DYNAMICS OF MALARIA TRANSMISSION AND ITS EPIDEMIOLOGY AMONG CHILDREN POPULATION OF KILIFI DISTRICT, COAST PROVINCE, KENYA.

BY

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THESIS SUBMITTED IN FULFILMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN MEDICAL ENTOMOLOGY OF THE UNIVERSTY OF NAIROBI

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1994

DECLARATION

This is my original work and has not been presented for a degree in any other University.

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DEDICATION

To my wife Gicuku, for her love and dedication; our children: Munene and Makena, for their bravery and endurance; and my parents for moral support.

Acknowledgement

I am grateful to several people for the role they have played to enable me undertake and accomplish these studies. In particular, I extend my deepest appreciation to my University supervisor Prof. Canute P.M. Khamala for his unwavering support and ethusiasm during the planning and execution of field experiments, his guidance, patience and availability. Without his constant encouragement and generous criticism of the manuscript this work would not have been accomplished. Dr. Lucie M. Rogo also greatly assisted me with planning in the initial stages.

I owe a big debt of gratitude to Dr. John C. Beier for his scientific and technical guidance throughout the course of research, data analyses and writing. I am also under special obligation to Drs. Bob Snow and Kevin Marsh for the insights I have gained from their critical evaluation of the manuscript in getting it ready for submission to the University. I am also happy to acknowledge the aid of Dr. Gregory Gurri-Glass and Dr. Dayo Forster who generously helped me with statistical analysis of the data.

I am most grateful to my colleagues, Mr E.W. Kabiru (Parasitologist/entomologist), S.K. Muiruri (technologist), and Joseph M. Nzovu (technician) for their expert technical support in the execution of field and laboratory experiments.

Finally, I wish to acknowledge the constant help of my wife Ruth Gicuku Mwaniki and the cheerful interest of my son Dennis Munene and daughter Doris Makena whose time I borrowed to do this project. I am thankful to my parents, brothers and sisters for their moral support.

These studies could not have been undertaken without financial assistance from the World Health Organization's Special Programme for Research and Training in Tropical Diseases, to me through the Kenya Medical Research Institute which provided the facilities and study leave in order to carry out the research.

Abstract

Studies on the malaria parasite transmission, identification of vector-related risk factors, and their relationships with the incidence of severe malaria in children aged 1 to 4 years were undertaken in the the rural and peri-urban areas around Kilifi District Hospital in the Coast Province of Kenya. Intensive mosquito sampling in the rural area of Sokoke and Kilifi town for one year through all-night human biting catches, elevated bednets, and day resting indoor collections yielded three anopheline mosquitoes, namely, *Anopheles gambiae s.l., An. funestus* and *An. coustani.* Of the three, *Anophelesgambiae s.l.* was the most predominant and comprised 87.9% and 97.9% of the total collected at Sokoke and Kilifi town respectively. The mosquitoes were examined for *Plasmodium falciparum* parasite infections by enzyme-linked immunosorbent assay (ELISA). The proportion of *An. gambiae s.l.* with *P. falciparum* comprised 4.1% (20/491) at Sokoke village and 2.2% (3/138) at Kilifi town, indicating low infection rates. No sporozoite infections were detected in *An. funestus* and in *An. coustani* suggesting that their role in malaria transmission was minimal. Identification of the members of the *An. gambiae* complex by the polymerase chain reaction (PCR) technique reve'aled three species; *An. gambiae s.s., An. cirabiensis,* and *An. merus.* Together with *An. funestus*, they form the malaria vectorial system in Kilifi District.

Evaluation of entomological inoculation rates (EIR), which is calculated as

the product of the human-biting rate and the sporozoite rate, indicated that residents were exposed to only 8.0 infective bites per year at Sokoke village and 1.5 infective bites per year at Kilifi town. This implies that residents at Sokoke are subjected to significantly more infective bites that their counterparts at Kilifi town. Transmission of malaria in children aged 1 to 4 years, the target group studied, was detected only during the months of June to September and December to January at Sokoke and during January, June and September at Kilifi town despite the fact that the vectors were active throughout the year in both sites. Monthly patterns of malaria transmission corresponded closely with the incidence of severe infections. The yearly incidence of severe *P. falciparum* infections in this age group of children was 24.1 per 1,000 children in Sokoke and 4.2 per 1,000 in Kilifi town. This indicates that children at the rural site of Sokoke frequently contract severe malaria infections than those at Kilifi town.

