others is well known and has been comprehensively reviewed by Boyd (1940). It has also been shown that there is a high degree of specificity in the relationship between the mosquito and parasite (MacDonald, 1967) and that susceptibility of an individual mosquito is genetically determined (Huff, 1963).

In nature, many species of *Anopheles* will feed on any large mammal that is available. In tropical Africa 80% of the *Anopheles* species behave in this way (Gillies, 1972). In the Garki project, conducted in Northern part of Nigeria, the arrival of migrant families in an area with

their herds of cattle was responsible for the increase in population of *An. arabiensis* (Molineaux and Gramacia, 1980). The host preference by a particular species of mosquitoes is also likely to be influenced by environmental conditions. In a study by Gillies (1964) in Muheza, a coastal lowland in Tanzania, the predominant species was *An. gambiae* s.s. which is known to be anthropophilic. When a calf was introduced in a hut where people also slept, it became apparent that there were two sub-species with different host preferences. Some of the mosquitoes were strictly zoophilic while others were anthropophilic. In the human population, the defensive behaviour of adults influences successful feeding by mosquitoes (Smith, 1955; Port *et al.* 1980). In the work by Port *et al.* (1980) children less than 18 months received more bites than the older children and it was the greater tolerance of small babies to bites that was thought to contribute to the feeding success of mosquitoes.

The biting habits of mosquitoes differ greatly depending on adaptations. *An. merus* and *An. arabiensis* found in some parts of East Africa bite more often outdoors than indoors (Highton *et al.* 1979; Mutero *et al.* 1984) while

An. gambiae s.s bites more often indoors (Highton *et al.* 1979). This biting pattern influences host preference. The indoor biting *An. gambiae s.s* and outdoor biting *An. merus* are highly anthropophilic but *An. arabiensis* is more zoophilic. In Kano plain, Kisumu district, Kenya, 92% of *An. gambiae s.s* sampled fed on man whereas 59% of *An. arabiensis* fed on cattle (Highton *et al.* 1979). Once a mosquito has fed and depending on its resting preference, it may remain at the site of feeding. Most often it is filled with blood and may not fly far from the feeding source. Otherwise it may shift to another habitat. Some vectors may, after an exophagic meal prefer to rest indoors in contrast to the exophilic group which prefer to rest outside the dwelling quarters (Boreham *et al.* 1982). Movement from hut to hut within a compound by an engorged mosquito has also been observed (Boreham *et al.* 1978; 1979; Bryan and Smalley, 1978).

The daily survival rate of a vector mosquito is an important factor that is known to influence malaria transmission (Ross, 1910; Garret-Jones, 1964). This is because small changes in survival rate can have a major effect on vectorial capacity. The longevity of a vector species determines the probability of its survival

through to the time required to produce viable sporozoites. On assessing survival rates in Papua New Guinea, Charlwood *et al.* (1985) estimated the daily survival rate of *An. farauti* to be 0.32-0.64 in wet season and 0.49-0.73 in the dry season. These rates are based on mean parity rate of many collections over a specific period (Gillies and Wilkes, 1963). Parous rates have also frequently been utilised to determine the age structure of mosquito populations. A population with a large proportion of parous females is an indication of aging and potentially contains individuals that have had an opportunity to become infected with the parasites. The parity rate in a population is known to be influenced by the method of collection, periodicity of blood feeding, the duration of the gonotrophic cycle as well as weather (Service, 1976). Gillies and Wilkes (1963) using human baits in Muheza, Tanzania, observed that the proportion of parous female mosquitoes (69.9%) caught at night was higher than the proportion (57.3%) collected resting inside houses during the day.

The effect of malaria parasites on the survival of mosquito vectors is potentially a limiting factor in malaria transmission. Experiments carried out have shown contradicting results on the effect of malaria parasites

on mosquito survival. Freier and Friendman (1987) and Klein *et al.*(1982 and 1986) reported that the infected mosquito lifespan was reduced. In the study by Klein *et al.* (1986) the effect of malaria parasites on the survival of mosquitoes was observed only when the mosquito contained more than 10 oocysts. Chege and Beier (1990) on the other hand, observed that the daily survival rate of caged wild-caught *Anopheles* was the same for mosquitoes which were infected or non-infected with malaria parasites indicating that the presence of parasites in natural infections had no effect on survivourship.

It is generally known that the distribution of malaria is not homogeneous within human populations in endemic areas. Among the factors that could account for clustering of malaria in individuals or households are unequal susceptibility to disease among the individuals which could be due in part to innate or acquired immunity (Armstrong, 1978; Miller and Cater, 1976). Additionally it could be due unequal risks of contact with the vector mosquito (Dye and Hasibeder, 1986; Burkot, 1988). Small area variation between village populations in the transmission of malaria have been reported (Greenwood, 1989). In Kataragana, Southern Sri-Lanka, malaria

attacks were found to be clustered and occurred more frequently in inhabitants with poorly constructed houses (Gamage-Mendis *et al.* 1991). There were significantly higher numbers of indoor resting mosquitoes collected from poorly constructed houses compared to well constructed ones. This suggested that the higher malaria risk associated with poorly constructed houses was at least partly due to higher human-vector contact among inhabitants.

2.2.2 Vectorial capacity

The vectorial capacity expresses the capability of a vector population to transmit malaria in terms of potential number of secondary inoculations originating per day, from an infective person. The primary case is usually the original case from where the mosquito gets the infection. A formula using this model was given by Macdonald (1957).

The formula is depicted as:

$C = ma^2p^n/-\log_e p$ where,

C = vectorial capacity

m = density of vectors in relation to man (man-biting rate)

a = number of blood meals by vector per day (feeding habits/frequency)

p = probability of daily survival of the vector population

n = duration of sporogony in days.

The formula assumes that:
1) that the vector is susceptible to the infection but is not affected by it and 2) that the death rate is independent of age and probability of feeding on man is equal in all members of one vector species. Changes in any element of the model will affect the vectorial capacity. The magnitude is more sensitive to change in probability of survival of the vector population (p) as was demonstrated by Macdonald (1952) in his expression for endemic levels of malaria. The human-biting rate (m) would also significantly affect the transmission potential. Vectorial capacity may vary from a few

infective bites to hundreds of bites per person per year. This entomological inoculation rate (EIR) may determine the occurrence of severe malaria but does not always mean that a high EIR ends up with more severe cases of malaria.

2.3 Sporozoites

2.3.1 Biology

Sporozoites are spindle-shaped organisms measuring 11 μm in length by 1 μm in diameter. It is the last stage in the sporogonic cycle within the mosquito vector. When a mature oocyst ruptures it releases these numerous motile organisms into the haemocoel (Robert *et al.* 1988; Golenda *et al.* 1990). A *Plasmodium falciparum* oocyst may release up to 10,000 sporozoites (Pringle, 1965). When these sporozoites are released they migrate either actively or passively into the salivary glands. However, not all sporozoites find their way into the salivary glands. A large number are lost in the mosquito's body tissues during their sojourn in the mosquito (Porter *et al.* 1954).

As already described in section 1.1.2 man is infected with malaria parasites when he is bitten by an infective female *Anopheles* mosquito. The sporozoites are injected into man when the mosquito bites to take a bloodmeal. Studies done have demonstrated that inoculation doses differ from species to species of the transmitting vector but usually <20 sporozoites are released at any one time (Rosenberg *et al.* 1990; Beier *et al.* 1991a, 1991b, 1992a; Ponnudurai *et al.* 1991). It is not always easy to correlate the number of sporozoites discharged by the mosquito and the total salivary gland load. For example Beier *et al.* (1991d) showed that some mosquitoes with low sporozoite counts (<100) discharged sporozoites while others with many sporozoites (>100,000) did not. At the same time, some of the inoculated sporozoites are re-ingested by the feeding mosquito. When the ingested sporozoites are counted from mosquitoes which have fed within 10 hours, they give the minimum number of sporozoites that may have been discharged (Beier *et al.* 1992). Although infected mosquitoes probe more times in an attempt to feed, this may increase the chance of infection, but not all bites will produce an infection. Observations by Pull and Grab (1974), showed that only 1:20% of the bites from salivary gland-positive mosquito led to an infection.

Vanderberg (1977) using a rodent model showed that only 1% of the *P. berghei* sporozoites inoculated in hamsters gave rise to pre-erythrocytic hepatic development. In addition, skin factors may determine the number of the infective sporozoites reaching the blood (Vanderberg, 1977). A study by Ponnudurai *et al.* (1991) showed that more sporozoites were ejected into the skin than into the blood. The sporozoites, however, disappeared from the site of bite within 2 hours after feeding.

The infection status of mosquito is determined by dissection and examination of salivary glands for sporozoites. It has been suggested that the size of the sporozoite inoculum may influence the severity of the disease and this has been reviewed by McGregor (1964 and 1965) and Greenwood (1991). It has also been suggested that parasite strain may be a determinant of severe malaria (Marsh, 1988 and, Lines and Armstrong, 1992).

The sporozoite dose may also be related to the number of the sporozoites in the salivary glands (Shute *et al.* 1965; Pringle, 1966; Vanderberg, 1977). It should be appreciated that under natural conditions it is difficult to determine the number of sporozoites inoculated during blood-feeding. In malaria endemic areas many children

experience bouts of malaria but it is only in a few that the disease progresses to life-threatening situations. It is not yet clear how the numbers of sporozoites injected into a child determines the risk of the occurrence of severe malaria.

Dissection techniques for the removal of salivary glands have been described by a number of workers (Blacklock and Wilson, 1942; Hunter *et al.* 1945; and WHO, 1975). The quantitation of sporozoites was done in a number of studies (Nicol *et al.*, 1927; Boyd, 1940; Shute, 1945; Greenberg *et al.* 1950; Pringle, 1966; Vanderberg and Gwardz, 1980, Beier *et al.* 1991a and 1991d). In a study by Shute *et al.* (1965) the salivary glands were dissected into a drop of saline on a platform formed by a small coverslip which was cemented on a slide with Canada balsam. The glands were crushed by pressure applied on an overlying coverslip. The preparation was dried and stained with Giemsa solution. Out of about 3,500 mosquitoes dissected they found 36 with salivary gland sporozoites. Using the same technique, Pringle (1966), dissected 11,500 mosquitoes and 174 of them were found infected with sporozoites. Beier *et al.* (1991d) used a haemocytometer to enumerate the total number of sporozoites in the salivary glands. The glands were

removed and ground in tissue grinders. A small aliquot was used to charge a counting chamber. Using phase contrast microscopy, 4 corners of the chamber were examined and the sporozoites were counted. Kawamoto (unpublished data) centrifuged mosquitoes to harvest the sporozoites. She put the mosquitoes head first in an open-ended Eppendorf conical tube. She placed this narrow tube inside a bigger conical tube, centrifuged the tubes at about 500x force of gravity and harvested the sporozoites. It was not clear whether all the sporozoites in the salivary glands were harvested.

The sporozoite densities in the salivary glands differ greatly. They range from <10 to >200,000 in Afrotropical anophelines (Pringle, 1966; Wirtz *et al.* 1987 and Beier *et al.* 1991d) but the majority will be between 1,000 to 4,000 (Pringle, 1966). Many factors influence the number of sporozoites in the salivary glands. Host antibodies against gametocytes, for example, may influence the number oocyst in infected *Anopheles* and ultimately the sporozoite load (Ponnudurai *et al.* 1987). Antimalaria drugs may also influence the number of oocysts in infected *Anopheles* mosquitoes. Studies have shown that when *An. stephensi* (Ramkaram and Peters, 1969) and *An. balabacensis* mosquitoes (Wilkinson *et al.* 1976) were fed

on chloroquine-resitant gametocytes they produced more oocysts as compared to mosquitoes which were fed on chloroquine-sensitive gametocytes. However Chutmongkonkul *et al.* (1992) demonstrated the inhibition of gametocyte development in the mosquitoes after therapy with chloroquine, halofantrine and pyrimethamine. Other factors that influence the number of sporozoites in the salivary glands are a progressive depletion in number and a decline in infectiousness of sporozoites during their stay in the mosquito which comes as a result of repeated feeds (Porter *et al.* 1954).

In the vector, sporozoites remain viable for several weeks. The mechanisms by which they survive and maintain their infectivity during their long stay in the vector are not well understood. They release circumsporozoite (CS) protein which facilitates their gliding movement. This CS protein usually occurs on the sporozoite surface (Mack and Vanderberg, 1978; Stewart and Vanderberg, 1991). CS protein is first detected during the development of the oocyst (Boulanger *et al.* 1988).

Sporozoites release CS protein as they migrate and settle in the salivary glands (Posthuma *et al.* 1989; Golenda, 1990). Though this CS protein is found abundantly in the

anterior part of the mosquito it can be detected anywhere in the mosquito (Lombardi *et al.* 1987; Robert *et al.* 1988; Beier *et al.* 1991). When in the salivary glands, the sporozoites regulate the CS protein output through feedback mechanisms which is dependent on sporozoite density (Beier *et al.* 1992).

It is not possible to identify sporozoites according to parasite species microscopically. Zavala *et al.* (1982) and Collins *et al.* (1984) first developed immuno-radiometric assay (IRMA) using *Plasmodium* specific monoclonal antibodies to detect sporozoites. Later, enzyme linked immunosorbent assays (ELISA) were developed and standardised for *P. falciparum* (Burkot *et al.* 1984; Wirtz *et al.* 1987b), *P. vivax* (Wirtz *et al.* 1985), *P. malariae* and *P. ovale* (Beier *et al.* 1988). Sporozoite rates by dissection give a measure of the proportion of female *Anopheles* mosquitoes with sporozoites in the salivary glands while sporozoite immunoassays give a measure of the presence of CS protein in the mosquito. Since not all sporozoites reach the salivary glands, high prevalence of sporozoite rates by ELISA may not correspond to the infectivity of the vectors. Beier *et al.* (1990) found that 45.4% of ELISA positive *Anopheles* did not have salivary glands sporozoites. It is

suggested that performing ELISA on the head and thorax only might reduce sporozoite rate to levels that could be reasonably real (Beier *et al.* 1990).

The strength of an ELISA reaction is usually measured with a spectrophotometer using an ELISA reader. The advantage of using peroxidase-based ELISA is that the results can be read visually (Beier and Koros, 1991b). This is well suited to field condition where mosquitoes may simply be classified as negative and positive. Despite the shortcomings of the peroxidase-based ELISA, it is useful in epidemiological studies if it is intended to measure the pattern and the intensity of malaria transmission in a given endemic area.

2.3.2 Clinical disease outcome due to sporozoite inoculum

It has been suggested that the size of the sporozoite inoculum may influence the outcome of infection with malaria parasites (Marsh, 1992). Assuming that all inocula are multiplying at the same rate, the heavier sporozoite dose will result in a clinically patent parasitaemia earlier than a lighter one. Allowing that the parasites are multiplying all the time in the host, a level comes when the host response against the parasite

is elicited. It is assumed that host response begins to appear after 4 cycles of asexual application and that efficiency of the response is doubled in each subsequent cycle (Marsh, 1992). When this response is mounted the increase in parasite ceases to be linear but forms a plateau, and may even decline depending on the degree of immunity developed.

2.4 Malaria antibodies

2.4.1 Asexual stage antibodies (ASAB)

Antibodies against the parasites in the blood stage are also detected in persons who have been exposed to malaria infections. These are the sexual and asexual stage antibodies being referred to here as asexual stage antibodies (ASAB). Few studies have been conducted on asexual stage antibodies. Asexual stage antibodies are used as a measure of the degree of malaria prevalence in a given area. An example is in a Kenyan site, in Kisumu district, where the parasite rate was over 80% and where 95% of the population was shown to have antibodies against *P. falciparum* asexual stage (Spencer *et al.* 1987). Although levels of CSAB and ASAB can act as

indicators for intensity of malaria transmission, studies done so far have shown that the presence or titres of ASAB do not necessarily correlate with CSAB (Del Giudice *et al.* 1990; Hoffman *et al.* 1986). In another study by Del Giudice *et al.* (1987a), all children tested had anti-*P. falciparum* ASAB irrespective of age or presence of CSAB. At present, there is evidence to indicate that naturally acquired protective immunity is developed against the blood stage of malaria parasites and this may account for the decreased susceptibility to malaria observed in adults living in malaria stable areas (Cohen, 1977, Hoffman *et al.* 1986, Del Giudice *et al.* 1990).

The serology of CSAB and ASAB in this study was intended to establish the relative intensity of malaria transmission in study sites which had varying incidence of severe malaria.

2.4.2 Circumsporozoite antibodies (CSAB)

As stated earlier in section 1.1.2, malaria is transmitted to man by inoculation of sporozoites through the bite of an anopheline mosquito. After repeated exposure to these bites, human beings in malaria endemic areas develop detectable levels of antibodies against the

sporozoite (Hoffman *et al.* 1986; Nardin *et al.* 1979; Nussenzweig and Nussenzweig, 1985). The prevalence of anti-sporozoite antibodies is age-dependent and reflects cumulative inoculation by infective vectors (Del Giudice *et al.* 1990) as well as the level of recent exposure to transmission by mosquito vectors (Nardin *et al.* 1979). Studies in Got Nyabondo and Aboch, Kisumu district, Kenya, by Campbell *et al.* (1987a) demonstrated that the prevalence rate of circumsporozoite antibodies (CSAB) increased from 40% in children under 4 years to 85% in adults above 20 years of age. The absence of evidence for a con-concomitant immunity mainly to sporozoites implies that the memory for boosting of this immune response is short lived. This phenomenon has been shown to be the case with naturally acquired immunity to sexual stage of *Plasmodium vivax* (Ranawaka *et al.* 1988). It is also in agreement with observations by Webster *et al.* (1987) that CSAB are short-lived and are rapidly lost soon after a malaria attack. Despite their brief existence in circulating blood, sporozoites induce a strong immune response that is species and stage specific (Cochrane, 1980). Observations by Druilhe *et al.* (1986) on primary attacks of malaria suggested that a single infection did not induce detectable CSAB, regardless of sporozoite dose.

The membrane of the infective sporozoite is covered by a poly-peptide called the CS protein which uniformly surrounds the external coat (Yoshida *et al.* 1980). The CS protein of *P. falciparum* contains a large immunodominant central domain consisting of tandemly repeated tetrapeptides. The availability of synthetic and recombinant peptides has allowed the development of ELISA (Hoffman *et al.* 1986; Del Giudice *et al.* 1987b); Campbell *et al.* 1987a). This has facilitated investigations on antibody response to *P. falciparum* sporozoites in populations which are naturally exposed to malaria infections. The reason for the use of repetitive peptides in the analysis of the antibody response to malaria is based on observations that the CS protein is the only antigen so far characterised on the malaria parasite sporozoites (Nussenzweig and Nussenzweig, 1986) and the majority of the synthetic and naturally induced CSAB recognises the repetitive region (Zavala *et al.* 1983).

There have been a number of epidemiological studies examining the relationship between the occurrence of CSAB and the prevalence and incidence of malaria. Many of these studies have compared areas of different malaria

endemicity (Hoffman *et al.* 1986). In spite of comparable risks of exposure to infectious bites, marked inter-house and clustering of CSAB has been reported (Del Guildice *et al.* 1987). In Madang, Papua New Guinea, two groups of women living under comparable conditions showed that one group had higher CSAB levels (Brabin *et al.* 1988). This suggested that there may have been factors other than exposure to infection with malaria parasites that account for some individuals developing a higher antibody response to the CS protein.

Factors governing the acquisition of CSAB and the protective role of these antibodies are not adequately understood. It is known that even in holoendemic areas where adults experience daily challenge from infected mosquitoes, high levels of CSAB do not necessarily protect against *P. falciparum* malaria (Marsh *et al.* 1988; Burkot *et al.* 1989). It is suggested that it could also simply be a function of dose (Greenwood *et al.* 1991) requiring at least 50 to 100 cumulative infective bites for 50% of the population to develop measurable levels of CSAB (Hoffman *et al.* 1986).

In areas with comparatively low human-biting rates and sporozoite rates, the entomological parameters of

transmission may be rendered valueless. In such areas the detection of CSAB in the human population may probably be a good indicator for assessment of the degree of malaria transmission.

2.5 Human malaria antibodies in mosquito bloodmeals

Repeated exposure to the bites of infected mosquitoes in malaria endemic regions lead to development of antibodies against sporozoites (Nardin *et al.* 1979). Due to frequency of biting, *Anopheles* mosquitoes usually ingest anti-sporozoite antibodies in blood meals. Ingestion of human antibodies against malaria sporozoites by *Anopheles* vectors in nature may affect the dynamics of malaria transmission. There has been contradicting observations of the effect of anti-sporozoite antibodies on the sporozoites. Contact between sporozoites and circumsporozoite antibodies (CSAB) has been shown to decrease sporozoite infectivity as measured by *in vitro* sporozoite invasion of hepatoma cells (Hollingdale *et al.* 1984). It has been shown that the anti-sporozoite antibodies do not alter infection rates and that enhancement of sporozoite numbers occurs only in the mosquitoes that have already acquired infections (Vaughan

et al. 1988). In a study by Vaughan *et al.* (1988) in which rat anti-sporozoite antibodies were given to *P. falciparum* infected mosquitoes, the numbers of salivary glands sporozoites in the anti-sporozoite-antibody-fed mosquitoes were 2-3 times greater than the corresponding controls. Also, *P. falciparum* infections in *An. gambiae*, fed on an infected individual from western Kenya, were not affected by either human circumsporozoite antibody positive sera or *P. falciparum* specific monoclonal antibodies and naturally acquired CSAB did not reduce sporozoite rates or sporozoite loads in *P. falciparum*-infected *Anopheles* mosquitoes (Beier *et al.* 1989). Other studies in mice by Egan *et al.* (1987) demonstrated that high levels of anti-sporozoite antibodies protect only against low numbers of challenging sporozoites.

Naturally infected *Anopheles* mosquitoes feed repeatedly on hosts with CSAB and contact between sporozoites and CSAB in the mosquito haemocoel does not block salivary glands invasion of sporozoites (Beier *et al.* 1989). The host antibodies against sporozoites remain detectable in the blood meals of mosquitoes for at least 24 hours after feeding (Service *et al.* 1986; Beier *et al.* 1988). Although the volumes of blood in the fed mosquitoes is

small (1-3 μl), host antibodies can be detected by simple immunoassays.

Indirect immunofluorescent assay and ELISA have been used to detect human malaria antibodies in mosquitoes (Vaughan *et al.* 1988, 1990; Beier *et al.* 1989; Contreras and Beier, 1992). The detection of anti-sporozoite antibodies provide information on intensity of exposure to transmission by vector populations. In almost all cases, human sera have been used for these studies. A possible alternative to these surveys that require blood collection from human populations would be the detection of malaria antibodies in the blood meals of human-blood-fed mosquitoes. It is important to confirm the presence of human immunoglobulins in the blood meals of mosquitoes before proceeding to determine the presence of malaria-specific antibodies in order to be sure that the mosquitoes have fed on human being (Beier *et al.* 1989).

CHAPTER 3

STUDY DESIGN AND METHODOLOGY

3.1 The study area

The study was conducted in Kilifi District, Coast Province, Kenya (figure 3.1). It lies on the coastal plain which is a narrow belt varying in width from 3 to 20 km. It is an area with dense forest as well as savanna type of vegetation. The altitude is between 0 to 400 m above sea level.

The specific study sites were within a region in which a recent case-control study on risk factors associated with development of severe malaria had taken place. The area was subdivided into enumeration zones (EZ). These EZ were defined using the National Census Bureau's divisions. Nine EZ were selected for entomological sampling. The selection was based on the incidence of severe malaria between 1989 to 1991. The frequency of severe malaria varied in different sites. The specific

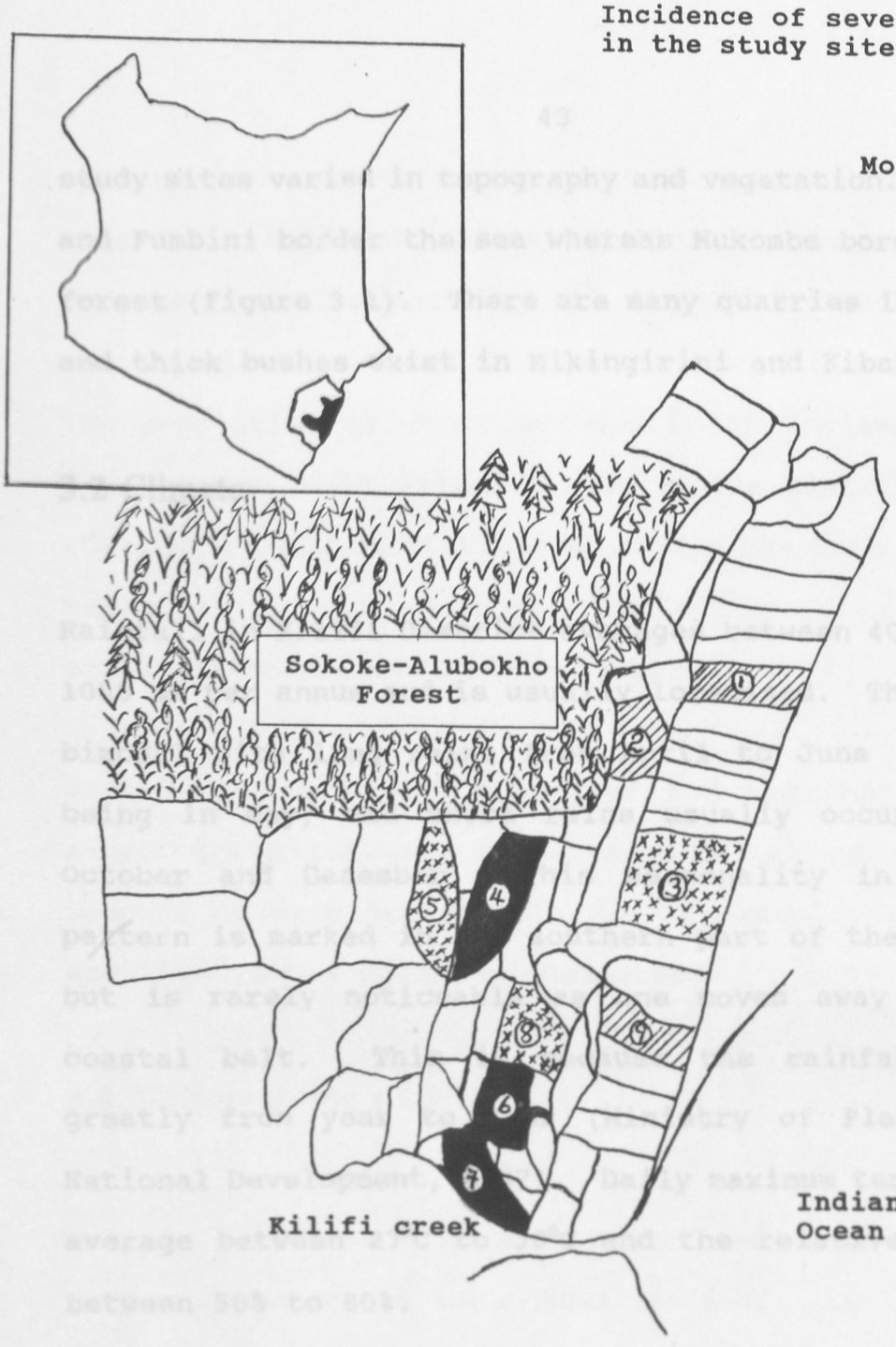


Figure 3.1:Sketch map showing the study sites with some geographical features. Inset:sketch map of Kenya showing Kilifi district and the study area.

Sites	1:Ufuoni	2:Kaoyeni	3:Zowerani
	4:Mukombe	5:Kambi ya Wari	6:Kibarani
	7:Fumbini	8:Mikingirini	9:Mtondia

study sites varied in topography and vegetation. Mtondia and Fumbini border the sea whereas Mukombe borders the forest (figure 3.1). There are many quarries in Mtondia and thick bushes exist in Mikingirini and Kibarani.

3.2 Climate

Rainfall in Kilifi District averages between 400 to over 1000 mm per annum and is usually localised. The rain is bimodal with long rains from April to June (the peak being in May) and short rains usually occur between October and December. This seasonality in rainfall pattern is marked in the southern part of the district but is rarely noticeable as one moves away from the coastal belt. This is because the rainfall varies greatly from year to year (Ministry of Planning and National Development, 1987). Daily maximum temperatures average between 27^0C to 30^0C and the relative humidity between 50% to 80%.

3.3 Study population

The inhabitants of this area are mainly peasants, growing maize and cassava for subsistence and coconuts,

mangoes and cashew nuts as cash crops. There are comparatively fewer domestic animals in the study area compared to other parts of the district. Goats, cattle and occasionally sheep are kept for domestic consumption. The population is comprised mostly of Giriama people. The average adult literacy rate in the District is 48% (Central Bureau of Statistics, 1988), but this could now be higher due to adult literacy classes introduced in parts of the district since 1985.

Most houses have walls made of mud and thatched with *makuti* (palm leaves). The compounds are usually large because of extended families. For example, sons marry and live in the ancestral homestead.

3.4 Malaria in the study area

Malaria is a serious problem in the study area and transmission occurs throughout the year. It is bimodal with the two peaks corresponding with the rains (Division of Vector Borne Diseases (DVBD), 1987). Fever accounts for 50% of all outpatient attendances and the percentage becomes higher during the peak malaria transmission during which fever may account for up to 80% of all

attendances. Three species of *Plasmodium* infecting man have been recorded from the area: *P. falciparum* which accounts for more than 90% of all malaria infections, *P. malariae* and *P. ovale* (DVBD, 1987).

There are no organized malaria control activities in the area except for few individual efforts. The use of mosquito nets as a control measure is minimal as only 6.2% of a sampled population had a mosquito net in their house before the commencement of this study (Snow *et al.*, 1992). During periods of high mosquito densities, the inhabitants use smoke repellents by burning commercial pyrethrum coils and/or specific trees and shrubs. The local repellents included boabab fruit, leaves of *Mkilifi* (Neem) tree and coconut husks (Snow *et al.*,1992).

Treatment seeking behaviour for the resident population has been reported by Snow *et al.* (1992). Most mothers bought drugs from shops or kiosks as their first step in treatment. During the time of interviews, no treatment was sought for 14% of the sick children in the study sites (Snow et al. 1992).

3.5 Selection of the specific study sites and houses for entomological sampling

Between 1989 and 1991 the Oxford/KEMRI group at Kilifi Research Unit had identified specific sites within the main study area with varying degrees of incidence of malaria (Snow *et al.* 1993). These sites were identified as having low, medium or high incidence of severe malaria (figure 3.1). The incidence rates were 37-41/1000 children for the high, 14-18/1000 for the medium and 0-12/1000 for the low areas. For the nine sites used for entomological sampling, three of each were at same level of incidence (figure 3.1). Before recruiting and selecting households, a meeting was held with the local residents on each study site. These meetings were organized through the village elders and other local leaders (plate 1). Each site was initially divided into 4 quadrants. In each quadrant, a compound (a group of houses under one head) was randomly selected. The main criterion for recruitment of the compound was that there should be at least two children ≤ 5 years of age since most severe cases of malaria occurred in children in this age bracket. When consent was given by the head of the compound, one house in which a child ≤5 years of age

Plate 1. Meeting with community members. A series of such meetings were organized to discuss the study.

slept was recruited for entomological sampling. In each site, 4 houses were selected for night biting catches. Around each of the houses where night collection of mosquitoes was done, 5 other houses from different compounds were selected for collection of day resting mosquitoes. This gave the total number of houses in each quadrant to 6.

3.6 Recruitment and training of mosquito collectors

Mosquito collectors were recruited from the local communities. They were recruited with the help of either the village elders or the local chiefs. Each study site had its own mosquito collectors. The mosquito collectors did not have formal employment. The collectors were trained on the collection techniques and mosquito identification. In order to minimise bias the mosquito collectors in each site were swopped each time.

3.7 Mosquito sampling techniques

The mosquito sampling techniques were done according to the World Health Organization standard methods (WHO,

1975). The techniques used in this study are described below. The sampling (except for pyrethrum spray catches) was done once a week in each house.

3.7.1 Night Biting Catches Indoors (NBC Indoors)

The mosquito collectors assembled at specific meeting places on each day of collection. They were issued with flashlights, aspirators and paper cups. They were then either driven to their respective collection houses or they walked if the house of collection was near. They collected mosquitoes from 6.00 pm to 6.00 am. Collections were done for half an hour in each hour. The collectors sat in the corridors of the houses with their legs bare. They regularly shined the flashlights over their legs at intervals of about 20-30 seconds, without moving their legs. Any mosquito that landed on their legs or arms was sucked into the aspirator and placed in the pint-size paper cups.

3.7.2 Night Biting Catches Outdoors (NBC Outdoors)

The NBC outdoors was done in the same way as the NBC indoors. The collectors sat about 10 meters from any

occupied house. NBC outdoors was done in one compound per site per week.

3.7.3 Day Resting Indoors (DRI)

DRI collections were done from 6.30 am to about 9.30 am (plate 2). The mosquitoes resting inside the houses were collected by mechanical aspiration by two mosquito collectors for 15 minutes in each house. The mosquitoes were collected from the walls, under the beds and any other place where the mosquitoes could hide.

3.7.4 Pyrethrum Spray Catches (PSC)

PSC was done in a total of 574 houses in all the 9 study sites. All the sites were equally sampled except in Mtondia where due to large numbers of mosquitoes collected by other methods, it was felt unnecessary to do PSC.

The floor of the house to be sampled was entirely covered with white sheets. The windows, if any were closed. The house was then sprayed with pyrethrum extract from outside to inside. The pyrethrum consisted of 1 ml pyrethrum (25% extract) in 1 litre of kerosene plus 1.2

Plate 2. Day resting indoor mosquito collection.

ml piperonyl butoxide (as a synergist). The door was then closed. After 10 minutes the sheets were examined for mosquitoes that had dropped on the sheets. The mosquitoes were placed in wet Petri-dishes and transported to the laboratory in cooler-boxes.

3.8 Supervision of mosquito collection

Supervision was carried out by two teams. Each of the team consisted of two technical officers and a driver (plate 3). They visited all sample houses. In case of night catches the supervisors visited the houses 3 or 4 times throughout the night. Their responsibilities included replacing torch cells and blown-up bulbs, keeping records of field activities and events which included number of collectors, houses sampled and general weather conditions.

3.9 Collection of rainfall data

Between June 1992 and May 1993 a rain gauge was placed in a homestead in each of the nine sites. A responsible person was trained on how to collect and record the daily rainfall data. The rain gauge was read between 8.00

Plate 3. The supervisory team. The team was divided into 2 groups.

am and 9.00 am each morning. The rain water which had accumulated over a 24 hour period was recorded (in mm) in a register provided for the purpose. The rain gauge was then drained, shaken dry and reset to be read the next morning.

3.10 Laboratory investigations

When the mosquitoes were brought to the laboratory they were sorted into anophelines and culicines. The culicines were dried with copper sulphate and later identified anatomically. The anophelines were initially identified into species based on external morphology according the method of Gillet (1972). They were also grouped according to their physiological status, i.e whether blood-fed, half gravid, gravid or empty as described by WHO (1975). The relevant information was entered in forms specially developed for the purpose (see appendix 2 and 3).

3.10.1 Dissection and estimation of sporozoite loads

The method of dissection recommended by WHO (1975) and the quantitative method for estimation of sporozoite

numbers in mosquito salivary glands described by Beier *et al.* (1991) were used.

The mosquito to be dissected was registered in designed forms which were specifically developed for the purpose (see appendix 4). The data entered for each mosquito included the collection site and the specific sampling house. The mosquito was then placed near a drop of M-199 which was the dissecting medium (Gibco Life Technologies Inc., Grand Island, New York). Using a 26 gauge needle the head was pulled away from the main body. When the salivary glands were pulled out, they were identified and nicked to detach them from the head. The salivary glands were picked using a 10 µl capillary pipette (VWR Scientific Inc. - USA) and placed in a glass tissue grinder (Kontes Glass Company, NJ. -USA) into which 50 µl of M-199 had been placed. The salivary glands were ground for 20 seconds. Using a 10 µl capillary pipette, 10 µl of the homogenate was withdrawn and transferred into a haemocytometer (Hausser Scientific Partnership, Horsham, PA. - USA).

The preparation was allowed to settle in a moist chamber for 3 minutes. The sporozoites were counted in each of the 0.1 µl corner squares of the counting chamber using

phase contrast microscopy. The total number counted in the 4 corner squares was multiplied by a factor of 125 to give the total sporozoite load of mosquitoes which were positive. To the remaining 40 µl of the salivary gland suspension, 50 µl of grinding solution and 50 µl of blocking buffer were added. The blocking buffer was prepared by boiling 5 gm Casein (Sigma Chemical Company, St. Louis, Mo. - USA) in 100 of 0.1 NaOH. Nine hundred ml of Phosphate Buffered Saline (Dulbecco's PBS, Sigma Chemical Company) were then added. When this preparation had cooled to room temperature the pH was adjusted to 7.4. Thimerosal (0.1 gm) and phenol red (0.02 gm), both from Sigma Chemical Company, were then added (see also appendix 4). The grinding solution was prepared by adding 200 µl of Nondet P-40 (Sigma Company, St. Louis, Mo., USA) to 40 ml of blocking buffer.

The salivary gland material which was not used for estimation of sporozoite loads was stored at -20^{0}C and later tested for sporozoites using ELISA.

3.10.2 Estimation of number of ingested sporozoites

Anopheles mosquitoes which were blood-fed (those that had visible blood in their guts and were also positive for salivary gland sporozoites) were further dissected to estimate the minimum number of sporozoites injected from the salivary glands during the time of feeding as described by Beier *et al.* (1992). The tergal and the sternal plates of the first abdominal segment were gently cut before removing the abdominal cuticle. Care was taken not to contaminate the gut contents with the sporozoites from the haemolymph.

Holding the mosquito from the wings or by the thorax the stomach was rinsed twice in fresh PBS (pH 7.4) in a depression slide (50 µl each rinse). The stomach was separated from the alimentary system by cutting the anterior region of the pharynx. The stomach was then suspended in 10 µl PBS (pH 7.4) after which it was punctured and the gut contents allowed to flow out. The contents were mixed well and spotted on indirect fluorescent assay (IFA) slides. The slides were air dried and stained with Fluorescein isothiocynate-conjugated (FITC) monoclonal antibody 2A10 as follows: Twenty (20) µl FITC-2A10 mixture were placed on IFA spot and incubated at room temperature in a moist chamber for

30 minutes. (The monoclonal antibodies were reconstituted in glycerine and water in the ratio of 1:1 and used at a concentration of 2 µg/5 ml PBS (5 µl stock antigen solution/250 µl PBS).

The liquid was withdrawn from the IFA spots using a Pasteur pipette and the slides rinsed once with 25 µl PBS. The slides were blotted dry and examined under a fluorescent microscope at high dry power to identify fluorescing sporozoites.

3.10.3 Dissection for parity and oocysts

The midguts of *Anopheles* mosquitoes were dissected for oocysts and /or parity depending on the physiological status of the mosquito. Blood-fed and empty mosquitoes were dissected for parity and oocysts but the gravid ones were dissected for oocysts only. The gravid mosquitoes were aken to be parous and were classified as ovarian stage 5.

After each mosquito was dissected to remove the salivary glands, the stomach section was placed in a drop of water and the second last segment was nicked at the top and

bottom using a dissecting pin. With one pin anchoring the mosquito at the chest, a fine pair of forceps was used to pull out the internal organs of the mosquito. The ovaries were detached from the midgut and the ovarian stage of eggs immediately determined. The ovaries were allowed to dry in air and then the parity determined. If the ovaries contained tracheoles with formed skeins the mosquitoes were nulliparous whereas if the tracheoles were smooth the mosquitoes were parous. As for the oocysts, a drop of 2% mercurochrome (Sigma, Chemical Company, Mo. -USA) diluted in distilled water, was placed on the midgut. A coverslip (9 x 9 mm) was mounted and the preparation examined for oocysts. If the mosquito was blood-fed, a portion of blood was saved for bloodmeal analysis. The remaining material was dried with copper sulphate (Drierite - W. A. Hammond Drierite Company, Xeina, Ohio. USA). The head and thorax region was saved for sporozoite ELISA and the remaining material (stomach, wings and legs) was saved for polymerase chain reaction (PCR) to identify the species within the *Anopheles gambiae* complex. Ovaries for half gravid mosquitoes were removed to determine the species of *An. gambiae* complex.

If the mosquito was not dissected, it was dried for 3 days in copper sulphate. Later it was transversely cut

to separate the head-thorax and abdominal sections. The head-thorax was tested for sporozoite ELISA and the abdomen segments saved for PCR to determine species of *Anopheles gambiae* complex.

3.10.4 Sporozoite ELISA

Before being tested for sporozoite antigens, the head-thorax specimens were prepared for ELISA by homogenising in 50 µl of grinding solution and then adding 200 µl of blocking buffer (see appendix 5). *Plasmodium falciparum* and *P. malariae* sporozoite ELISA tests were done using the techniques described by Wirtz *et al.* (1987b) and Beier *et al.* (1991b). Lyophilized monoclonal antibodies (Mab were reconstituted in a mixture of glycerine and water diluted in the ratio of 1:1. Working concentrations of the Mab were diluted in PBS in the ratio of 1:125.

3.10.4.1 *Plasmodium falciparum* sporozoite ELISA

The 96-well microtiter plates (Dynatech, Sullyfield Circle, Chantilly, Virginia, USA) were labelled appropriately. Twenty (20) µl of *P. falciparum*

monoclonal antibody (2A10-Kirkgaard and Perry, Gaitherburg, MD. USA) was diluted in 5 ml PBS. In each microtitre well, 50 µl of the monoclonal antibody was placed and incubated for 30 minutes before the contents were aspirated. The plate was then banged dry on soft tissue paper. Without washing the unbound reactive sites were blocked with 200 µl blocking buffer. After 1 hour of blocking, the contents were aspirated and the plate dried again. The plates were then charged with 50 µl of the mosquito triturate. Both negative and positive controls were included. The negative control consisted of four male *Anopheles* mosquitoes which were homogenised in 1 ml of PBS. The positive control consisted of commercially prepared recombinant *P. falciparum* CS protein (Wirtz *et al.* 1987a). The plates were incubated for 2 hours. The contents were aspirated and the plates washed twice with PBS-Tween 20. The solution for washing was prepared by adding 0.5 ml Tween 20 (Sigma, Chemical Company) to 1 litre PBS. Fifty (50) µl horseradish peroxidase-conjugated Mab (Kirkgaard and Perry) were added to each well. The plates were then washed 3 times with PBS-Tween and incubated for 1 hour after which 100 µl of peroxidase substrate (2.2 azino-di[3-ethyl-benzethiazoline] sulphonate) - [ABTS] was added. The

plates were read visually after 30 minutes using the technique described by Beier and Koros (1990).

3.10.4.2 *Plasmodium malariae* sporozoite ELISA

Plasmodium malariae sporozoite ELISA was carried out as already described for *P. falciparum* ELISA (see section 3.10.4.1). Thirty (30) µl of *P. malariae* Mab (453 and 88-44 [K & K]) per 5 ml PBS were used.

3.10.4.3 Determination of entomological inoculation rate (EIR)

The EIR was monitored for the period between June 1992 and May 1993. The EIR was the product of man-biting rate and the sporozoite rate. The daily man-biting rate (MBR) was computed as the number of *Anopheles* mosquitoes collected per night per collector. The daily MBR was then multiplied by the number of days for the specific month to give the monthly MBR. The monthly EIR was the product of the monthly MBR and monthly sporozoite rate both by dissection and sporozoite ELISA techniques. The cumulative monthly EIRs gave the annual EIR which in this text is referred to simply as EIR.

3.10.5 Determination of index of dispersion for the *Anopheles* mosquitoes

The index of dispersion to assess the spatial pattern for *Anopheles* mosquitoes was derived using the formula given by Ludwig and Reynolds (1988). The formula stipulates that:

Index of dispersal = variance/mean.

The index of dispersion indicates how the population varies from 1, which is the equilibrium of dispersion. If variance is less than mean, then the dispersal is regular and if variance is greater than mean, then the organisms are clumped together.

3.11: Cross-sectional survey of parasitology, serology, spleen rate and haemoglobin levels

3.11.1 Recruitment and collection of blood

During specific periods of the year, the population in the study sites migrate to other areas to prepare land for planting. When this exercise is over, the people return to their residential area (the study area) to wait

for the planting season. A cross-sectional collection of blood was done between 15th and 26th February 1993 in the entomological sampling compounds, at a time when most adult family members are settled in the study area. In this study two hundred and sixteen (216) compounds were sampled. A total of 2387 persons (including 1082 children ≤ 10 years of age) were screened and their blood samples taken. Before blood collection, all the households were visited and the heads of households informed of the intended cross-sectional blood sample collection (plate 4). The main reason given was that having collected mosquitoes in their houses for almost one year, we wished to collect a small amount of blood, analyze it, and find out whether there was any correlation between the sporozoite rate and the parasites that they may be carrying in their blood. The households were visited again and informed of the specific dates that the sampling would take place in their respective sites. A timetable was drawn for bleeding the population in the 9 sites. On the actual day for screening, the residents were requested to assemble in specific compounds. Those who were far away were provided with transport.

Plate 4. Discussing the study with a household head. Consent for recruitment of a house depended mainly on the household heads.

Two teams were used for blood collection. Each team was made up of a driver, field assistant (one of the mosquito collectors who was conversant with the location of all the entomological sampling houses), record entry personnel, interpreter, clinician and laboratory personnel for blood collection (plate 5).