Blood meal samples were tested by the ELISA method for 534 *An. gambiae s.l.* and 76 *An. fun.est.us* collected from 25 sites by light traps, aspiration inside houses and pyrethrum spray collections. Human Immunoglobulin G (IgG) was detected in 94.4% of the *An. gambiae s.l* and in 90.8% of the *An. funestus.* No samples were positive for cow and only a few were positive for goat the commonly kept livestock in the homes. Both species fed predominantly on humans irrespective of availability of other ho^ts,. Inference was drawn that at these sites on the Kenya Coast, the high degree of human-feeding by malaria vectors accounted for the efficiency of

the malaria transmission. These studies also demonstrated that in Kilifi District in the Coast Province of Kenya, the temporal incidence of severe malaria infections in children was associated with low-level *P. falciparum* transmission by this vector population.

Environmental factors associated with vector abundance were evaluated. This was done by monitoring mosquito abundance for 4 days in households of children with severe malaria infections admitted to the Kilifi District Hospital and comparing with households of children reporting to the out-patient clinic with mild malaria. Intensive mosquito sampling using all-night biting catches, CDC light traps and aspiration inside houses was done for 119 pairs of households throughout the study area. These findings were then related to a total of 75 environmental, demographic, behavioural and animal husbandry variables characterized for each household. The overall abundance of anopheline mosquitoes (OR=1.5) and *P. falciparum* sporozoite rates (OR=1.5) were not significantly different (P>0.05) between households of children with severe malaria and those of children with non-severe (mild) malaria. The risk of severe disease in children increased in households with 3 or more adults and when more than two goats were found in the compound. These investigations suggest that the risk of developing severe malaria in Kilifi District is independent of vector abundance and transmission intensity of the local vectors implying other possible host and parasite factors are more important.

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CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Malaria has pre-occupied man for centuries. The Chinese described it as far back as 5000 B.C. Recent estimates from the World Health Organisation (WHO) reported the magnitude of the current problem and claimed that 1.7 billion or 30% of the world's population is at risk from malaria, while 90% or approximately 445 million of the world's population are infected (WHO, 1990). Of this latter group, 100 million cases occur in Africa South of the Sahara. The greatest burden of morbidity in Africa is suffered by children, with approximately one million dying annually as a result of *falciparum* infection (Greenwood, 1990a). These figures are approximations since they were compiled from data fraught with inadequacies of record keeping and under reporting (Ruebush *et al.,* 1986), lack of reliable data on the importance of malaria as a cause of death (Snow *et al.,* 1992a), and the inherent difficulties of making a clinical diagnosis (Trape *et al*., 1985).

There are four types of human malaria caused by parasitic species of the genus *Plasmodium* namely: *P. falciparum, P. ovale, P. uiucoc* and *P. mcdariae.* The most widely distributed of the four species is *P.*

uivax whereas the most clinically dangerous is *P. falciparum* that is wide spread in Africa South of the Sahara and the world's tropics (Wernsdorfer & McGregor, 1986). Among 50-60 mosquito species of the genus *Anopheles* are responsible for the transmission of Plasmodia which affect man causing malaria. The transmission of *P. falciparum* is highly variable and is associated with severe disease and death for the persons with little or no acquired immunity, such as infants, when the immunity gained through maternal antibodies during gestation has waned (McGregor, 1964; Greenwood, 1991). Non-immune and semi-immune persons such as immigrants from malaria-free areas, travellers and individuals who lose their acquired immunity also suffer severe illness often requiring urgent clinical care.

Malaria presents an increasing challenge in many endemic areas mainly due to failures in previous control strategies. These failures were a result of rapidly emerging insecticide resistance in mosquitoes, emergence of antimalarial drug resistant parasite populations, poor compliance within control programmes, and economic and -social disturbances. Malaria control effort's first focused on eradication using either residual insecticide or mass drug administration. It was soon realised by the 1960's that eradication was impossible and control of disease was the only possible option for areas such as Africa (Prothero, 1961; WHO, 1974).

Despite the current focus of malaria programmes on the control of morbidity and mortality, our knowledge of how malaria as a disease relates to its transmission is surprisingly incomplete. The vector population in the sub-Saharan Africa is uniquely effective, with the six species of *An. gambiae* complex being the most efficient vectors of human malaria (Coluzzi, 1984). In Africa, these vectors have been incriminated as the most effective in transmitting the malaria parasites to humans in both urban and rural areas (Coluzzi, 1970).

In the first half of this century, field scientists made important attempts to examine malaria parasite transmission in terms of morbidity and mortality among host populations (Garnham, 1929, 1949; Walton, 1947). However, during the following 30 years this emphasis was noticeably absent in epidemiological studies of the transmission. There is a resurgence of interest in the epidemiology of malaria as a disease for two main reasons. First, improved understanding of the biological complexities and diversities of *falciparum* malaria have highlighted the importance of focusing on markers of disease rather than parasitization for vaccine and drug development (Marsh, 1992). Secondly, the belated acceptance that the control of disease rather than parasite eradication was the only plausible goal in public health especially in Africa. International donors of control programmes in Africa are increasingly demanding strategic plans and targets for malaria-specific mortality reductions

(WHO, 1993). However, there is paucity of empirical data on malaria mortality and no models to predict what would happen to either severe malaria morbidity and mortality following reductions in *falciparum* transmission from different starting endemicities. There is limited data available on the relationship between transmission and malaria-specific childhood mortality in Africa.