Before taking blood samples, each recruited individual, was physically examined by the clinician and their splenic status recorded (see section 3.11.4 for details). Those who required simple medications were treated on the spot whereas those who required further investigations and more drugs were referred to Kilifi District Hospital. Those who looked very sick were referred and taken to Kilifi District Hospital immediately. After the general examination, the individuals had their finger pricked and their blood smears were made (plate 6). About 300 µl of capillary blood was also collected in plain microtainers^{R} (Becton Dickson and Company, Rutherland, New Jersey, USA). For children up to 10 years of age, 5 µl of blood were collected in a capillary tube for haemoglobin estimation. The 5 µl blood was diluted in 2 ml Drabkin's solution and later transported to the laboratory in Kilifi.

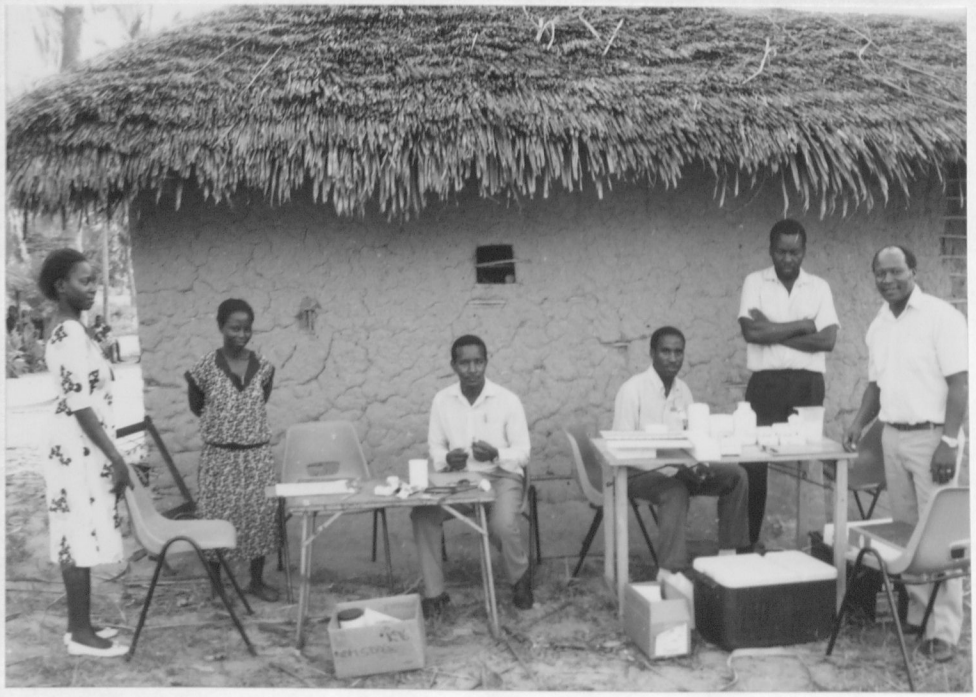


Plate 5. Members involved in the cross-sectional survey.

Plate 6. Blood collection in progress.

In the laboratory, the specimens were sorted and matched with the individual's form. The microtainers were centrifuged and an aliquot of serum (100μl) was separated for *Plasmodium malariae* and *P. ovale* studies by other researchers. The remaining serum in the microtainer was stored at -20^{0}C until when they were tested for CSAB and ASAB.

3.11.2 Examination of malaria parasites

Thick and thin blood smears were made on the same slide. The thin smear was fixed in absolute methanol. Care was taken not to fix the thick smear. Once dry the smears were stained in 4% Giemsa solution for 30 minutes. Two hundred (200) microscopic fields were examined for asexual stages of *P. falciparum*. For gametocytes of *P. falciparum* and all stages of *P. malariae* and *P. ovale*, 400 fields were examined. The conversion of parasite density per μl of blood was worked out as per formula in appendix 6.

3.11.3 Estimation of haemoglobin

Haemoglobin (Hb) for children up to 10 years was estimated using cyanmethemoglobin technique according to Daicie and Lewis (1991). Five (5)μl of blood were collected in a heparinised capillary tube. The blood was then mixed with 2 ml Drabkin's solution. The tubes were kept in a cooler box until when they were tested in the laboratory. The Hb levels were estimated by spectrophotometry at 540 nanometers (nm).

3.11.4 Screening for spleen rates

The subjects were screened by a clinician for enlarged spleens while standing. For young babies the screening was done while they were being held by their mothers. The splenic region was palpated and the results were recorded as "yes" for enlarged spleens at all degrees and "no" if the spleen was normal. No attempt was made to classify the spleen size as in Hacket's grading (Hacket, 1944).

3.11.5 Detection of circumsporozoite antibody

The technique described by Wirtz *et al.* (1987b) was used with some modifications as outlined below. The test was standardised at a serum dilution of 1:100 and the conjugate at 1:5000. R32LA antigen, obtained through John Beier (Johns Hopkins University), was reconstituted in distilled water. This formed the stock antigen used.

Fifty (50) µl of solution B (see appendix 5) were placed in odd numbered wells of an Immulon 2 "U" microtitre plate, labelled B (Dynatech, York, PA, USA) as shown in appendix 7. Fifty (50) µl of solution A were placed in even numbered rows of the wells, labelled A. The plates were covered and allowed to incubate overnight at room temperature.

The contents were aspirated and each well was blocked with 250 µl of solution C (see appendix 5). After 1 hour of blocking the contents in the wells were aspirated and 50 µl of test samples, diluted 1:100 in solution C were added. Positive control serum (from known Kilifi residents) and negative control serum (a battery of sera supplied by Oxford University, England through Kevin

Marsh) were added at this stage. The plates were incubated for 2 hours.

After 2 hours, the contents were aspirated and the plates washed 2 times with PBS-Tween (see appendix 5). Fifty (50) μl of goat anti-human conjugate, diluted 1:5000 in solution C were placed in each well. The plates were incubated for 1 hour.

The plates were then washed 3 times with PBS-Tween. One hundred (100)μl of ABTS substrate was then added into each well. After incubation for 30 minutes the absorbance was read using a spectrophotometer (Titertek Multiskan^{R} MCC) at 412 nm. The samples were run in duplicate and the mean optical density (OD) of the 2 wells without antigen was subtracted from the mean OD of the two wells with the antigen and this taken as the OD value for the sample. The cut-off value for positive ODs was taken as the mean negative absorbance values of all the plates plus three standard deviations. The seropositive values were used to obtain seroconversion rates using the model formulated by Knight (1975) in which 2 assumptions are made: 1) that the rate of gain or loss of infection and seropositivity can each be

represented by a simple rate constant and 2) that superinfection may occur but this does not affect the duration of infection. Sixteen age brackets were used to calculate the α (which was the constant for seropositive rate) whereas the smallest chi square test result obtained after a series of trials, was used for analysis (see appendix 8).

3.11.6 Indirect fluorescent antibody test (IFAT) for asexual stage antibodies

3.11.6.1: Standardization of IFAT

To standardize IFAT serial dilutions of serum were ran against serial dilutions of the conjugate. The serum dilutions were 1:10, 1:20, 1;50 and 1:100. Similar dilutions were made for the conjugate. The negative sera used were from individuals from Oxford University, England, who had no prior exposure to malarial infections. The positive sera used were from individuals known to have had malaria from Kilifi, coastal Kenya, and Kisumu in western Kenya. A serum dilution of 1:20 and conjugate dilution of 1:50 gave the best results and these dilutions were thereafter used throughout the rest of the study.

13.11.6.2 Indirect immunofluorescent antibody test

Serum was tested for *P. falciparum* asexual stage antibodies using IFAT. The antigen slides which had whole asexual stage parasites of *P. falciparum* were prepared from *in vitro* culture as described in appendix 9. The technique described by Contreras *et al.* (1988) was used with some modifications as outlined below.

Immediately before testing, the slides were removed from -20^{0}C and thawed quickly by use of a hot air blower. The slides were then dehaemoglobinised with 0.5% Hydrochloric acid for 5 minutes. They were rinsed in distilled water and PBS, pH 7.4, for 5 minutes in each change. They were then blotted dry using soft tissue paper. Ten (10) µl of the test serum diluted 1:20 in PBS were placed on each spot. The slides were allowed to react for 30 minutes in a moist chamber.

After 30 minutes, the slides were rinsed in PBS for 5 minutes. They were then incubated with 5 µl of 1:50 dilution of FITC anti-human conjugate (Kirkgaard and Perry, MD) for 30 minutes in a moist chamber. A positive and negative control (same sera used in CSAB assay) were

spotted on each slide. The slides were then rinsed in PBS for 5 minutes. After blotting the slides dry, a coverslip (20 x 40 mm) was mounted with glycerine. The slides were examined using a fluorescent microscope at high dry power. They were reported as "Yes" for positive if fluorescing antibodies were seen or "No" for negative results.

3.12 Identification of severe malaria

Children aged between 1 and 59 months who presented with severe malaria were admitted in the paediatric ward in Kilifi District Hospital. These children were resident within a defined study area. Every admission was registered and a description of the locality of their homes recorded. The child's clinical and laboratory results were reviewed and the primary cause of admission recorded. The primary diagnoses of malaria were further defined as severe or not severe by the criteria suggested by the Wellcome Trust/Kilifi epidemiological researchers (Snow *et al.*, 1990). The child was considered to be having severe malaria if it did not localise pain, or had Hb of less than 5.1gm/dl with a peripheral parasitaemia of at least 10,000 parasite/mm^3, or was prostrated

(unable to sit or stand unaided), or had two or more generalised convulsions within 24 hours prior to admission.

3.13 Heterogeneity of *Anopheles* mosquitoes

Household, socio-demographic and environmental survey

All the 216 entomological sampling households were visited. The purpose for conducting the interview was explained to the heads of each household before commencement of the interview.

An interview was then conducted in the presence of the head or any other responsible person in the homestead. A physical check was done to verify some items e.g. the presence or absence of mosquito nets, the number of windows and the number of doorways. The interviewer also inspected the compound to obtain information on house environment. A questionnaire formulated for the purpose was completed (see appendix 10).

3.14 Human malaria antibodies in blood-fed mosquitoes

3.14.1 Mosquito collection

Mosquitoes for detection of human malaria antibodies were collected between March and June, 1993. Since mosquito populations were not high during most of the year more emphasis was put on PSC collections. All species of mosquitoes collected were brought into the laboratory and sorted out according to blood-fed or not. Mosquitoes with up to 4 segments filled with blood qualified to be categorised as blood-fed. These blood-fed mosquitoes were dried with copper sulphate for 3 days to facilitate the separation of the thorax from the abdomen. They were then identified according to genus and if possible, according to species. The thorax was separated from the abdomen and the abdomen homogenised in 500 µl PBS. Information on each mosquito species processed was entered in the appropriate entomological forms.

3.14.2 Human bloodmeal ELISA

The technique described by Beier *et al.* (1988) was used with a few modifications as outlined below. The purpose

of doing bloodmeal ELISA was to identify the mosquitoes which had fed on human beings as opposed to those which had fed on other animals.

A 96-well microtitre plate (Dynatech) was labelled appropriately. Into column 1, in row A was placed 50 µl of human sera (positive control) diluted 1:100 (5 µl/500 µl) in PBS. In rows B, C, D and E of the same column was placed 50 µl of sera from chicken, dog, cat and goat respectively, representing the animals which are commonly seen in the study area. Negative controls (50µl) were placed in column 2. The negative controls were male mosquitoes ground in PBS at 1 mosquito for 250 µl PBS. Homogenised mosquito bloodmeal samples (50µl) were placed into the other wells. The plates were incubated at room temperature overnight. They were then washed 2 times with PBS-Tween and dried on soft tissue paper.

Five (5)ml of enzyme diluent (100 ml blocking buffer and 25 µl Tween 20) 2.5 µl of horseradish antihuman conjugate was placed in a test tube and 10 µl of sera from chicken, dog, cat and goat were added. Fifty (50) µl of this preparation was introduced into each well after which the plate incubated for 1 hour. The plates were then washed 3 times with PBS-Tween. After drying, 100 µl of ABTS

substrate was added to each well. The plates were incubated for a further 30 minutes. The reaction was read visually as described by Beier and Koros (1991b) and these were recorded as positive for human blood if the colour in the well turned blue/green or as negative if there was no colour change.

3.14.3 Human malaria antibodies

The technique described Beier *et al.* (1989) was used with a few modifications as outlined below. The technique was standardised at a conjugate dilution of 1:5000.

The samples that were human blood positive from the blood meal analysis were tested for human malaria antibodies. R32LA antigen obtained through John Beier (Johns Hopkins University, USA) was used.

Fifty (50) µl of Solution B (see appendix 5) were placed in odd numbered wells of Immulon 2 "U" plates labelled B (appendix 7). Fifty (50) µl of solution A were placed in even numbered rows labelled A. The plates were covered and incubated overnight at room temperature.

The contents were aspirated, the plates dried, and each well was blocked with 250 µl of solution C (see appendix 5). After 1 hour the contents in the wells were aspirated and the plates dried. Fifty (50) µl of the mosquito triturate were added in each well.

Positive and negative controls (same as the ones used in CSAB ELISA) were used. The plates were incubated at room temperature for 2 hours. After 2 hours the contents were aspirated and the plates washed 2 times with PBS-Tween. 50 µl of goat-antihuman conjugate diluted 1:5000 in solution C were placed in each well. The plates were then incubated for 1 hour.

After incubation the plates were washed 3 times with PBS-Tween and 100 µl of ABTS substrate added. Absorbance was read using a spectrophotometer at 412 nm. The samples were run in duplicates. The mean OD of the 2 wells with antigen less the mean OD of the corresponding wells without antigen was taken as the OD of the sample. The cut off value for the positive OD was taken as the mean negative absorbance value for all the plates plus 3 standard deviations.

CHAPTER 4

RESULTS

4.1 Malaria in the study population

4.1.1 Infections with malaria parasites

During the cross-sectional survey, 1,229 (52.0%) of 2,365 blood slides examined, were positive for malaria parasites. Parasite positive rates varied from site to site (X^2 = 16.56, df = 8, p < 0.001) (table 4.1.1). Three species of *Plasmodium* were identified: *P. falciparum* (1,195 asexual stages), *P. malariae* (134 cases) and *P. ovale* (46 cases). In this sub-section, *P. falciparum* refers to asexual stage only whereas *P. malariae* and *P. ovale* refers to all stages, unless otherwise indicated. A total of 164 cases were mixed infections out of which 105 cases were positive for *P. falciparum* and *P. malariae*, 41 cases positive for *P. falciparum* and *P. ovale*, and 12 cases positive *P. falciparum*, *P. malariae* and *P. ovale*. There were 29

Table 4.1.1: Summary of malaria parasites and *P. falciparum* gametocyte rates in the study population.

Site	Number examined	Malaria parasites +ve		Gametocytes +ve	
		Number	%	Number	%
1	275	125	45.5	29	23.2
2	233	108	46.2	41	38.0
3	249	139	55.8	35	25.2
4	210	108	51.4	17	15.7
5	289	143	49.5	37	25.9
6	272	143	52.6	46	32.2
7	278	140	50.3	36	25.7
8	312	157	50.3	44	28.0
9	247	166	67.2	39	23.4
Total	2365	1229	52.0	324	26.4

cases of *P. malariae* alone and 5 cases of *P.ovale* alone. The parasite positive rate increased from 22.5% in children up to one year of age to 70.2% in the 5 to 9 years age group (figure 4.1.1). The rate gradually dropped to 25.1% in individuals over 40 years of age.

4.1.2 Density of malaria parasites

Parasite densities ranged from 2 to 294 parasites per microliter (μl) for *P. ovale*, 2 to 903 parasite per μl for *P. malariae* and from 2 to 160,480 parasites per μl for *P. falciparum*. The overall geometric mean density for *P. falciparum* was 153 parasites per μl (standard deviation (sd) = 149) and varied significantly among sites ($F = 2.655$, $df = 8,1185$, $p < 0.005$) (table 4.1.2).

Children up to one year of age had a mean density of 228 (sd = 267) parasites per μl. Children between 2 and 4 years of age had a geometric mean of 518 parasites per μl (sd = 504). The mean densities declined gradually with age to individuals 15 years and above who had a mean density of 42 parasites per μl (sd = 35).

Fig. 4.1.1: Positive rate for *P. falciparum* asexual stage parasites and gametocytes

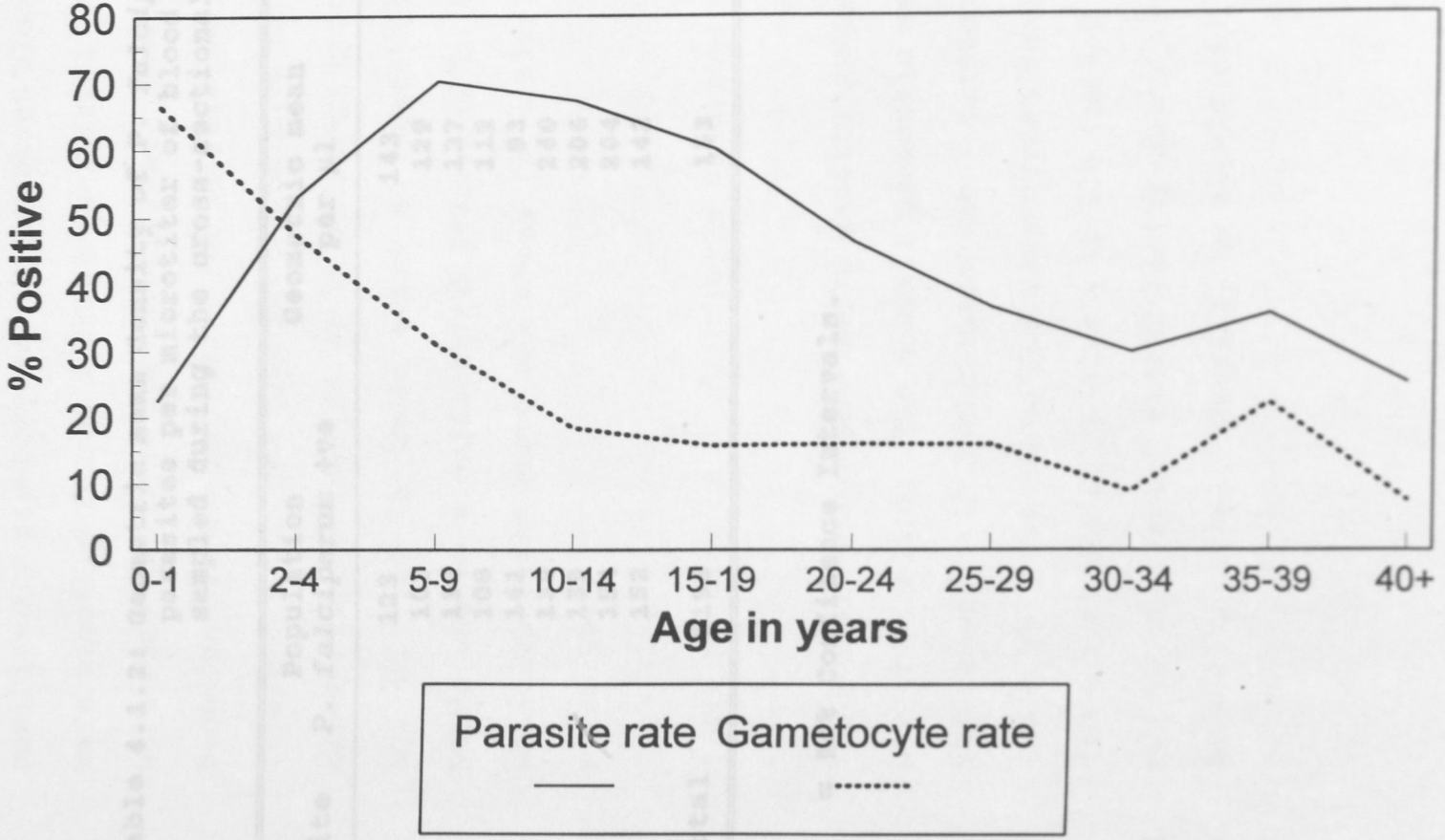


Table 4.1.2: Geometric mean density of *P. falciparum* asexual parasites per microtiter of blood in volunteers sampled during the cross-sectional study.

Site	Population *P. falciparum* +ve	Geometric mean per µl	95% CI[$]
1	123	143	97 - 212
2	103	129	83 - 201
3	136	137	92 - 205
4	108	112	75 - 167
5	141	93	65 - 141
6	139	240	167 - 346
7	139	206	135 - 285
8	154	204	148 - 312
9	152	142	102 - 198
Total	1195	153	135 - 174

[$] = 95% Confidence Intervals.

4.1.3 *Plasmodium falciparum* gametocyte carriers

Out of 1,229 *P. falciparum* positive slides, 277 were positive for gametocytes. Forty-seven blood smears which were negative for *P. falciparum* asexual stage parasites were found positive for gametocytes. Overall 324 (26.4%) blood smears were positive for *P. falciparum* gametocytes (table 4.1.1). The gametocyte positive rate in the study sites dropped from 66.7% in children up to one year of age to 7.5% in those above 40 years (figure 4.1.1). There was no significant differences in the gametocyte positive rates among the sites (X^2 = 13.77, df = 8, p > 0.09).

4.1.4 Density of *P. falciparum* gametocytes

The *P. falciparum* gametocyte density ranged from 1 to 109 per μl of blood. The overall geometric mean density for the gametocytic individuals was 3 gametocytes per μl (sd = 2). The means for gametocytes ranged from 2.6 (sd = 1.5) per μl in site 4 to 4.4 (sd = 2.7) gametocytes per μl and varied significantly among sites (X^2 = 2.1, df = 8, p <0.04). Overall, no significant difference in

geometric means occurred among age groups (≤1 year, 2-4 years, 5-9, 10-14 years and 15 years and above) ($F = 1.17$, $df = 4, 1189$, $p > 0.14$).

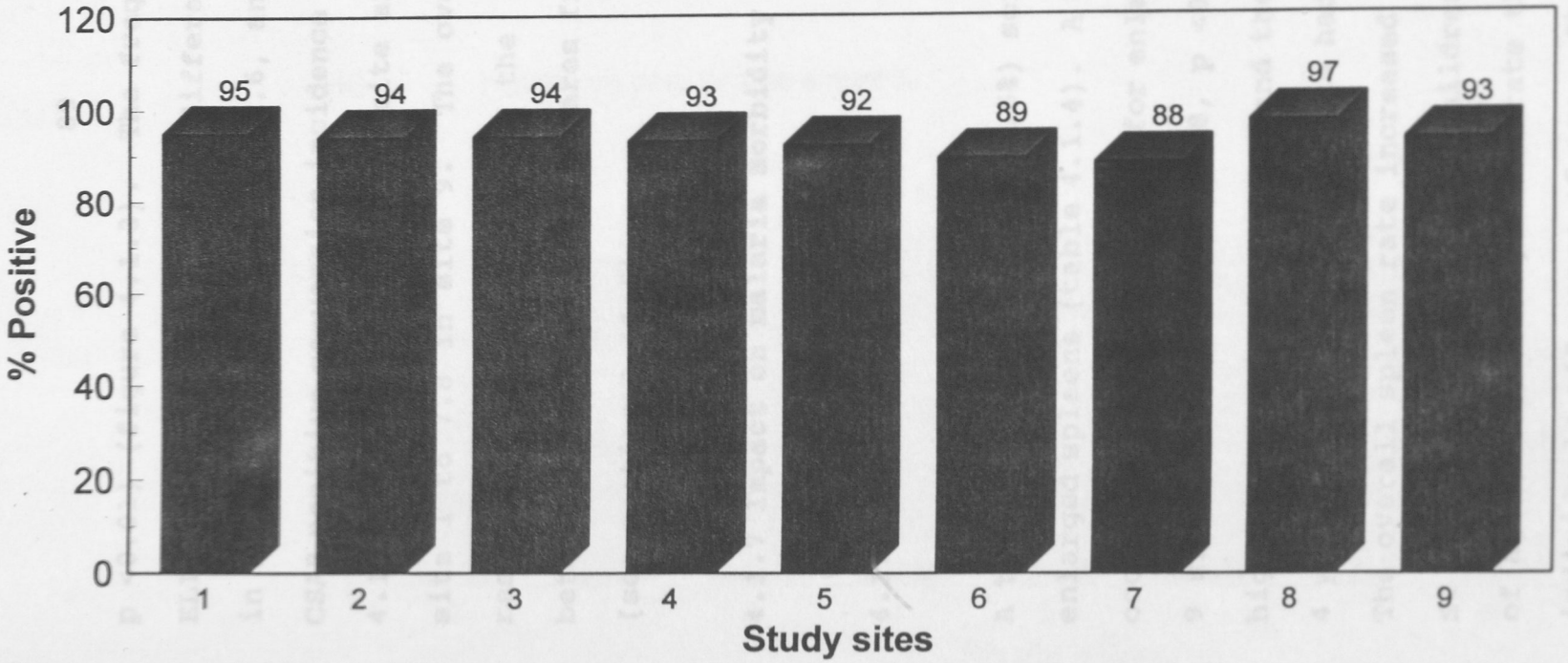
4.1.5 Infection exposure: Asexual stage antibodies (ASAB)

Indirect fluorescent antibody tests for asexual stage antibodies were done on 2,366 serum samples. Two thousand one hundred and ninety seven (92.9%) samples were positive for ASAB (figure 4.1.2). The positive rate ranged from 88.4% to 96.5% in all the 9 study sites. There was a significant difference in asexual stage positive rates among the 9 sites ($X^2 = 20.8$, $df = 8$, $p < 0.01$).

4.1.6 Sporozoite exposure: circumsporozoite antibodies (CSAB)

A total of 2,365 serum samples were tested for CSAB. The ELISA cut-off optical density (OD) for positivity was 0.12 (mean of negative controls plus 3 standard deviations). Out of 2,365 samples tested, 1,104 were positive, giving an overall positive rate of 46.7% which varied significantly among sites ($X^2 = 25.2$, $df = 8$,

Figure 4.1.2 : Prevalence of asexual stage antibodies detected in human sera from nine study sites.



Figures on bars denote actual %

p <0.01) (figure 4.1.3). The frequency distribution of ELISA absorbance values for different age groups is shown in figures 4.1.4, 4.1.5, 4.1.6, and 4.1.7. The annual CSAB positive conversion incidence rate as shown in table 4.1.3, varied from site to site and ranged from 2.7 in site 1 to 7.8 in site 9. The overall chi square test result per site indicated the level of discrepancy between the computed chi squares from a series of trials (see section 3.11.5).

4.1.7 Impact on malaria morbidity

4.1.7.1 Spleen rate

A total of 427 of 2,291 (18.6%) screened individuals had enlarged spleens (table 4.1.4). A significant difference occurred in positive rates for enlarged spleens among the 9 sites (X^2 = 20.06, df = 8, p <0.05). Site 4 had the highest spleen rate (26.2%) and the children between 2 to 4 years of age in the same site had spleen rate of 45.8%. The overall spleen rate increased from 14.2% in children ≤1 year of age to 29.7% in children between 5 to 9 years of age (table 4.1.5). The rate then dropped to 5.2% in individuals >19 years of age. The population with

Figure 4.1.3: CSAB positive rate from human sera in the study sites.

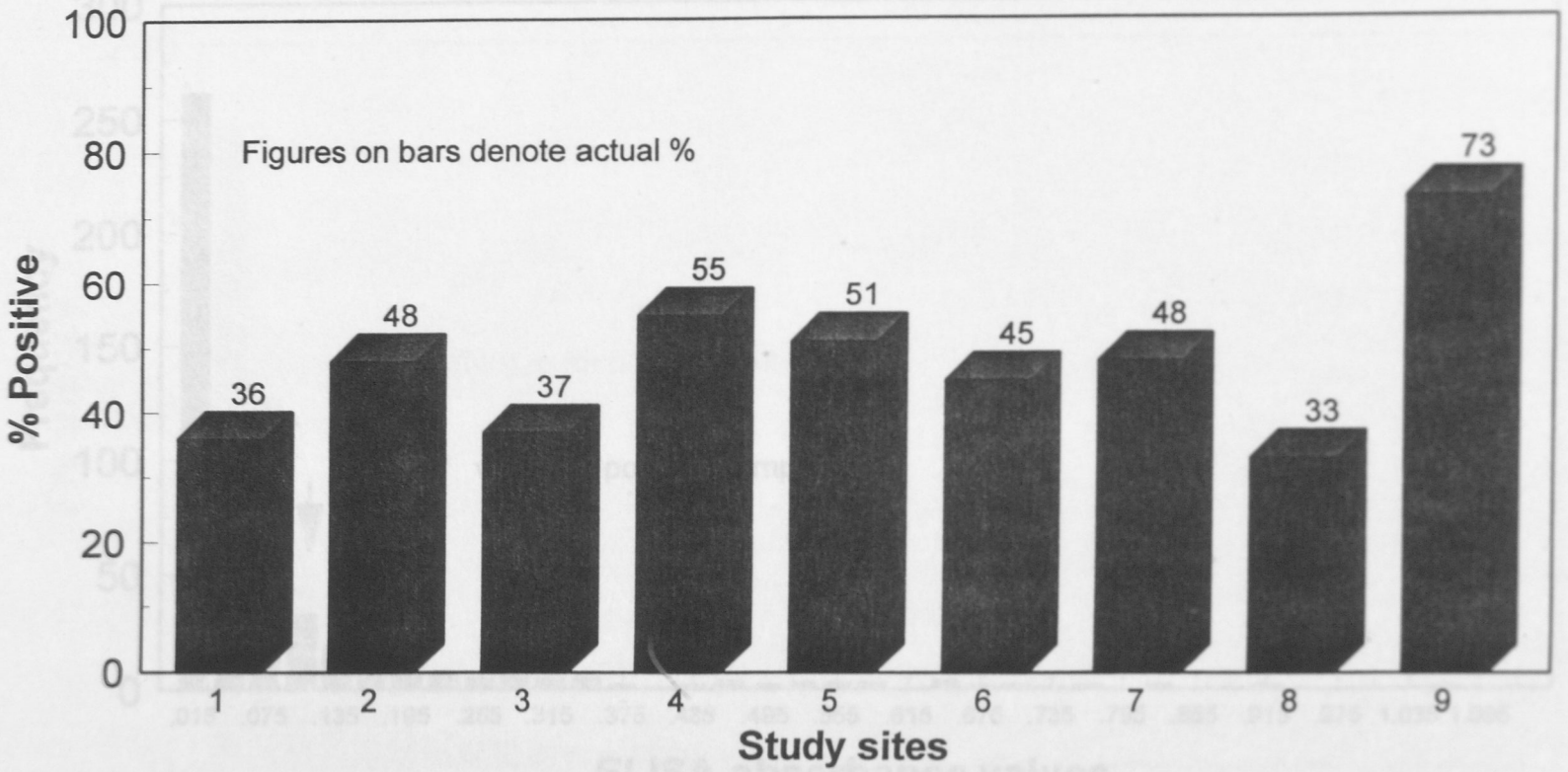


Figure 4.1.4: Frequency distribution of CSAB absorbance values for children up to 5 years of age.

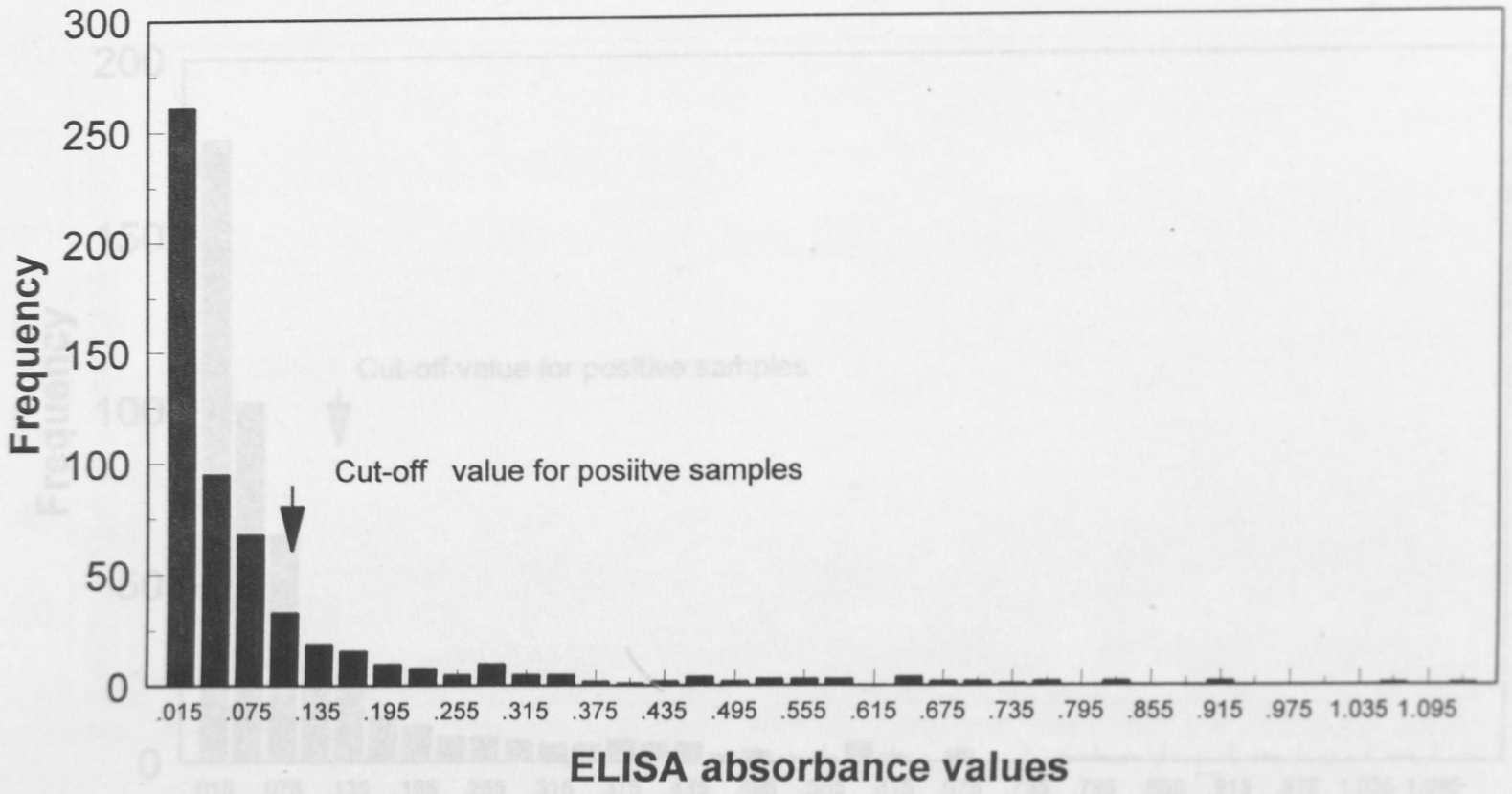


Figure 4.1.5: Frequency distribution of CSAB absorbance values for individuals 6 to 10 years of age.

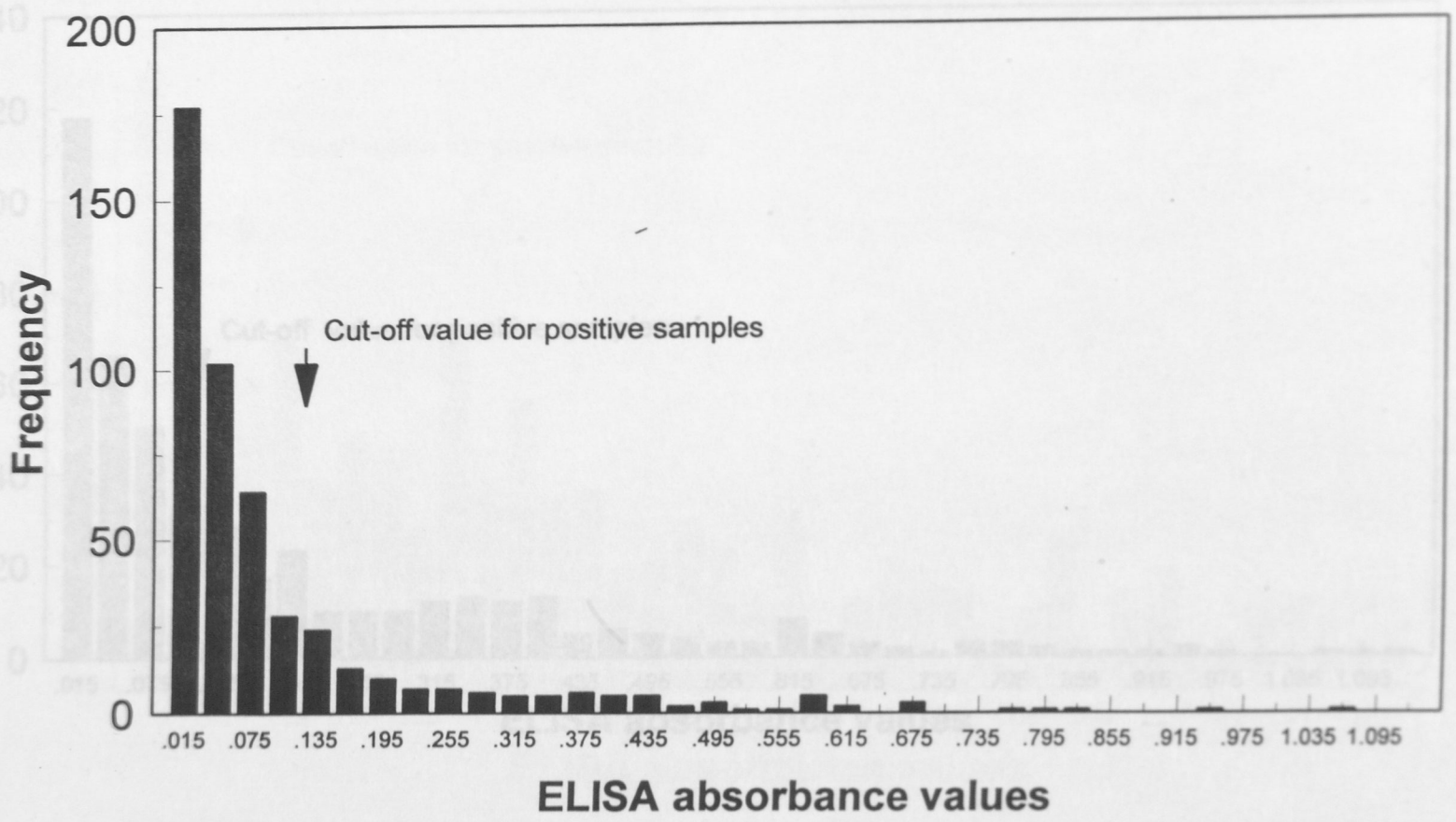


Figure 4.1.6: Frequency distribution of CSAB absorbance values for individuals 11 to 19 years *of age*.

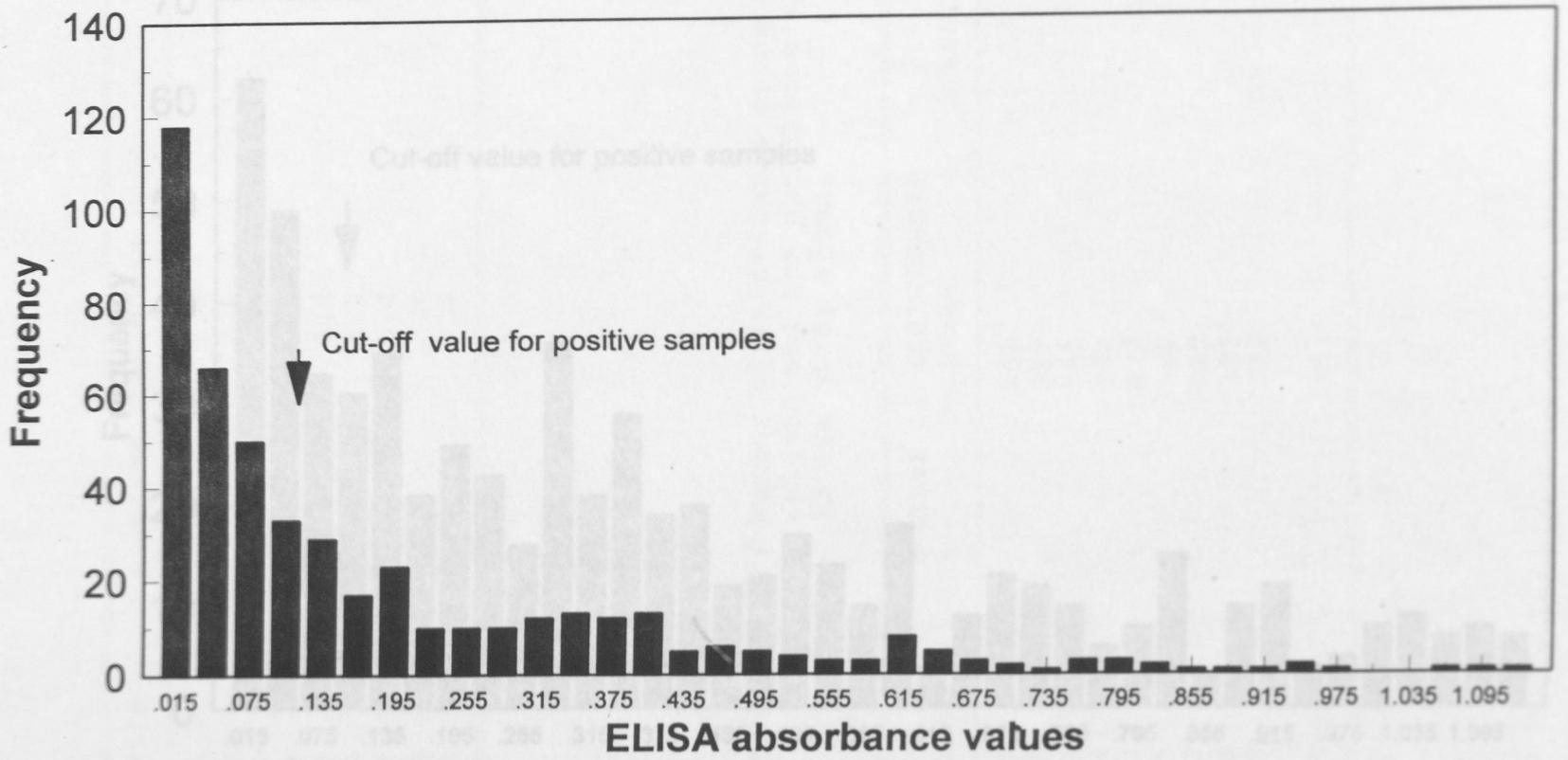


Figure 4.1.7: Frequency distribution of CSAB absorbance values for individuals 20 years of age and above.

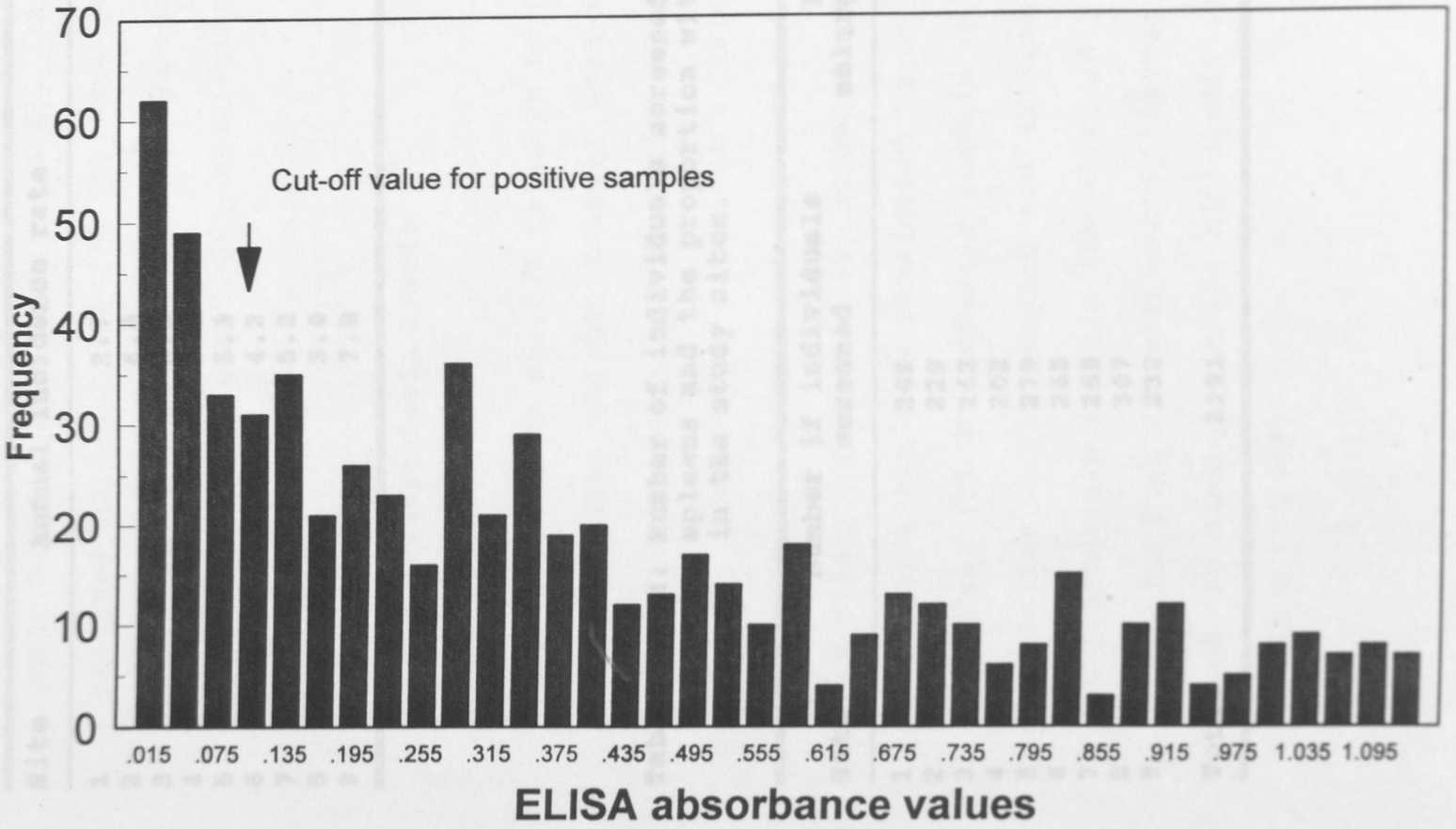


Table 4.1.3: Circumsporozoite antibody conversion rates in the study area according to sites (p=0.05).

Site	Annual incidence rate	Chi square
1	2.7	19.67
2	4.8	27.50
3	3.3	27.36
4	5.4	31.40
5	5.3	22.31
6	4.2	20.87
7	5.2	44.23
8	3.0	31.31
9	7.8	59.84

Table 4.1.4: Number of individuals screened for enlarged spleens and the proportion with splenomegaly in the study sites.

Site	Number if individuals screened	Number with enlarged spleens (%)
1	269	45 (16.7)
2	229	43 (18.8)
3	243	47 (19.3)
4	202	53 (26.2)
5	279	68 (24.4)
6	265	38 (14.3)
7	265	44 (16.6)
8	307	50 (16.3)
9	232	39 (16.8)
Total	2291	427 (18.6)

enlarged spleens was also examined in relation to malaria parasite infections. Out of 427 individuals with enlarged spleens 316 (74.0%) had malaria parasites in their blood smears (table 4.1.6). Out of 864 without enlarged spleens, 315 (34.6%) were parasite positive. Thus, the relationship between parasite infection and enlarged spleens was highly significant (X^2 = 176.01, df = 1, p <0.001).

4.1.7.1 Haemoglobin levels

Haemoglobin (Hb) of 1076 children up to 10 years of age was estimated and the overall mean Hb level was 9.73 gm/dl with a standard deviation of 3.16 gm/dl (table 4.1.7). There was no significant difference in the means of Hb levels between the sites (F = 1.294, df = 8, p > 0.242). Twenty (1.9%) screened children had Hb below 5.1 gm/dl and were considered to be severely anaemic. Fourteen (71.0%) severely anaemic children had malaria parasites. Six hundred and sixteen (58.3%) of 1,056 children with anaemia (hb <7.1gm/dl) were parasitaemic implying that the relationship between parasite infection and the haemoglobin was not significant (X^2 = 1.521, df = 1, p > 0.214).

Table 4.1.5: Summary of spleen rates in the study sites according to age groups and site, in a cross-sectional survey done in February 1993.

Site	Age in years						Total
	≤1	2-4	5-9	10-14	15-19	20+	
1	0.0	8.3	16.1	12.0	20.0	8.0	16.7
2	10.0	22.0	40.3	15.2	16.0	2.6	18.8
3	25.2	24.1	35.2	18.0	18.8	2.7	19.3
4	20.0	45.8	41.0	33.3	15.9	5.5	26.2
5	37.5	18.9	30.6	24.5	4.3	3.3	24.4
6	0.0	25.5	28.1	7.9	4.3	4.7	14.3
7	14.3	16.7	27.8	10.8	0.0	7.0	16.6
8	5.0	30.0	25.0	10.0	0.0	7.4	16.3
9	9.0	31.0	35.9	12.2	0.0	5.1	16.8
Total	14.2	25.0	29.7	16.1	8.7	5.3	18.6

Table 4.1.6: Number of individuals screened in the study sites who had enlarged spleens and were also parasitaemic.

Site	Number of individuals with malaria parasites	% with enlarged spleen
1	125	36
2	140	31
3	143	33
4	157	34
5	108	63
6	166	23
7	139	32
8	108	46
9	143	27
TOTAL	1229	35

Table 4.1.7: Average haemoglobin levels in the children ≤10 years in the study sites.

Site	Number of children	Mean haemoglobin gm/dl	Standard deviation
1	116	9.43	3.87
2	113	9.87	3.36
3	113	10.12	2.87
4	79	9.75	3.05
5	135	9.76	3.16
6	130	10.07	2.78
7	141	9.27	3.59
8	148	9.78	2.67
9	101	9.59	3.18
Total	1076	9.73	3.73

4.1.8 Incidence of severe malaria

The rate of severe malaria in the study sites varied from site to site and from year to year. Table 4.1.8 shows the incidence of severe malaria cases by site. Between June 1992 and May 1993 (study period), 37 cases of severe malaria were identified in the nine entomological study sites (table 4.1.8). The profile of the severe malaria cases were retrieved from the hospital records. Thirteen (35%) of the cases came from 12 houses where entomological studies were done. Of the 37 severe malaria cases, 12 (32.4%) were admitted into the hospital in June 1992 (table 4.1.9). Site 4 produced two cases from one house and two houses in site 5 had two cases each. All three pairs of children were admitted in the hospital at different times.

4.1.9 Relation of malaria parasite infections to severe malaria

The parasite density for the children with severe disease from the nine study sites ranged from 1,492 to 1,240,000 parasite per µl. The geometric mean parasite density for severe cases was 61,958 per µl and was highly significantly different from the cohort group which was

Table 4.1.8: Number of severe cases of malaria identified between June 1989 and May 1993 and the incidence of severe malaria per 1000 children ( ) in the study sites.

Site	Population 1-5 years	89/90	90/91	91/92	92/93	Total	Level[L]
1	199	3 (15.08)	1 (5.03)	3 (15.08)	3 (15.08)	10 (12.56)	Low
2	233	3 (12.88)	0 (0)	0 (0)	2 (8.58)	5 (5.36)	Low
3	231	3 (9.06)	3 (9.06)	7 (21.15)	3 (9.06)	16 (17.31)	Moderate
4	292	8 (27.41)	4 (13.70)	16 (54.79)	6 (37.67)	34 (29.11)	High
5	202	2 (9.09)	1 (4.95)	7 (34.65)	7 (34.65)	17 (21.04)	Moderate
6	216	4 (18.52)	4 (18.52)	11 (50.93)	4 (18.52)	23 (26.62)	High
7	156	6 (38.46)	3 (19.23)	7 (44.87)	2 (12.52)	18 (28.85)	High
8	259	1 (3.88)	3 (11.58)	8 (30.88)	6 (23.17)	18 (17.37)	Moderate
9	105	0 (0)	1 (9.52)	1 (9.52)	4 (38.10)	6 (5.71)	Low

Source of data: Snow, *et al.* (1993)

L = level previously set for the different degrees of incidence of severe malaria prior to June 1992 when the entomological studies began.

Table 4.1.9: Summary of % of severe cases admitted per month between June 1992 and May 1993 and the % of *An. gambiae* positive for salivary glands sporozoites and monthly rainfall during the same period

Year/ month	Number of severe cases admitted	*An. gambiae* with sporozoites	Amount of rainfall
Jun 1992	32.4	12.5	115
Jul	16.2	20.8	112
Aug	10.8	12.5	25
Sep	2.7	0.0	27
Oct	0.0	2.1	28
Nov	0.0	10.4	83
Dec	5.4	0.0	81
Jan 1993	2.7	16.7	56
Feb	16.2	10.4	1
Mar	8.1	0.0	5
Apr	0.0	2.1	29
May	5.4	10.4	189

446 parasites per μl for children up to 5 years of age ($F = 157.5$, $df = 1, 1229$, $p = <0.001$). There was no significant difference in the overall CSOD between the populations living in the houses where severe cases came from and the houses where the cohort group of children lived ($F = 0.88$, $df = 1, 1093$, $p > 0.439$).

The overall spleen rate varied among sites and among age groups (table 4.1.4 and 4.1.5). No significant association occurred between the spleen rate and occurrence of severe malaria (Spearman's $r = 0.56$, $p > 0.32$). The children with severe malaria had significantly lower Hb mean of 7.10 gm/dl (sd = 2.66 gm/dl) compared to the 0 to five years of age cohort group which had mean Hb of 9.56 gm/dl (sd = 4.12) ($F = 21.168$, $df = 1, 1118$, $p <0.001$).

4.1.10 Risk factors of contracting malaria infection

4.1.10.1 Population density

The size of the homesteads varied from site to site. The number of houses in a given homestead ranged from 1 to 15. The number of individuals living in the index houses (houses within the compound where entomological studies

were done) varied from two to 16 with a mode of five. There was no significant difference in the number of individuals living in the index houses in the nine sites (X^2 = 0.8, df = 8, p <0.01). There was no significant difference in the rates of severe malaria in the houses in the study sites irrespective of population density (F = 1.055, df = 15, 200, p > 0.401). Also, there were no significant differences in the occurrence of severe malaria between the houses with more than five individuals or those with less than 5 (F = 0.349, df = 1, 214, p >0.555).

4.1.10.2 Behavioural factors

In 212 (98.1%) of the homesteads, the residents sat outside their houses regularly in the evenings upto 2100hrs and occupants of 69% of the homesteads went to bed at or after 2100hrs. There were few vector control measures in the study area. During the preceding six months of the study, six homesteads had their houses sprayed with insecticide at least once. Of 216 houses surveyed 186 (86.1%) of the households never used mosquito coils, 23 (10.6%) used the coils occasionally, while only 5 (2.3%) used the repellents every night

during the peak mosquito density. The mosquito coils included the commercially manufactured pyrethroids and other repellents from the local shrubs and trees. Six homesteads (2.8%) used insecticide aerosols. Fourteen (6.5%) of the households used mosquito nets. The use of insecticides, repellents or mosquito nets played no role in preventing severe malaria (table 4.1.10).

Table 4.1.10: Possible effect of practised control measures in prevention of occurrence of severe malaria in the study houses.

Variable	Insecticide spray	Insecticide repellents	Mosquito nets
Used:			
No. units[U] with severe malaria	0	2	0
No. units without	6	28	14
Not used:			
No. units with severe malaria	12	10	12
No. units without	198	176	190
Significance for variable in preventing severe disease(p)	ns 0.546	ns 0.418	ns 0.348

u - No. of units refers to number of houses.

4.2 MALARIA TRANSMISSION

4.2.1 *Anopheles* species

Out of 4,961 *Anopheles* mosquitoes collected, 4,929 (99.3%) were *Anopheles gambiae s.l* (table 4.2.1) and 21 (0.4%) were *An. funestus*. Other species identified were *An. nili* (2), *An. squamosus* (1) and *An. coustani* (8). *An. funestus* was collected only in sites 7, 8 and 9.

4.2.2 Density and distribution of *Anopheles* mosquitoes

Table 4.2.2 shows the number of *Anopheles* mosquitoes collected at different sites between February 1992 to June 1993. Overall 946 (19.1%) of the 4,961 *Anopheles* mosquitoes were collected from sites 1 to 8. Site 5 had the lowest number of mosquitoes collected (42) while site 9 had the highest number (4,015).

4.2.3 Mosquito collection by technique

A total of 3,234 *Anopheles* mosquitoes were collected by night biting catches (indoors) and 261 mosquitoes by NBC

Table 4.2.1: Distribution of *Anopheles* species in the study area.

Site	number of *Anopheles* by species			
	An. gambiae s.l	*An. funestus*	others[c]	Total
1	47	0	3	50
2	80	0	1	81
3	137	0	1	138
4	84	0	3	87
5	41	0	1	42
6	294	0	2	296
7	187	2	2	191
8	60	1	0	61
9	3998	16	1	4015
Total	4928	19	14	4961

c:Other species include:

An. coustani
An. nili
An. squamosus

Table 4.2.2: Number of *Anopheles* collected by different sampling techniques in the study sites.

Site	Number of *Anopheles* by collection technique				
	NBC(I)	NBC(O)	DRI	PSC	Total
1	10	6	9	25	50
2	29	5	11	36	81
3	108	3	17	10	138
4	35	8	14	30	87
5	24	3	8	7	42
6	76	29	8	183	296
7	72	1	21	97	191
8	22	2	9	28	61
9	2858	204	927	26[M]	4015
Total	3234	261	1024	442	4961

NBC(I) - Night biting collection indoor
NBC(O) - Night biting collection outdoor
DRI - Day resting indoor collection
PSC - Pyrethrum spray collection

m - Due to relatively large number of mosquitoes collected by NBC and DRI, PSC collections were done infrequently at site 9.

(outdoors) (table 4.2.2). Day resting (indoors) collected total of 1,024 mosquitoes.

4.2.4 Seasonal distribution of *Anopheles* mosquitoes

Between June 1992 and May 1993, an average of 756.8 mm of rain was received in the 9 study sites (figure 4.2.1). During this observation period, February 1993 was the most dry month with a monthly mean of 1 mm of rain while May 1993 was the most wet month with an average of 189mm. The highest amount of rain received during the wettest month (May 1993) was 245 mm in site 1. Although 7 sites did not receive any rain for one or two months (table 4.2.3), the overall amount ranged from 532 mm in site 8 to 966 mm in site 2.

The highest numbers of *Anopheles* mosquitoes were collected in the months of May and June 1992 (table 4.2.4). A total of 1,736 and 1,092 mosquitoes were collected in May and June 1992, respectively. When the months were grouped according to rainfall pattern (figure 4.2.2), no association was observed between rainfall and the number of vectors collected (Spearman's $r = 0.252$, $p > 0.15$).

Figure 4.2.1: Monthly average rainfall in the study sites

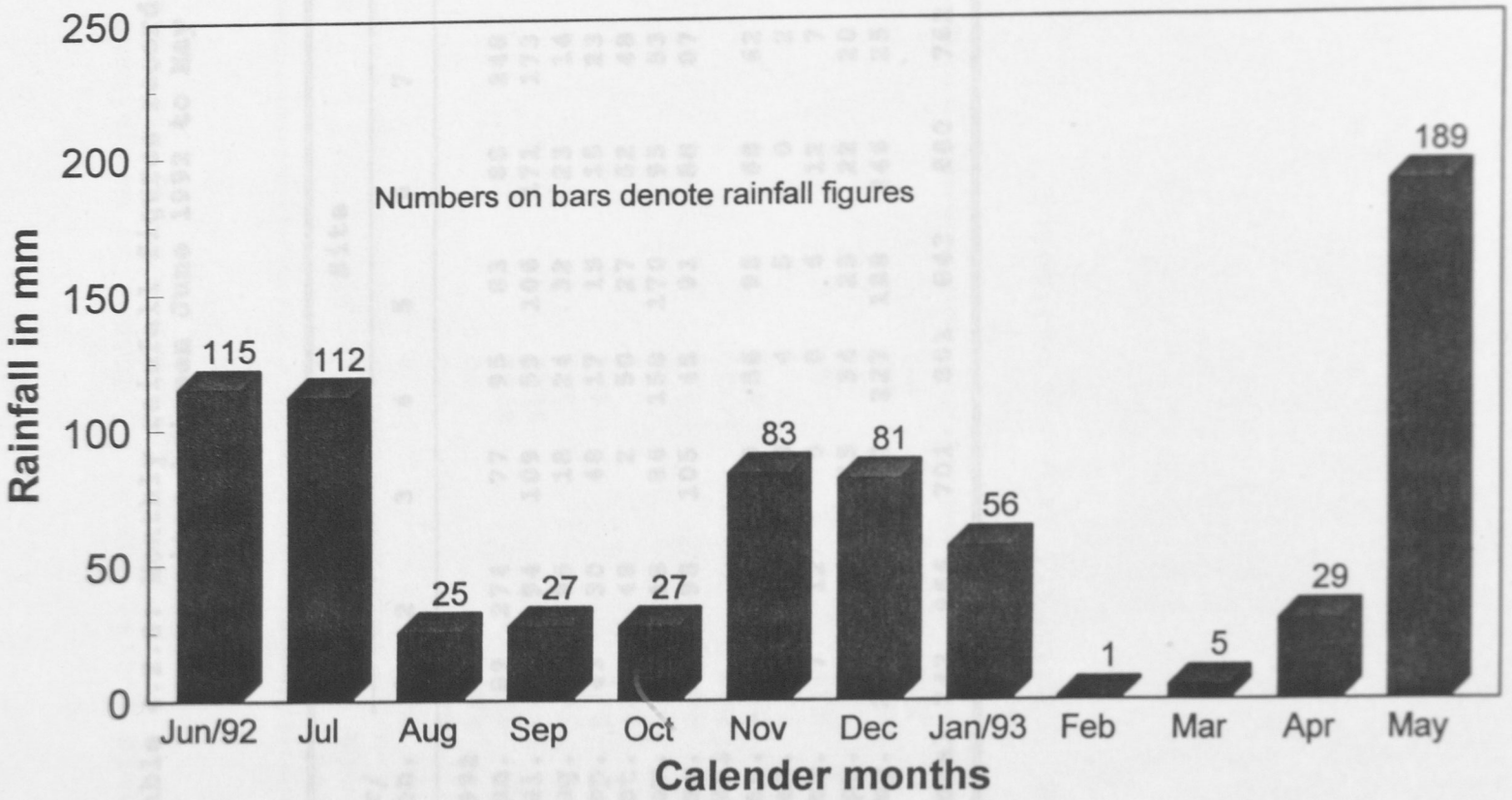


Table 4.2.3: Monthly rainfall figures recorded in the study sites between June 1992 to May 1993.

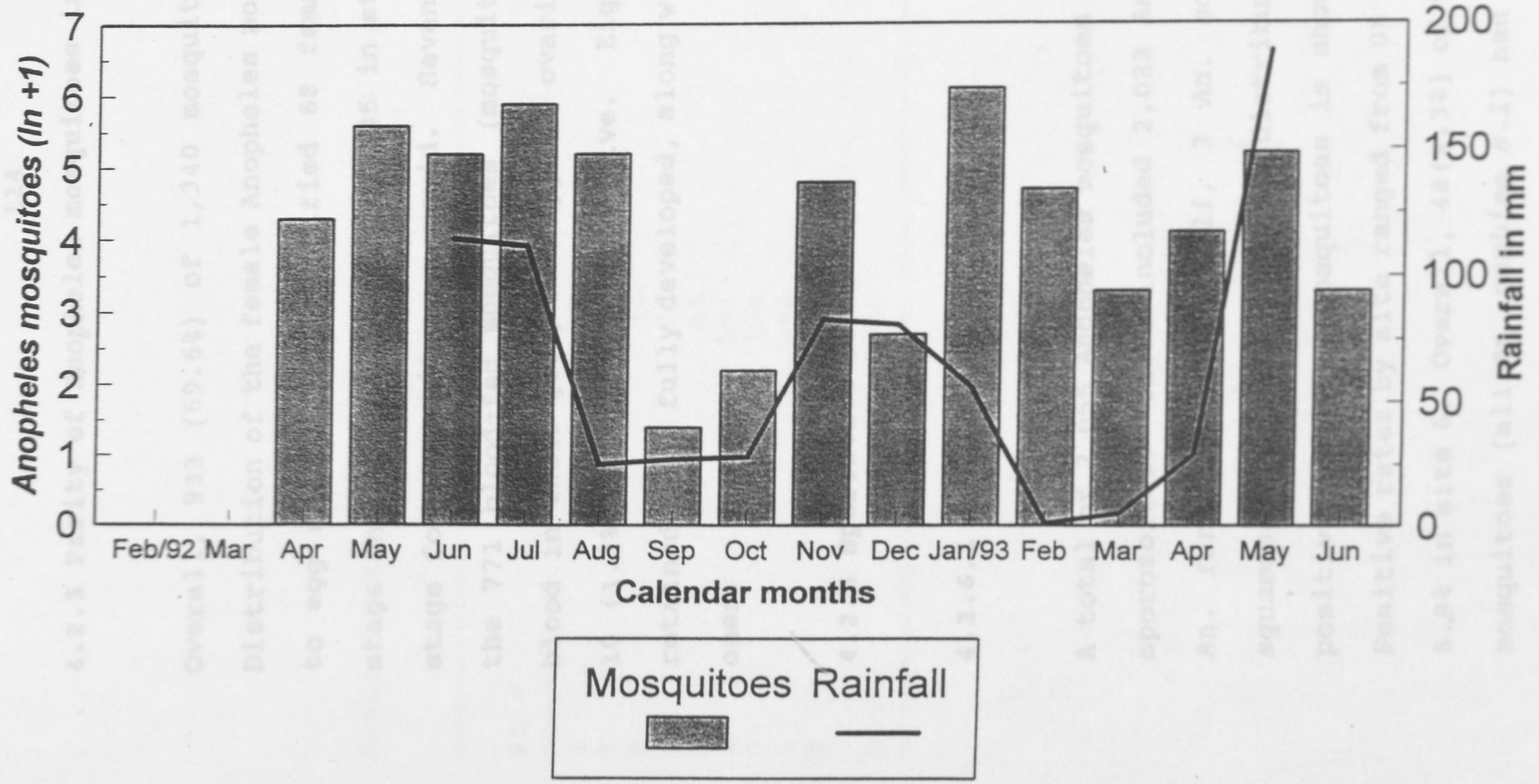
Yr/ mon.	Site 1	2	3	4	5	6	7	8	9	mean
1992										
Jun.	89	274	77	95	83	88	248	41	36	115
Jul.	96	94	109	59	106	171	173	106	95	112
Aug.	26	96	18	24	32	23	14	24	9	25
Sep.	43	30	48	17	15	15	23	31	41	27
Oct.	9	48	2	50	27	52	48	12	2	28
Nov.	74	18	86	158	170	95	53	21	76	83
Dec.	85	98	105	48	91	88	87	51	73	81
1993										
Jan.	48	70	39	86	95	68	62	10	25	56
Feb.	0	0	0	4	5	0	2	0	0	1
Mar.	7	12	0	0	6	12	7	0	0	5
Apr.	25	46	45	34	25	22	20	23	19	29
May.	245	180	172	227	188	246	25	212	204	189
Total	747	966	701	801	843	880	762	532	579	751

Table 4.2.4: Number of *Anopheles* mosquitoes collected per month and mean monthly rainfall in the study sites.

Year/ momth	Number collected	Mean monthly rainfall in mm
1992 Feb	0	nc
Mar	0	nc
Apr	72	nc
May	1736	nc
Jun	1092	115
Jul	634	112
Aug	434	25
Sep	7	27
Oct	9	28
Nov	120	83
Dec	15	81
1993 Jan	439	56
Feb	107	1
Mar	29	5
Apr	58	29
May	175	189
Jun	28	nc

nc - rainfall data not collected

Figure 4.2.2: Monthly number of *Anopheles* collected relative to rainfall in the study sites between June 1992 to May 1993



4.2.5 Parity of *Anopheles* mosquitoes in the study area

Overall, 933 (69.6%) of 1,340 mosquitoes were parous. Distribution of the female Anopheles mosquitoes relative to egg development identified 68 female with eggs in stage one, 340 in stage two, 135 in stage three, 35 in stage four and 200 were gravid. Seventy-four (9.6%) of the 771 blood-fed mosquitoes (mosquitoes with visible blood in their guts) had eggs in ovarian stage four and 10 (1.3%) of them in stage five. Eight mosquitoes had retained eggs, fully developed, along with less developed ones.

4.2.6 Sporozoite rates

4.2.6.1 By dissection

A total of 2,055 *Anopheles* mosquitoes were dissected for sporozoites. These included 2,023 *An. gambiae s.l*, 19 *An. funestus*, 8 *An. nili*, 3 *An. coustani* and 2 *An. squamosas*. The site-specific distribution of sporozoite positive *Anopheles* mosquitoes is shown in table 4.2.5. Positive rates by site ranged from 0% in sites 1 and 8 to 8.8% in site 6. Overall, 48(2.3%) of the 2,055 *Anopheles* mosquitoes (all *An. gambiae s.l*) had sporozoites in the

Table 4.2.5: Sporozoite positive rates for 2,055 *An. gambiae* according to study sites.

Site	Number dissected	Number positive (%)
1	48	0 (0.0)
2	75	2 (2.7)
3	54	1 (1.9)
4	70	4 (5.7)
5	31	1 (3.2)
6	186	16 (8.8)
7	176	2 (1.1)
8	54	0 (0.0)
9	1262	22 (1.7)
Total	2055	48 (2.3)

salivary glands. No other mosquito species was found positive for salivary gland sporozoites.

Table 4.2.6 summarises sporozoite positive mosquitoes according to collection techniques. No significant differences in positive rates of mosquitoes by collection technique was observed among NBC (indoors), DRI and PSC (X^2 = 0.08, df = 2, p <0.01). NBC outdoor, however, had a significantly lower rate than the other collection techniques (X^2 = 3.8, df = 3, p < 0.02).

4.2.6.2 By Sporozoite ELISA

A total of 4,961 *Anopheles* mosquito samples were tested for CS protein by ELISA (table 4.2.7). The *P. falciparum* ELISA rate ranged from 0% in site 8 (Mikingirini) to 10.8% in site 6 . The overall *P. falciparum* sporozoite rate was 2.5%. Thirteen samples were positive by *P. malariae* ELISA, producing an overall sporozoite rate of 0.2% (table 4.2.8).

A significant association occurred between the sporozoite rates by dissection and by ELISA for the 9 sites (Spearman's r = 0.803, p < 0.009).

Table 4.2.6: Salivary gland sporozoite positive *An. gambiae* by mosquito collection techniques.

Collection technique	Number dissected	Number positive	% Positive
PSC	441	12	2.7
DRI	722	15	2.1
NBC(I)	776	20	2.6
NBC(O)	116	1	0.9

PSC - Pyrethrum spray collection
DRI - Day resting indoor collection
NBC (I) - Night biting collection indoor
NBC (O) - Night biting collection outdoor

Table 4.2.7: *Plasmodium falciparum* sporozoite rate by ELISA for the *An. gambiae s.l* in the study sites.

Site	Number tested	Number positive	% positive
1	50	1	2.0
2	81	2	2.5
3	138	2	1.4
4	87	6	6.9
5	42	2	4.8
6	296	32	10.8
7	191	4	2.1
8	61	0	0.0
9	4015	74	1.8
Total	4961	123	2.5

4.2.7 Annual Entomological Inoculation Rate (EIR)

Tables 4.2.9 to 4.2.17 and figure 4.2.3 show the annual EIR by site. In sites 4 and 7, man biting rate (MBR) was observed in 4 months while EIR was observed for 2 months during the 12 months when EIR was estimated. In site 1, 2 and 3, EIR was recorded in 5 of the 12 months. In site 8 MBR was recorded for 6 months but EIR was not measurable in that site. Sites 5, 6 and 9 recorded MBRs in more than 6 of the 12 months when EIR was estimated. Overall, the EIR in the study sites averaged 6.7 bites (table 4.2.18). There was no association observed between the EIR and the occurrence of severe malaria.

4.2.8 Oocyst rate

Four (0.2%) of 2,055 Anopheles mosquitoes dissected were positive for oocysts (table 4.2.19). The number of oocysts per mosquito ranged from 1 to 4. In 2 of the mosquitoes, the sporozoite ELISA was positive whereas the sporozoite status by dissection was negative. For the other 2 mosquitoes, 1 was negative both for salivary gland sporozoites and sporozoite ELISA and the other one was positive both for salivary gland sporozoites and sporozoite ELISA.

Table 4.2.8. *P. malariae* sporozoite rates of *An. gambiae s.l* in the study sites.

Site	Number tested	Number positive	% positive
1	50	0	0.0
2	81	0	0.0
3	138	0	0.0
4	87	0	0.0
5	42	0	0.0
6	296	3	1.0
7	191	1	0.5
8	61	1	1.6
9	4015	8	0.2
Total	4961	13	0.2

Table 4.2.9: Entomological Inoculation Rate in Site 1, between June 1992 to May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	SP rate	Daily	EIR Mon.
Jun/92	20	3	0.15	0	0	0
Jul.	16	0	0	0	0	0
Aug.	16	2	0.13	0	0	0
Sep.	16	0	0	0	0	0
Oct.	16	0	0	0	0	0
Nov.	20	0	0	0	0	0
Dec.	8	0	0	0	0	0
Jan/93	15	4	0.27	0	0	0
Feb.	14	2	0.14	16.7	0.02	0.56
Mar.	18	1	0.06	0	0	0
Apr.	12	0	0	0	0	0
May	13	0	0	0	0	0
Annual EIR						0.56

Mon. Month
MBR Man biting rate
Sp Sporozoite

Table 4.2.10: Entomological Inoculation Rate in Site 2, between June 1992 to May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	Sp rate	EIR Daily	EIR Mon.
Jun/92	20	2	0.10	50.0	0.05	1.5
Jul.	14	2	0.14	0	0	0
Aug.	20	0	0	0	0	0
Sep.	16	0	0	0	0	0
Oct.	16	0	0	0	0	0
Nov.	20	0	0	0	0	0
Dec.	8	0	0	0	0	0
Jan/93	12	15	1.25	0	0	0
Feb.	14	1	0.07	0	0	0
Mar.	20	0	0	0	0	0
Apr.	10	1	0.10	0	0	0
May	20	0	0	0	0	0
Annual EIR						1.5

Mon. Month
MBR Man biting rate
Sp Sporozoite

Table 4.2.11: Entomological Inoculation Rate in Site 3, between June 1992 to May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	Sp rate	EIR Daily	EIR Mon.
Jun/92	18	13	0.72	0	0	0
Jul.	16	3	0.19	0	0	0
Aug.	16	3	0.19	0	0	0
Sep.	16	0	0	0	0	0
Oct.	16	0	0	0	0	0
Nov.	20	0	0	0	0	0
Dec.	8	0	0	0	0	0
Jan/93	16	13	0.81	0	0	0
Feb.	16	2	0.13	18.0	0.02	0.66
Mar.	24	0	0	0	0	0
Apr.	12	0	0	0	0	0
May	20	0	0	0	0	0
Annual EIR						0.66

Mon. Month
MBR Man biting rate
Sp Sporozoite

Table 4.2.12: Entomological Inoculation Rate in Site 4, between June 1992 to May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	Sp rate	EIR Daily	EIR Mon.
Jun/92	16	5	0.31	0	0	0
Jul.	19	1	0.05	0	0	0
Aug.	15	0	0	0	0	0
Sep.	15	0	0	0	0	0
Oct.	18	0	0	0	0	0
Nov.	15	0	0	0	0	0
Dec.	11	0	0	0	0	0
Jan/93	14	10	0.71	9.1	0.06	1.86
Feb.	16	1	0.06	100.0	0.06	1.68
Mar.	15	0	0	0	0	0
Apr.	16	0	0	0	0	0
May	14	0	0	0	0	0
Annual EIR						3.54

Mon. Month
MBR Man biting rate
Sp Sporozoite

Table 4.2.13: Entomological Inoculation Rate in Site 5, between June 1992 to May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	Sp rate	EIR Daily	EIR Mon.
Jun.	14	7	0.50	0	0	0
Jul.	19	2	0.11	25.0	0.03	0.93
Aug.	15	2	0.13	42.0	0.05	1.55
Sep.	16	0	0	0	0	0
Oct.	20	0	0	0	0	0
Nov.	16	0	0	0	0	0
Dec.	12	1	0.08	0	0	0
Jan.	16	11	0.69	9.7	0.07	2.17
Feb.	16	0	0	0	0	0
Mar.	16	0	0	0	0	0
Apr.	19	1	0.05	0	0	0
May	16	1	0.06	0	0	0
Annual EIR						4.65

Mon. Month
MBR Man biting rate
Sp Sporozoite

Table 4.2.14: Entomological Inoculation Rate in Site 6, between June 1992 May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	Sp rate	EIR Daily	EIR Mon.
Jun/92	15	4	0.27	0	0	0
Jul.	20	0	0	0	0	0
Aug.	15	0	0	0	0	0
Sep.	16	0	0	0	0	0
Oct.	20	0	0	0	0	0
Nov.	14	13	0.93	7.0	0.07	1.95
Dec.	12	1	0.08	22.2	0.19	0.57
Jan/93	15	10	0.67	9.6	0.06	1.98
Feb.	14	6	0.43	12.8	0.55	1.54
Mar.	13	17	1.31	0	0	0
Apr.	18	24	1.33	20.5	0.03	9.00
May	16	1	0.06	0	0	0
Annual EIR						15.04

Mon. Month
MBR Man biting rate
Sp Sporozoite

Table 4.2.15: Entomological Inoculation Rate in Site 7, between June 1992 to May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	Sp rate	EIR Daily	EIR Mon.
Jun/92	15	6	0.40	0	0	
Jul.	10	0	0.0	0	0	
Aug.	16	0	0.0	0	0	
Sep.	15	0	0.0	0	0	
Oct.	19	0	0.0	0	0	
Nov.	16	0	0.0	0	0	
Dec.	12	0	0.0	0	0	
Jan/93	16	51	3.19	2.7	0.086	2.67
Feb.	16	1	0.06	2.4	0.002	0.04
Mar.	16	0	0.0	0	0	
Apr.	20	1	0.05	0	0	
May	16	0	0.0	0	0	
Annual EIR						2.71

Mon. Month
MBR Man biting rate
SP Sporozoite

Table 4.2.16: Entomological Inoculation Rate in Site 8, between June 1992 to May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	Sp rate	Daily	EIR Mon.
Jun/92	16	7	0.44	0	0	0
Jul.	19	2	0.11	0	0	0
Aug.	16	2	0.13	0	0	0
Sep.	16	0	0	0	0	0
Oct.	20	0	0	0	0	0
Nov.	16	0	0	0	0	0
Dec.	12	0	0	0	0	0
Jan/93	15	9	0.60	0	0	0
Feb.	16	1	0.06	0	0	0
Mar.	16	0	0	0	0	0
Apr.	20	0	0	0	0	0
May	15	1	0.07	0	0	0
Annual EIR						0.0

Mon. Month
MBR Man biting rate
Sp Sporozoite

Table 4.2.17: Entomological Inoculation Rate in Site 9, between June 1992 to May 1993.

Mon/ yr	Man-bite nights	*An. gambiae* collected	MBR	Sp rate	EIR Daily	EIR Mon.
Jun/92	19	857	45.11	1.2	0.54	16.2
Jul.	16	347	21.69	1.5	0.33	10.3
Aug.	20	357	17.85	0.5	0.89	2.8
Sep.	16	3	0.19	8.0	0.02	0.6
Oct.	16	0	0	0	0	0
Nov.	16	0	0	0	0	0
Dec.	8	2	0.25	7.0	0.02	0.6
Jan/93	16	11	0.69	4.2	0.03	0.9
Feb.	16	4	0.25	0	0	0
Mar.	19	1	0.05	0	0	0
Apr.	12	15	1.25	0	0	0
May	20	131	6.55	0	0	0
Annual EIR						31.4

Mon. Month
MBR Man biting rate
Sp Sporozoite

Figure 4.2.3: Annual entomological inoculation rates according to site between June 1992 to May 1993

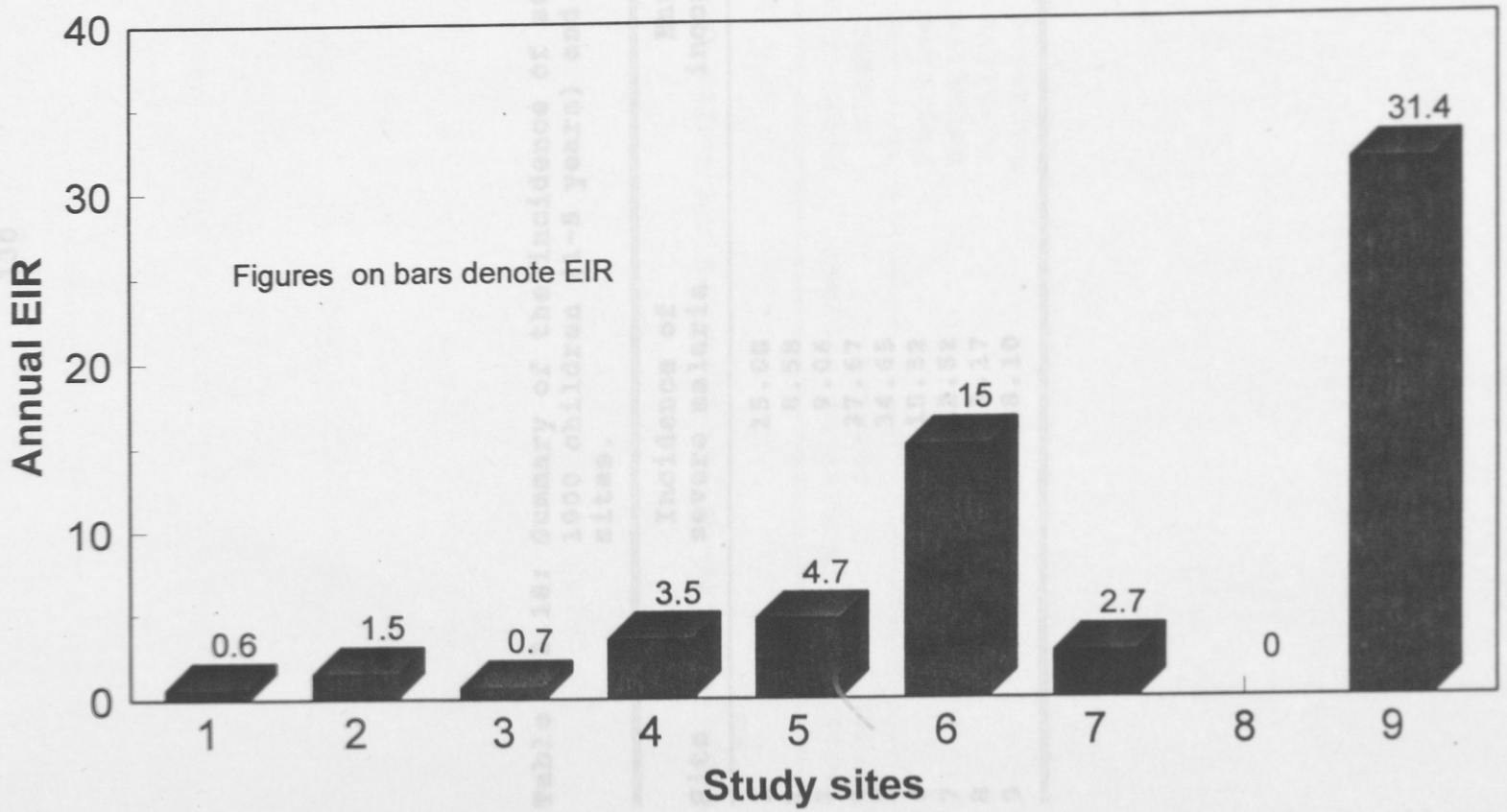


Table 4.2.18: Summary of the incidence of severe malaria per 1000 children (1-5 years) and EIR according to sites.

Site	Incidence of severe malaria	Entomological inoculation rate
1	15.08	0.56
2	8.58	1.50
3	9.06	0.66
4	37.67	3.54
5	34.65	4.65
6	18.52	15.04
7	12.52	2.71
8	23.17	0.00
9	38.10	31.44

Table 4.2.19: Oocyst-positive *Anopheles* mosquitoes in study sites.

Specimen number	Number of oocysts counted	Sporozoite status	Sporozoite ELISA
1	1	Negative	Negative
2	2	Negative	Positive
3	2	Positive	Positive
4	4	Negative	Positive

4.2.9 Vector heterogeneity

4.2.9.1 Distribution by type of house construction

Out of 216 index houses in the study area, 179 (82.9%) had mud walls, seven (3.2%) had walls of *makuti*. Twelve (5.6%) of the houses had concrete (total stone) walls. Vector mosquitoes were collected in 9 of the 12 houses with concrete wall. Seventeen (7.9%) houses had stone walls and were made up of mud into which pieces of stone were embedded. Vector mosquitoes were collected in 8 of these 17 stone houses. One house had a wall made of sisal bags. However, no vector was collected from this house. No significant difference existed between means of mosquitoes collected in a house with different types of walls (table 4.2.20.1) ($F = 1.09$, $df = 3, 214$, $p > 0.36$).

Two hundred and five (94.9%) houses were thatched with *makuti*. Eleven (5.1%) were roofed with iron sheets. Vector mosquitoes were collected in 7 of the houses roofed with iron sheets.

Eight (3.7%) of the study houses had ceilings and most of these ceilings were made of mud. There was no

Table 4.2.20.1: Summary of number of houses in the study area where *Anopheles* mosquitoes were collected relative to the major type of house construction and significance levels of mean mosquito densities by type of construction.

Character type	Mud wall	Makuti roof	Ceiling	Eaves
Present				
No. units with mosquitoes	89	100	2	99
No. units without mosquitoes	90	105	6	94
Absent				
No. units with mosquitoes	18	7	103	13
No. units without mosquitoes	19	4	105	10
Significance of geometric mean densities for character type (p)	ns 0.36	ns 0.47	ns 0.67	ns 0.09

significant difference in means of mosquito vector between the houses with or without ceiling ($F = 0.180$, $df = 1, 214$, $p > 0.621$). One hundred and ninety three (89.4%) of the houses had eaves ranging from small to very large passages. The presence of eaves did not influence mean number of *Anopheles* mosquitoes collected ($F = 2.83$, $df = 1, 214$, $p > 0.09$). In spite of the houses being made up of mud, only 70 (32.4%) of the houses had large openings to the exterior. All openings with a diameter of over 10 cm were considered large. Most of the houses with large openings were in sites 5, 8, and 9. No significant difference in vector density occurred between the houses with or without openings (table 4.2.20.2) ($F = 0.135$, $df = 1, 214$, $p > 0.713$).

Fifty one (23.6%) of the 216 houses had windows (table 4.2.20.2). The size and number of windows was dependent mainly on the size of the house. *Anopheles* mosquitoes were collected in 25 of the houses with windows. There was no significant difference in mosquito density between the houses with any number of windows or those without ($F = 0.72$, $df = 6, 209$, $p > 0.59$). Sixty five (30.1%) of the 216 houses surveyed, did not have bolted doors. They either had curtains or *makuti* mats as doors. Of these 65 houses without bolted doors, *Anopheles* mosquitoes were

Table 4.2.20.2: Summary of number of houses in the study area where *Anopheles* mosquitoes were collected relative to the major type of house construction and significance levels of mean mosquito densities by type of construction.

Character type	Holes	Window	Closable doors
Present			
No. units with mosquitoes	39	26	71
No. units without mosquitoes	31	25	80
Absent			
No. units with mosquitoes	68	81	36
No. units without mosquitoes	78	84	29
Significance of geometric mean densities for character type (p)	ns 0.71	ns 0.69	ns 0.31

collected in 29 of them. No significant difference in vector density was observed between houses with any number of bolted doors or those without such kind of doors ($F = 1.05$, $df = 4, 211$, $p > 0.31$).

4.2.9.2 Distribution by type of house environment

One hundred and thirty six (63%) of the houses were constructed within 10 meters from the nearest bushes and thick vegetation. There was no significant difference in the vector density between the houses in the open spaces and the rest of the houses (table 4.2.21) ($F = 0.18$, $df = 1, 214$, $p > 0.67$). Presence of a farm within 20 meters of the houses had no impact on vector density ($F = 1.50$, $df = 1, 214$, $p > 0.22$).

Four water bodies were found in the study area; two in site 6 (two pools and one pond), one pool in site 7 and one swamp in site 9. During the study period only 7 *Anopheles* mosquitoes were collected from one house adjacent to the swamp in site 9. No *Anopheles* mosquitoes were collected from the other two houses in site 7 and site 6 which were adjacent to the pools but the house next to the pond in site 6, provided 90.5% of the total number of mosquitoes collected from that site (site 6).

Table 4.2.21: Summary of the number of houses in the study sites where *Anopheles* mosquitoes were collected in relation to house environment and significance levels of mean mosquito densities for type of environment (see appendix HHC).

Type of environment	Open spaces	Farm	Water bodies	Burrow pits
Present				
No. units with mosquitoes	70	25	2	54
No. units without mosquitoes	66	28	2	52
Absent				
No. units with mosquitoes	37	82	105	53
No. units without mosquitoes	43	81	107	57
Significance of geometric mean densities for type of environment (p)	ns 0.67	ns 0.22	ns 0.38	ns 0.58

One hundred and six burrow pits were identified in the study sites. Two homesteads had 4 pits each, four homesteads had 3 pits each, 19 homesteads had two pits each and 81 homesteads had one pit each. These pits were used to excavate special kind of soil to make the mud walls. None of these burrow pits had water during the study period as the clay-sandy soil in the study area had no water retention capabilities. No significant difference in the means of vector mosquitoes existed between the houses with any number of burrow pits or those without ($F = 0.722$, $df = 4, 211$, $p > 0.09$).

4.2.10 Index of dispersal (ID)

The arithmetic mean densities and variance for *Anopheles* mosquitoes according to site is shown in table 4.2.22. The ID (see section 3.10.5) indicated how dispersion varied from 1.00 which represents normal dispersion, and it varied significantly between sites ($X^2 = 56.3$, $df = 8$, $p = <0.01$). In all sites, the variance was greater than the mean densities indicating that mosquitoes were congregated within sites. For example, 90.6% of the *Anopheles* mosquitoes were collected from one household in site 6 and 66.5% of the mosquitoes from site 9 were

Table 4.2.22: Arithmetic mean densities, variance and index of dispersion for *Anopheles* mosquitoes according to site.

Site	Number of mosquitoes collected	Mean density per house	Variance	Index of dispersion
1	50	5.6	69.0	12.3
2	81	6.8	145.4	21.4
3	138	17.3	570.8	33.0
4	87	5.6	36.1	6.4
5	42	3.8	9.0	2.4
6	296	24.7	5876.6	238.0
7	191	13.6	814.2	59.2
8	61	7.3	107.0	14.7
9	4015	154.4	146055.9	946.0

collected from only two houses.

4.2.11 Relation of malaria transmission to severe malaria

Thirteen of the 37 severe malaria cases were identified in 12 houses where entomological studies were done. In one of these houses there were two severe cases. The geometric mean density in houses with severe cases was 25 mosquitoes while the mean for the houses without cases was 5 giving a significant difference in the means for the *Anopheles* mosquitoes ($F = 7.200$, $df = 1, 105$, $p < 0.008$). However, no significant association was found on overall *Anopheles* density in relationship with severe malaria in the study sites (Spearman's $r = 0.412$, $p > 0.243$).

For all 9 sites, the EIR averaged 6.7 infective bites per person per year (table 4.2.18) and when compared to incidence of severe malaria during the study period (table 4.2.18) there was no association noted (Spearman's $r = 0.533$, $p > 0.139$).

4.3 Sporozoite loads

4.3.1 Overall sporozoite loads and frequency distribution

Sporozoite loads for 48 *An. gambiae* ranged from 125 to 79,875. The frequency distribution of sporozoite loads is shown in figure 4.3.1. The highest counts of 79,875 and 36,250 were from mosquitoes collected from site 9. The geometric mean number of sporozoites for the 48 positive mosquitoes was 1,743 with a standard deviation of 1,160. Twenty two of 48 (45.8%) sporozoite-infected *Anopheles* mosquitoes had loads of <1,000 per mosquito. Only 8 (16.6%) mosquitoes had loads above 10,000 sporozoites.

4.3.2 Ingested sporozoites (sporozoite in mosquito bloodmeals)

Five of 7 blood-fed mosquitoes with salivary gland sporozoites also had ingested sporozoites in their bloodmeals (table 4.3.1). The number of ingested sporozoites increased with the number of sporozoites in the salivary glands (Spearman's $r = 0.97$, $p < 0.001$).

Figure 4.3.1: Frequency distribution of sporozoite loads determined for 48 *An. gambiae*.

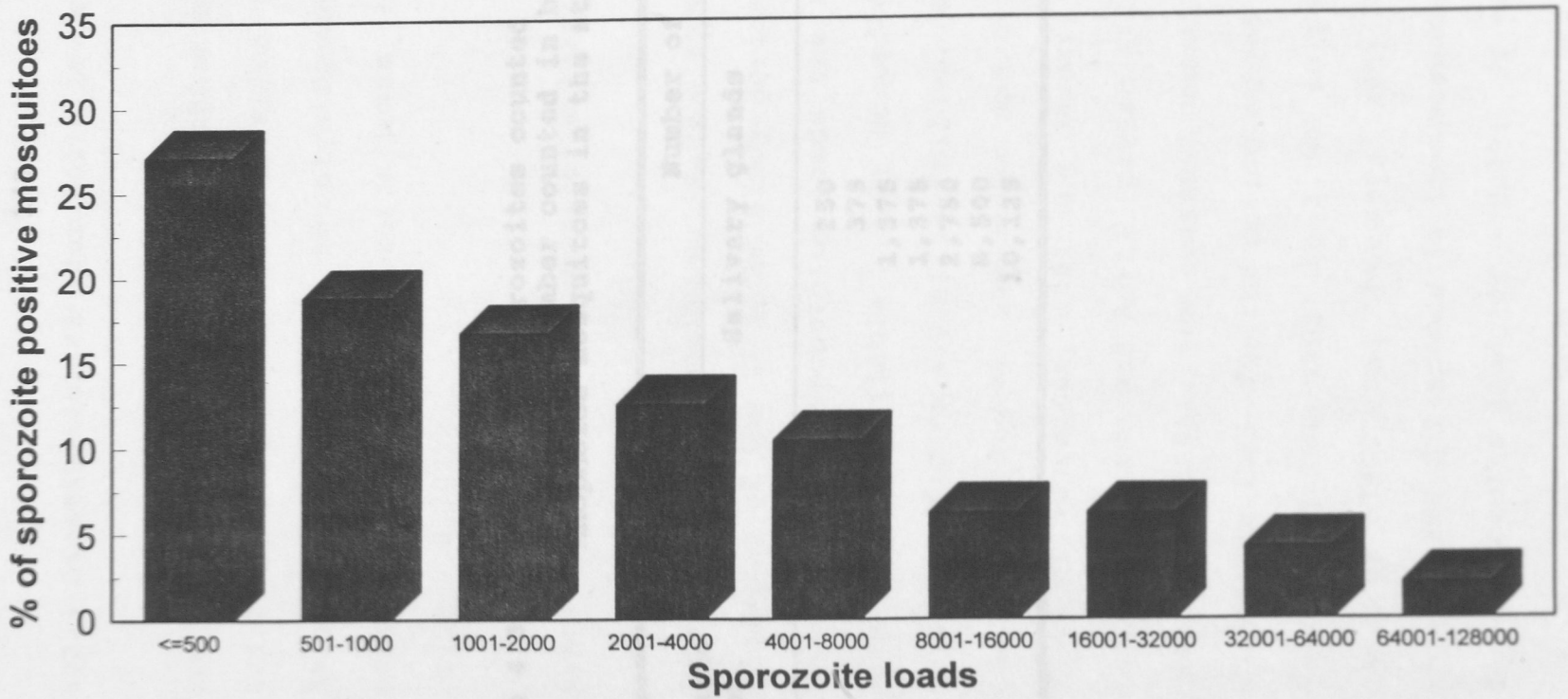


Table 4.3.1: Number of sporozoites counted in the salivary glands and number counted in bloodmeals of *Anopheles* mosquitoes in the study sites.

Mosquito Number	Number of sporozoites	
	Salivary glands	Bloodmeal
1	250	0
2	375	0
3	1,375	3
4	1,375	7
5	2,750	5
6	8,500	13
7	10,125	17

4.3.3 Distribution of sporozoite loads by sites

Sporozoite loads for 48 *An. gambiae* are shown in table 4.3.2. From sites 6 and 9 which had >15 positive *An.gambiae*, there was no significant differences in geometric means in sporozoite loads ($F = 0.148$, $df = 1, 36$, $p > 0.702$).

4.3.4 Distribution of sporozoite loads by season

Sporozoites in mosquito salivary glands were detected in 10 months of the 17 month study period. To examine the seasonality of sporozoite loads the study was divided into 5 periods (table 4.3.3) according to patterns of rainfall (figure 4.2.2) as follows: May, June and July (period 1), August, September and October (period 2), November, December, 1992 and January 1993 (period 3), February, March and April (period 4) and May and June, 1993 (period 5). The seasonal geometric means for the sporozoite loads for the period ranged from 991 in period 5 (May and June 1992) to 3,062 in period 3 (November, December 1992 and January 1993). There was no significant difference in the seasonal geometric means for sporozoite loads ($F = 1.701$, $df = 4, 10$, $p > 0.226$).

Table 4.3.2: Geometric mean sporozoite loads for 48 *Anopheles gambiae s.l.* according to study sites.

Site	Number dissected	Number positive (%)	Sporozoite loads: Geometric means
1	48	0 (0.0)	-
2	75	2 (2.7)	1,972
3	54	1 (1.9)	5,370
4	70	4 (5.7)	1,594
5	31	1 (3.2)	501
6	186	16 (8.8)	1,961
7	176	2 (1.1)	617
8	54	0 (0.0)	-
9	1262	22 (1.7)	1,778
Total	2055	48 (2.3)	1,743

Table 4.3.3: Seasonal geometric means of sporozoite loads by dissection between May 1992 to June 1993.

Period	Number dissected	Number positive (%)	Sporozoite loads: Geometric means
May-Jul 1992	819	17 (2.1)	1,302
Aug-Oct 1992	195	7 (3.6)	1,019
Nov 92-Jan93	573	13 (2.3)	2,252
Feb-Apr 1993	189	6 (3.2)	1,346
May-Jun 1993	202	5 (2.5)	991

4.3.5 Validation of ELISA

In order to assess the validity of the ELISA technique, salivary gland material from the 48 mosquitoes which had sporozoites, was tested for *Plasmodium falciparum* CS protein. Forty three (89.6%) of the 48 sporozoite-positive specimens were ELISA positive. Twenty eight (1.4%) of the 2007 salivary gland sporozoite-negative specimens were also sporozoite ELISA positive (table 4.3.4). A total of 1,579 samples of thoracic material were also tested by *P. falciparum* sporozoite ELISA. The salivary glands had been removed from these samples. Thirty six of 43 (83.7%) salivary glands sporozoite positive specimens tested positive for *P. falciparum* ELISA. Twenty one of 1536 (1.4%) sporozoite-negative specimens were ELISA positive (table 4.3.4).

Considering dissection as the standard method, the specificity of ELISA was calculated as the percentage of both the thoracic and salivary glands samples which were sporozoite ELISA positive and were also positive on dissection. The sensitivity was calculated as the percentage of both the negative thoracic and salivary glands samples which were negative with sporozoite ELISA and were also negative on dissection. Therefore, the

Table 4.3.4: ***Plasmodium falciparum*** **CS protein in salivary glands and thorax samples from dissected** ***An. gambiae s.l.***

Sample tested	Dissection result	N	% Positive by ELISA
Salivary gland	Positive	48	89.6
	Negative	2,007	1.4
Thorax	Positive	43	83.7
	Negative	1,536	1.4

specificity and sensitivity of ELISA was 89.6% and 98.6% respectively.

4.3.6 Relation of sporozoite loads to severe malaria

From 216 entomological sampling houses, 13 cases of severe malaria were identified. Two of these cases were reported from 2 houses in which mosquitoes had sporozoites in the salivary glands. One of the two houses was in site 6 and the other one in site 9. Fifteen (98.8%) of the 16 sporozoite positive vectors in site 6 came from one house whereas nine (42.9%) of the 21 positive vectors in site 9 were also from one house. Whereas severe malaria cases were identified from each of the nine sites, sites 1 and 8 did not have any *Anopheles* mosquito with salivary glands sporozoites. No significant association was found when the geometric means of the sporozoites were examined for each site in relation to the incidence of severe malaria (Spearman's $r = 0.368$, $p > 0.330$).

4.4 Human malaria antibodies in mosquito bloodmeals.

4.4.1 Mosquito collection

A total of 1,303 blood-fed mosquitoes were collected from the 9 study sites between March and July 1993 (table 4.4.1). These included three genera: *Culex* (n = 1,098 mosquitoes), *Anopheles* (n = 190) and *Aedes* (n = 15). The *Culex* included two main species. *Culex bitaeniorhynchus* emerged immediately after the rains but numbers declined considerably soon after the rains. *Culex quinquefasciatus* occurred in small numbers in the community centres.

4.4.2 Human Blood Index (HBI)

Out of 1,216 mosquitoes tested for human blood by ELISA, 1,135 (93.3%) were positive (*Culex* 93.1%, *Anopheles* 94.8% and *Aedes* 100%).

Table 4.4.1: Summary of the number of mosquitoes collected, the CSAB positive rate for the mosquitoes and the CSAB positive rate for the human sera for individuals <20 years of age.

Site	Mosquitoes collected	% CSAB +ve	Positive rate for sera in human population <20yrs
1	175	30.5	20.7
2	74	37.5	30.8
3	16	6.3	25.0
4	124	42.1	37.2
5	12	66.7	33.5
6	138	40.3	28.0
7	462	54.3	33.9
8	46	35.7	20.2
9	256	32.2	61.7
Total	1303	42.0	30.7

4.4.3 Circumsporozoite antibodies (CSAB)

The cut-off value for positive optical density (OD) was 0.072 (mean of negative controls plus 3 standard deviations). Out of 1,216 mosquitoes tested for CSAB, 511 (42.0%) had positive CSAB OD values (table 4.4.1). The CSAB positive rate varied significantly from site to site from 6.3% in site 3 to 66.7% in site 5 (X^2 = 58.6, df = 8, p <0.001). No significant difference was noted between the CSAB positive rate in all the human sera examined (46.7%) and CSAB positive rate in mosquitoes (42.0%) (X^2 = 5.256, df = 1, p > 0.102).

Further, each study site was divided into 4 quadrants and bloodmeal CSAB were examined in relation to the total 36 quadrants. Twelve of the quadrants had 10 mosquitoes and above and the positive rate varied significantly (X^2 = 7.00, df = 11, p < 0.001). The proportion of individuals under 20 years in each site was over 60% (60.3% in site 9 to 70.4% in site 3). When the overall CSAB positive rates in human sera were compared by CSAB positive rates in the mosquitoes by site, no significant association was observed (Spearman's r = 0.521, p>0.428). When, again, the CSAB positive rate in the human sera for

individuals under 20 years of age was compared to CSAB positive rate in the mosquitoes by site (table 4.4.1) there was no correlation (Spearman's r = 0.376, p >0.317).

4.4.4 Circumsporozoite antibodies by genera of mosquito

The CSAB positive rate for *Aedes* (6.7%) was significantly lower than *Anopheles* and *Culex* (table 4.4.2). *Culex* with a positive rate of 44.2% was also significantly different from *Anopheles* (48.8%) (X^2 = 8.13, df = 1, p <0.001).

Table 4.4.2: Circumsporozoite antibody positive rates by genera of mosquitoes.

Genus	Number tested	% positive
Culex	1,021	44.2
Anopheles	180	48.8
Aedes	15	6.7
Total	**1,216**	**42.0**

CHAPTER 5

DISCUSSION

5.1. Malaria in study population

5.1.1 Malaria parasites

The overall parasite infection rate (52.0%) in the study sites in Kilifi district was consistent with parasite rate (50% - 74%) for the coastal region (Roberts *et al.* 1974, Division of Vector Borne Diseases, (DVBD) unpublished data). *Plasmodium falciparum* is the only species considered to be of importance in relation to malaria and accounts for 97.3% of all malaria parasite infections in the study area. *P. falciparum* generally accounts for 96.0% of all malaria infections in Kenya (DVBD, unpublished data). In the study sites the children aged between 5 to 9 years had the highest parasite infection rate which is similar to a study in Kisumu district, Kenya, where transmission is relatively higher (Githeko *et al.* 1992) and in Papua New Guinea (Cattani *et al.* 1986). The geometric mean parasite

density for the study sites was 153 parasites per μl of blood. The infection rate and parasite densities observed in Kilifi study sites are comparable to those observed in Papua New Guinea where parasite infection rate range between 45% to 57% and the parasite densities are usually < 201 parasites per μl (Cattani, *et al.* 1986).

The prevalence of *P. falciparum* gametocytes in the study sites was 26.4%. Twenty seven individuals who were negative for asexual stage parasites, were positive for *P. falciparum* gametocytes. This probably indicates indiscriminate use of antimalarial drugs in the area. Chloroquine is the most widely used antimalarial drug in Kenya as a whole, and although it is a good schizontozidal drug, it does not usually affect the mature gametocytes of *P. falciparum* (WHO, 1990b). The drug eliminates the asexual stages but the gametocytes are left circulating in the blood.

The mean parasite density for the children with severe malaria in the study sites was significantly higher than the mean for the cohort group of children up to 5 years of age ($p < 0.001$). This could suggest that parasite density is a risk factor for the occurrence of severe

malaria. However, one of the criterion for a severe case in this study was parasite density of >20,000 per μl. Eleven of the 37 (29.7%) severe malaria cases identified between June 1992 to May 1993, had parasitaemia of <20,000 parasites per μl providing evidence that high parasite density was not responsible for at least 30% of the severe cases. When parasite densities were analysed in relation to incidence of malaria between sites, no significant association occurred ($p < 0.001$)

It has been suggested by that malaria parasite density is an important factor in the occurrence of severe malaria (Marsh, 1988; Greenwood *et al.* 1991), and that superinfection triggers illness (Lines and Armstrong, 1991). The results from this study indicate that no significant association was found between malaria parasite density and severity of malaria ($p < 0.001$). Sites with high parasite infection rates do not necessarily have high incidence of severe malaria.

5.1.2 Infection and sporozoite exposure

Although transmission is low in the study sites, 92.9% of the resident population had a *P. falciparum* infection by

their first birthday. In Saradidi, Nyanza Province, Kenya, where parasite rate in the community is 88% and transmission is more intense, Spencer *et al.* (1987) reported 100% asexual stage antibody positive rate by first birthday. This finding of Spencer, *et al.* (1987) is similar to results from Kilifi study sites.

Whereas 92.9% of the population >1 year of age in the study sites had measurable levels of asexual stage antibodies, only 46.7% of them had measurable levels of CSAB. Most of the residents in the study area have to live for 19 years to convert seropositive for CSAB (see figures 4.1.4, 4.1.5, 4.1.6 and 4.1.7). Twenty percent of the children up to 5 years of age were positive for CSAB as opposed to 79.4% positive rate observed in individuals above 19 years. This was in agreement with other studies by Campbell *et al.* (1987) and Deloron *et al.* (1989), in western Kenya, Hoffman *et al.* (1986), in Indonesia, Marsh, *et al.* (1988), in The Gambia and Esposito *et al.* (1988), in Burkina Faso, which showed that CSAB is associated with age. Individuals in an area of high transmission may have high levels of CSAB in earlier age groups as reported by Cattani *et al.* (1986) who showed that 80% of children aged 5 years and above in Papua New Guinea were positive. Giuldice *et al.* (1987)

working in Tanzania also reported 36.5% positive rate for children 2 to 5 years of age. In the study sites, the CSAB conversion rates varied significantly between sites ($p = <0.01$) and this indicated differing sporozoite challenge rates. Seroconversion from positive to negative for CSAB was negligible (R. Knight, personal communication) and this indicates a continuous challenge that is responsible for maintenance of CSAB positive rates.

5.1.3 Impact on morbidity

The overall spleen rate which was 18.6% varied significantly between and within sites ($p <0.05$). This indicates heterogeneity in transmission pressure on the population. Different levels of transmission challenge brought about by unequal risks of exposure (Dye and Hasiberder, 1986; Gamage-Mendis et al. 1991; Burkot, 1988) may have contributed to the differences in exposure and ultimately differences in spleen rate. The overall spleen rate in the study sites followed the pattern of malaria parasite infection (table 4.1.5) where the rate increased from 1.2% in children up to 1 year of age to 29.7% in children between 5 and 9 years of age. The significant association between the spleen rate and

parasite infection rate ($p < 0.001$) closely linked splenomegaly with malaria parasite infection in the study sites. No correlation, however, was observed between the spleen rate and the occurrence of severe malaria in this study ($p < 0.32$).

5.1.4 Occurrence of severe malaria

Severe malaria in the study sites varied significantly between the years and among sites from June 1989 to May 1993 (table 4.1.8). Between June 1992 to May 1993, a total of 37 severe malaria cases from the study sites were identified in Kilifi District hospital. All severe cases from the area may not have been identified as some of them may not have gone to the hospital. Herbal medicine is practised in the study area and interviews with the local residents during our field trips revealed that majority of them visit traditional healers before they attend the health facilities.

Most of the severe cases in the study area occurred in children between 1 to 4 years of age (Marsh, unpublished data). At the present moment there are no comparable data on incidence of severe malaria from other parts of Kenya. Trape *et al.* (1987 and 1993), working in Congo,

reported the occurrence of severe malaria in children of 2 years of age and individuals 7 to 14 years of age respectively, in two settings indicating that occurrence of severe malaria varies from region to region.

5.1.5 Risk factors in malaria transmission

5.1.5.1 Population density

Occurrence of severe malaria in the study sites was not significantly associated with population density of residents in any house since there was no significant difference in population density in houses from where the severe cases were identified and the houses without severe cases ($p < 0.01$).

Where transmission of malaria occurs, it can be assumed that in a homestead, all family members have an equal chance of being bitten by infective mosquitoes but Burkot (1988) has demonstrated that some individuals, under the same conditions, are more prone to mosquito bites that others. Furthermore, there is the unequal risk exposure by way of either defense mechanism (Pull and Grab, 1978) or by occupation (Rusell *et al.* 1963). This unequal risk of exposure may explain the intra-house differences in

malaria parasite infections as reported by Giuldice *et al.* (1987) in Tanzania and Gamage-Mendis *et al.* (1991) in Sri Lanka.

5.1.5.2 Behavioural factors

During the study period chemotherapy was the main form of malaria control strategy practised by the resident population. Vector control measures were used minimally. Residents in 186 of 216 (86.1%) index houses surveyed, did not practise personal protection measures to reduce the human-vector contact. From interviews with the people living in the study sites, mosquito nuisance was not considered a problem most part of the year, as the density was generally low. The low density of mosquitoes may have led to limited vector control measures in the area. Although there were no severe malaria cases identified from the houses where any form of control measure was practised, the absence of a control measure did not favour the occurrence of severe malaria. This is in agreement with the work done in Brazzaville, Congo, by Carme *et al.* (1994) who did not observe any significant difference in occurrence of cerebral malaria in households where mosquito nets and insecticides were used or not. In the study sites no

control measure was significantly associated with protection of members of a specific household from getting severe malaria (table 4.1.10).

5.2 Malaria transmission

5.2.1 Malaria vectors

The predominant malaria vector in the study sites was *Anopheles gambiae s.l* which accounted for 99.3% of all *Anopheles* mosquitoes collected. *Anopheles funestus* had been previously considered to be the main vector in the study area (DVBD, unpublished data). In Jaribuni, Kilifi district, an area close to the study sites, *An. funestus* has been reported to be 81.5% of the anophelines in the area (Wijers and Kiilu, 1977). In this study, *An. funestus* played a limited role in malaria transmission since none of the 21 *An. funestus* collected were positive for sporozoites, either by dissection or by sporozoite ELISA.

The *Anopheles* mosquito density varied significantly among and within sites, for example, in site 6, out of the 291 mosquitoes collected, 268 (90.6%) were from one house (table 4.2.1). The overall low mosquito density in the study sites may have contributed to these major differences in mosquito densities within small areas.

Out of the total 4,961 *Anopheles* mosquitoes collected, 261(5.3%) were collected outdoor using human bait. Out of the 261 mosquitoes collected outdoors, 0.08% were positive for sporozoite. This indicates the potential of outdoor malaria transmission. The population in the study area usually sat outside the houses, sometimes up to 11.00 o'clock at night. Biting activity in the study area starts from 8.00 pm and continues to 6.00 am the following morning with a biting peak between 11.00 am and 2.00 am (Charles Mbogo, personal communication). Although species of *An. gambiae* complex were not identified during this study, three species in the complex, *An. gambiae s.s.*, *An. merus* and *An. arabiensis*, have been identified on the Kenyan Coast (Muirhead-Thomas, 1951; White *et al.* (1972; Mosha and Petrarca 1983). In Jego, an area in South Coast of Kenya, the collection of *Anopheles gambiae s.s*, *An. merus* and *An. arabiensis* was 71.7%, 1.4% and 10.9% respectively (Mosha and Petrarca, 1980). It is reported by Highton *et al.* (1979) and Mutero *et al.* (1984) that in East Africa *An. merus* and *An. arabiensis* feed more often outdoors compared to *An. gambiae s.s.* which prefer to feed indoors.

The parity index for the Anopheles mosquitoes in the study sites was 0.7 indicating a large portion of parous females. These parous mosquitoes were aging and potentially containing members infected with malaria parasites. This high parous index suggests high life expectancy for *Anopheles* vectors but could not explain the low inoculation rate. Besides this, a few vectors in the study sites might make contact with many human hosts and hence maintain the transmission (Rossignol *et al.* 1985, Wekesa *et al.* 1992). Wijers and Kiilu (1977) recorded a parous index of 0.8 for the mosquitoes collected in Jaribuni and Mamburui coastal areas in Kilifi district. Boudin *et al.* (1993), in Barre, Burkina Faso, reported a low parity index of <0.6 for *An. gambiae s.l.* which attempted to explain the low inoculation rate in the human population.

At least 10% of the *Anopheles* mosquitoes collected in the study sites had a second bloodmeal before laying eggs. Out of 771 blood-fed mosquitoes, 74 (9.6%) had eggs in ovarian stage four or five. Although extensive search for breeding sites for *Anopheles* mosquitoes was carried out during the period of study, only four water bodies were identified (section 4.2.9.2). Lack of readily

available breeding sites for *Anopheles* mosquitoes might have contributed to the phenomenon of mosquitoes taking a second bloodmeal before laying eggs.

5.2.2 Type of house and surrounding environment

There was a strong correlation between type of house and socio-economic status. At least 95% of the population in the study sites were Giriamas who constructed similar houses of mud walls (82.9%) and *makuti* thatching (94.9%). The presence of eaves and lack of ceiling in most of the houses offered mosquitoes and other arthropods easy access in and out of the houses. The study sites differed in geographical locations and topography and mosquito densities were expected to vary between sites. No significant difference, however, was observed in mosquito densities between sites according to geographical locations or topography (table 4.2.21). For example, two of the sites (7 and 9) bordered the sea and 3.9% and 80.9% of mosquitoes were collected from these sites respectively. Differences in geographical locations and topographical settings in mosquito densities has been reported by Gamage-Mendis *et al.* (1991), in Sri Lanka and Boudin *et al.* (1993), in Burkina Faso.

No significant association was noted between the density of *Anopheles* mosquitoes and the type of housing construction or environment (table 4.2.21). This could have been brought about by the overall number of *Anopheles* mosquitoes collected which was low and may not have allowed for this difference to be observed. Furthermore, no significant association occurred between the mosquito density and the occurrence of severe malaria ($p < 0.243$).

5.2.3 Entomological inoculation rates

Overall sporozoite ELISA rate for *P. falciparum* was 2.5%. This rate was similar to results from Mwea irrigation scheme, in Central Province, Kenya. In this scheme, which is an area of seasonal malaria transmission Mukiama and Mwangi (1989) reported sporozoite rate of 1.3% for *An. gambiae s.l.* Higher sporozoite rates (18.6% and 15.6%) are reported in western Kenya by Beier *et al.* (1987 and 1991d), in an area of high malaria transmission. The low sporozoite rate contributed to the low inoculation rates in the study sites.

Residents in the study sites were exposed to an average of 6.7 infective bites per person per year. Significant

differences in EIRs occurred among sites (table 4.2.18). For example, no EIR was recorded in site 8 (table 4.2.18). No significant association occurred between EIRs and incidence of severe malaria ($p = 0.139$). For instance, in site 8 where no EIR was recorded, the incidence of severe malaria was 23.17 per 1000 children. Whereas evidence of malaria transmission in site 8 is demonstrated by the occurrence of severe malaria, the measurement techniques might have not been sensitive enough to pick the transmission.

As already referred earlier, most cases of severe malaria in the study sites occurred in children between 1 to 4 years of age. Different levels of incidence of severe malaria has been recorded for different transmission settings. For example, Trape *et al.* (1987), in Congo, reported peak admissions among children of 2 years of age in an area where EIR was 22 infective bites per year. In another part of Congo, Trape *et al.* (1991), reported more severe cases in individuals 7 to 14 years of age where EIR was 75 bites per year. In some parts of The Gambia, where malaria transmission is comparable to Kilifi district in Kenya, fewer cases were reported in areas with low EIR and more cases in areas with high EIR (Snow

et al. 1988, Lindsay, 1990). Results obtained in this study are not comparable to these observations as no significant association existed between EIRs and occurrence of severe malaria.

5.3 Sporozoite loads

The sporozoites loads for 48 *An. gambiae s.l* in the nine study sites ranged from 125 to 79,875 with a geometric mean of 1,743 sporozoites. Similar sporozoite loads are reported by Beier *et al.* (1991d) in Saradidi and Kisian in Kisumu district, Kenya, where sporozoite loads ranged from 1 to 79,732 with a geometric mean of 1,222 sporozoites. Although the sporozoite loads are comparable between the two study regions (Kilifi district in Coast Province and Kisumu district in Nyanza Province), it is important to note that whereas malaria transmission is low in Kilifi study sites, transmission is very high in Kisumu district. There is no comparable data on incidence of severe malaria from Kisumu district. Apart from this study in Kilifi district, there is no other field study which has been done to examine the relationship between sporozoite loads in wild *Anopheles* vectors and the occurrence of severe malaria.

It is hypothesised that inoculation dose of sporozoite would determine the outcome of an infection (Marsh 1988, Greenwood *et al.* 1989; Lines and Armstrong, 1992). No significant association was observed between the

sporozoite loads in the study sites and severe malaria ($p > 0.330$). It would be expected that sites with higher geometric means of sporozoites would correspondingly have a higher incidence of severe malaria cases. No significant differences in sporozoite loads within defined periods occurred (table 4.3.3) as reported for Kisumu district, Kenya by Beier *et al.* (1991d). They (Beier *et al.* 1991d) reported a 10 fold increase in number of sporozoites over an eight month period. Adungo *et al.* (1991), also working in Kisumu district, Kenya, reported significantly higher sporozoite rates during dry season than wet season. In June 1992, there was an upsurge of malaria admissions in Kilifi District hospital, but surprisingly no increase in sporozoite loads was noted during the same rainy period (table 4.1.9).

Anopheles mosquitoes collected in the study sites showed the same relationship of sporozoite re-ingestion as observed by Beier *et al.* (1992). There was a positive correlation between the sporozoite number in salivary glands and the bloodmeals (section 4.3.2). The number of re-ingested sporozoites confirmed that only a few sporozoites were transmitted by the infected vectors. Re-ingestion of injected sporozoites when the mosquito

feeds may also explain why some infective mosquitoes do not produce an infection. All the ingested sporozoites might be re-ingested ending with no sporozoites left in the host and in a way demonstrating the disparity of sporozoite inocula. In spite of the differences in the sporozoite densities, severe malaria occurred in all the sites.

5.4. Human malaria antibodies in mosquito bloodmeals.

Human CSAB was detected in mosquito bloodmeals of three genera (*Anopheles, Culex and Aedes*) by ELISA. The human blood index (HBI) for the mosquitoes collected in the study area was 0.93. In a study covering 25 sites in Kilifi district, Mbogo *et al.* (1993) reported an HBI of 0.95 for *Anopheles gambiae* and 0.91 for *An. funestus* which was comparable to HBI in the study sites. In another related study (unpublished data) we established that the three genera of mosquitoes collected in the study sites also fed on cows and goats. When the results were analysed at homestead level, it was demonstrated that over 90% of the mosquitoes collected in the study area fed primarily on human beings, irrespective of availability of other alternative hosts.

In this study, human CSAB were detected in *Culex, Anopheles* and *Aedes*. In previous studies, detection of human CSAB in mosquito bloodmeals was reported for *Anopheles* mosquitoes (Beier *et al.* 1989; Conteras and Beier, 1992).

The overall CSAB positive rate for mosquitoes in the study sites was 42.0%. In Western Kenya, Beier, *et al.* (1989) reported a 45.0% positive rate in bloodmeals for *An. gambiae* and *An. funestus* which is comparable to the results in this study. The three genera of mosquitoes in the study sites had significantly different CSAB positive rates (section 4.4.4). This was probably attributed to feeding preferences of the specific genus of the mosquito. Preferential feeding behaviour for *Anopheles* mosquitoes on different age groups of human beings has been reported in studies by Pull and Grab (1974), Port *et al.* (1980)and Boreham *et al.* (1978). *Aedes* mosquitoes may have fed on younger people (1-19 years) who may not have seroconverted positive for CSAB.

Distribution of blood-fed mosquitoes varied between and within sites (table 4.4.1) and when each site was divided into 4 quadrants, only 12 of the 36 quadrants had more than 10 blood-fed mosquitoes. Clustering of mosquitoes may not have been as a result of the type of house as the houses in the study sites were all similar. The occurrence of parasite infection clusters, coupled with clustering of human-blood feeding mosquitoes may have influenced the disparity of CSAB in the study sites.

This is not unusual considering that malaria is known to vary drastically within small geographical areas (Greenwood *et al.* 1989).

Although other studies have demonstrated human CSAB in mosquito bloodmeals (Vaughan *et al.* 1988 and 1990, Beier *et al.* 1989 and Conteras and Beier, 1992) these studies were not designed to determine the relation between the CSAB in mosquitoes in relation to severe malaria. This is the only study in which detection of human CSAB in bloodmeals of mosquitoes has been examined in relation to transmission of malaria. On analysing the CSAB results from human sera in the study population, it was apparent that the individuals sero-converted positive after 19 years. More than 60% of the study population were individuals <20 years. When the CSAB positive rates of this age group was analysed in relation to CSAB in mosquito bloodmeals, there was no correlation (table 4.4.1).

No significant difference occurred between the overall CSAB positive rates in the nine study sites in the human sera tested (46.7%) and mosquito bloodmeals (42.0%) ($p > 0.102$). However, no significant association occurred between the sites for the CSAB positive rates in human

sera and the CSAB positive rates in the mosquitoes ($p > 0.428$). This indicates that the detection of human CSAB in mosquito bloodmeals could be an indicator of malaria transmission in a given area.

CHAPTER 6

General discussion, conclusions and recommendations

6.1 General discussion and conclusions

The intensity of malaria transmission is dependent on EIR which is the number of infected bites per person per specified period. The higher the EIR the more intense the transmission is. The use of mosquito nets and other fabrics reduces the frequency of mosquito bites on human beings and consequently the vectorial capacity (Macdonald, 1957). In areas where malaria transmission is continuous, the frequency of bites by infected mosquitoes determines the rate of acquisition of immunity.

The immune status, among other factors, may determine the clinical outcome of a malaria parasite infection as suggested by Marsh (1992). As mentioned earlier (see section 1.1.2), it is during the schizogonic cycle in the blood phase that the clinical symptoms of malaria are

experienced. It has also been suggested that the duration between the onset of the disease and treatment (Greenwood, 1991) and the challenging dose of sporozoites (Marsh, 1992; Lines and Armstrong, 1992) influences the progression of the disease to life-threatening situation. But while many individuals, especially children, present with malaria symptoms, it is only in a few that the disease becomes severe. In Kilifi district, 9 sites were identified in which severe malaria was invariably reported. The incidence was classified as high, moderate or low (see setion 3.5). It was also assumed that the 9 sites had varying intensities of malaria transmission which would influence the occurrence of severe malaria. This study was conducted to find out whether the sporozoite challenge and malaria transmission pattern in the study sites influenced the occurrence of severe malaria. From the results of the study, the following conclusions were made.

1. The parasite rate and parasite density was comparable to densities found in other geographical areas but there is no comparable data on association of parasite infection rate and parasite density from other areas. Although overall over 92% of individuals from

the study area had a *P. falciparum* infection at one time during their lifetime, there was marked heterogeneity in parasite rates among the different study sites. However, no significant association was noted between the parasite rates or parasite densities and the occurrence of severe malaria. This implies that the parasite density was not the root cause of occurrence of severe malaria in the study sites.

2. *Plasmodium falciparum* gametocyte rate (26.4%) was high for the study sites, an area with low malaria transmission. This finding indicates that at least one in every 4 individuals had circulating gametocytes in their blood. It also suggests that the few *Anopheles* mosquitoes available were not many enough to deplete the gametocyte pool in the community (Clive Shiff, personal communication). This shows that the probability of *Anopheles* mosquitoes to ingest gametocytes was very high and the few vectors maintained year-round transmission.

3. The type of houses and their environment in the area were similar. The population behaviour towards mosquito control was also similar. This means that the risk for

malaria parasite infection for the population residing in the study area was the same. In spite of all this, there was significant variation in malaria transmission patterns in the study sites implying that different transmission patterns did not favour the occurrence of severe malaria.

4. Vector density was generally low and varied significantly among sites. The rainfall pattern varied significantly among sites but did not correlate with the density of *Anopheles* mosquitoes. The mosquito density was not significantly associated with the occurrence of severe malaria suggesting that vector density was not responsible for occurrence of severe malaria in the study area.

5. The sporozoite loads for the *Anopheles* vectors in the study sites were comparable to sporozoite loads from *Anopheles* mosquitoes in Kisumu, Kenya where malaria transmission is more intense. This shows that sporozoite loads are not influenced by transmission intensity. Whereas there are no comparable data on severity of malaria and sporozoite loads from Kisumu, no significant association was noted between sporozoite loads and the

incidence of severe malaria in the study sites. This dissociation means that sporozoite loads in salivary glands do not influence the occurrence of severe malaria.

6. Overall, sporozoite rate and entomological inoculation rate were relatively lower than in other areas in Kenya. Although sporozoite rates and EIRs varied significantly among study sites, they were not significantly associated with the incidence of severe malaria implying that intensity of transmission does not precipitate the occurrence of severe malaria.

7. No entomologically or ecologically related variables monitored in this study were associated with occurrence of severe malaria, and probably there are other factors responsible for this occurrence that are not vector or ecology related.

6.2 Recommendations for further work

1. Since no vector or ecologically related factors were associated with occurrence of severe malaria in the study sites, it is probable that the main risk factors are within the individuals who suffer from severe malaria. As such, studies to look at genetic variables and immunity in human beings would elucidate the factors associated with the occurrence of severe malaria.

2. It was possible to detect CSAB in bloodmeals of *Culex*, *Anopheles* and *Aedes* mosquitoes. The overall CSAB positive rates for human sera tested and mosquitoes from the study sites were comparable at main study area level but there was discordance at site level. This technique could be developed further as an alternative to blood collection from human beings and could be used as an indicator of malaria transmission in a given area.

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Appendix 1

Clinical features suggested by World Health Organization (1990) which are associated with severe malaria.

1. Unarousable coma
2. Severe normocytic anaemia
3. Renal failure
4. Pulmonary oedema
5. Hypoglycaemia
6. Circulatory collapse
7. Spontaneous bleeding
8. repeated generalised convulsions
9. Acidosis

Other manifestations include:

1. Prostration
2. Hyperparasitaemia
3. Jaundice
4. Hyperpyrexia

Appendix 2

KILIFI RESEARCH UNIT - ENTOMOLOGICAL FIELD FORM 1

DAILY RECORD SHEET - DRI/PSC

DATE:

SITE:_____ **ZONE I_I_I.**

	An. gambiae				*An. funestus*				Others	Total
Hse no.	E	B	HG	G	E	B	HG	B		

E - Empty
B - Blood-fed
HG - Half gravid
G - Gravid

Others - Specify:_____.

Appendix 3

KILIFI RESEARCH UNIT - ENTOMOLOGICAL FIELD FORM 2

DAILY RECORD SHEET - NBC

DATE:I_I_I_I_I_I_I

SITE:_____ ZONE I_I_I.

	An. gambiae					*An. funestus*				Others	Total
Hse number	E	B	HG	G		E	B	HG	B		
NBC In											
NBC Out											
NBC In											
NBC Out											
NBC In											
NBC Out											
NBC In											
NBC Out											

NBC - Night biting catches In = indoor Out = outdoor
E - Empty
B - Blood
HG - Half gravid
G - Gravid
Others - Specify:_____.

Appendix 4

KILIFI RESEARCH UNIT - ENTOMOLOGY SHEET 3

DISSECTION RECORD SHEET

Specimen #: I_I_I_I_I_I	Date (DDMMYY) I_I_I_I_I_I_I
Coll site _____	Zone code:I_I_I Hse #:I_I_I_I
Type of coll:**DRI/PSC/NBC**	Species: **AG/AF**
Blood stage: **E/B/HG/G**	Dissection: **Y/N**
Sporozoite: **Y/N**	Sporos Hemocytometre: I_I_I_I
Sporo load:I_I_I_I_I_I_I	Ingested sporozoites: I_I_I_I
Oocyst: **Y/N**	Oocyst #: I_I_I
Parity: **P/NP**	Ovarian stage: I_I
Ovaries saved: **Y/N**	Id: **Gambiae/ Merus/ Arabiensis**
PCR body saved: **Y/N**	PCR results: **G / M /A**
Wing units: I_I_I	Wing size: I_I_I_I_I
ELISA **H/T:PF Y/N, PM Y/N**	ELISA **SG: PF Y/N, PM Y/N**
BM ELISA saved: **Y/N**	Bld: **Human/ Cow/ Goat/ Others**

Note: Some of the parameters were not done.

Appendix 5

CSAB ELISA SOLUTIONS:

1. Phosphate buffered saline (PBS), pH 7.4. Add 1 bottle of Dulbeco's PBS to 1 litre of distilled water. Store in fridge. (Shelf life is 1 week).

2. Boiled cassein, 0.5% (BB).

Cassein	gm	5.00
0.1N NaOH	ml	100.00
PBS, 7.4	ml	900.00
Thimerosal	gm	0.10
Phenol red	gm	0.02

Suspend cassein in 0.1N NaOH and boil the contents. After cassein is dissolved, slowly add PBS, allow to cool and adjust the pH to 7.4 with Hydrochloric acid. Add phenol red and thimerosal. (Shelf life is 1 week).

3. Wash solution (PBS-Tween 20): Add 0.5 ml of poly-oxyethelene-sorbitan monolaurate (Tween 20) to 1 litre of

PBS. Mix well and store in the fridge. (Shelf life 2 weeks).

4. Solution A: Add 5 μl of 0.5% BB to 5 ml of PBS. Use immediately.

5. Solution B: Add 5 μl stock antigen (R32LA - 1mg/ml) per 2.5 ml of solution A. Use immediately.

6. Solution C: Add 100 μl of Tween 20 to 100 ml of BB. Mix well. (Shelf life 1 day in the fridge).

Appendix 6

Formula for conversion of parasite counts into densities per µl of blood

(a) *P. falciparum* asexual stage:

Normal thickness of thick blood film = 0.09mm

Volume of blood /field =(ii(diameter of field)^2)/2 x thickness.

Field diameter of microscope used = 0.18mm

Volume of 1 field = (ii(0.18)^2)/2 x 0.09 = 0.00229mm^3

Volume of 200 fields = 200 x 0.00229 = 0.458mm^3

Count per 200 fields into counts/mm^3 = count x 1/0.458

(b) *P. falciparum* gametocytes

Count per 400 fields x 1/0.916

Appendix 7

Labeling of microtitre plate for CSAB ELISA

	B	A	B	A	B	A	B	A	B	A	B	A
A												
B												
C												
D												
E												
F												
G												
H												
	1	2	3	4	5	6	7	8	9	10	11	12

Appendix 8

Age groups used for the computation of seropsitive conversion rates in the study sites

Code	Age group
1	1.0 - 2.9
2	3.0 - 4.9
3	5.0 - 6.9
4	7.0 - 8.9
5	9.0 -10.9
6	11.0 -12.9
7	13.0 -14.9
8	15.0 -16.9
9	17.0 -19.9
10	20.0 -24.9
11	25.0 -29.9
12	30.0 -34.9
13	35.0 -39.9
14	40.0 -44.9
15	45.0 -49.9
16	50.0 +

Appendix 9

Preparation of IFA slides

The *P. falciparum* antigens used here were either from *in vitro* continuous cultures carried on in Johns Hopkins University, USA and short term cultures carried on in Kilifi Research Unit, Kenya.

1. Dilute Poly-L-Lysine (Sigma Chemical Company, St. Louis, Mo. USA) 1:10 in distilled water.

2. Dip pre-cleaned IFA slides and let dry at room temperature on a rack.

3. Place 5 µl of whole *P. falciparum* culture (3-5% parasitaemia) on each spot.

4. Allow to dry flat on a bench. Store at -20^{0}C until used.

Appendix 10

Questionnaire for household characteristics

SITE _____ **HOUSENO I_I_I_I**

I_I

Name of head of household _____

PEOPLE IN THE HOUSE

1. How many houses are in the compound? ..... I_I

   How many people sleep in the index house? .....I_I

   How many children are under 5 years?.....I_I

   How many are from 5 to 9 years?.....I_I

   How many are from 10 to 15 years?.....I_I

   How many are over 15 years?.....I_I

BEHAVIOUR

2. How often do you sit outside in the evenings? (1) regularly (2) occasionally (3) never .....I_I

3. What time do the family go to bed? .....I_I_I_I_I

4. Are mosquito coils used (1) regularly (2) every night during peak insect abundance (3) occasionally (4) never? I_I

5. Are insect sprays in cans used (1) regularly (2) every night during peak insect abundance (3) occasionally (4) never?..... .I_I

6. Are mosquito nets used in your house? .....I_I

HOUSE CHARACTERISTICS

7. What is the main construction material for the walls? (1) mud (2) makuti (3) concrete (4) stone (5) sisal bags I_I

8. What is the main construction material for the roof? (1) makuti (2) iron sheets (3) tiles .....I_I

9. Does the house have a ceiling? Y/N.....I_I

10. Does the house have eaves? Y/N.....I_I

11. Do the walls have any large holes or openings to the outside except windows, eaves or doorways? Y/N .....I_I

12. How many windows are there in the house? .....I_I

13. How many outer doorways are there in the house? ....I_I

How many have doors?.....I_I

ENVIRONMENT

14. Does the house stand in open space (without bushes)?

Y/N .....I_I

15. Are there any large farms within 20 meters of the house?

Y/N.....I_I

16. What type of rubbish is found around the house?

Tins (Y/N) .....I_I

Broken pots.....I_I

Coconut husks.....I_I

Tyres.....I_I

Stones.....I_I

Others (_____).....I_I

17. Is there stagnant water within 20m of the house?

Y/N .....I_I

If yes, are they ponds? Y/N.....I_I

pools?.....I_I

swamps?.....I_I

other (_____).....I_I

19. How many burrow pits are within 20m of the house? .I_I