Epidemiologic studies on malaria in Africa have tended to focus on understanding the conditions under which uninfected humans become infected. Indeed, it has been shown that one of the most basic concepts is that there is a linear relationship between the intensity of malaria transmission as measured by entomologic inoculation rates (EIR) and the incidence of malaria infection (Molineaux, 1988). If it were the case, then decreasing transmission intensity by effective vector control would have an impact on infections. However, it is important to distinguish between infection and disease and to appreciate that malaria disease is a progressive spectrum of phases (non-infected, infected but asymptomatic, clinical malaria, severe malaria, and death) (Greenwood *et al.*, 1991). The factors that influence the progression from one stage to the next are poorly understood. In endemic areas, not everyone exposed to malaria sporozoites develops the severe phase of the disease or dies (Marsh, 1992). Therefore, the identification of risk factors that cause the severe disease leading to mortality is now a priority for malaria

epidemiologic studies in Africa (Oaks *et al*1991). These factors include combinations of host, parasite or vector-related phenomena which individually or in combination influences malaria outcome and this needs to be established.

These studies were therefore initiated to identify some of these factors, and their means of interaction, as a step towards the treatment and prevention of the severe disease in humans. Insight into whether decreases in transmission intensity would yield corresponding reductions in the incidence of the severe disease and mortality will provide strategic guidance for vector control programmes aimed at reducing the burden of morbidity and mortality. Hitherto, the difficulty to achieve this has been due to the limited field research that address natural relationships between transmission and disease. Given the heterogeneities of malaria transmission in Africa, where annual EIRs range from less than 1 to more than 500 infective bites per person, relationships between malaria transmission and disease may not be linear.

These studies are about the transmission of *P. falciparum* by its vectors on the incidence of the severe malaria disease in children in the Kilifi District on the Kenyan coast and were initiated with the objectives listed below in mind.

1.1.1 OBJECTIVES

The main objectives for initiating these studies were:-

1. To determine anopheline species composition and abundance in relation to malaria transmission intensity and illness at two ecologically distinct sites in the Kilifi District in the Coast Province of Kenya.

2. To evaluate entomological risk factors for severe malaria in households with children suffering from severe malaria as compared with households with children having mild malaria.

The secondary objectives were:-

3. To determine the blood-feeding and biting behaviour of anophelines in Kilifi District.

4. To determine the best sampling procedures for anophelines in areas of"low vector abundance but with high severe malaria incidence.

1.2 Literature Review

In this introductory chapter past studies on the transmission of the malaria parasites as evidenced by various aspects of field and laboratory studies are highlighted. This was in order to provide the basic information for better understanding of the possible vector-related risk factors associated with the development of malaria into a severe disease as will be analyzed in these investigations.

1.2.1 Factors Affecting Distribution of Malaria

Several factors affect significantly the distribution of malaria in space and time, between persons, and the resulting morbidity and mortality. Some of these factors include; the natural environment through its influence on vector populations, interaction between vector and parasite, parasite determinants and some of its genetically controlled characteristics, host-biological factors, behaviourial, social and economic elements.

Factors pertairfing to the natural environment are, for example, the availability of the breeding habitats for malaria vectors which influences the distribution of malaria in an area. Local rainfall produces rain pools favoured by most malaria vector species, for example, *An. gambiae s.s* and *An. arabiensis.* The slope of the land

and the nature of the soil are some of the other environmentally related factors which affect the type of surface water available and its persistence and subsequently the increase of local malaria vector populations. The optimal range of temperature and relative humidity for most malaria vectors is 20-30"C and 70-80% respectively (Wernsdorf & McGregor, 1986). Increasing temperature increases the growth of vector population by shortening the intervals from oviposition to adult emergence and vice versa. Biological factors such as immune response and genetics, as well as socio-economic status, living and working conditions, exposure to vectors, and human behaviour, all play a critical role in determining a person's risk of malaria infection and hence illness.

1.2.2 Clinical Disease

The clinical manifestations of *falciparum* infection are varied. The attack may progress episodically from shaking chills through to hot and cold flushes, to drenching sweat (Wernsdorfer & McGregor, 1986). These characteristics are, however, not universal and the symptoms may mimic other diseases, making clinical diagnosis difficult (Bruce-Chwatt, 1985; Trap*eetaL,* 1985; Bassette^a/., 1991; Rooth and Bjorkman, 1992; O'Dempsey *et al.*, 1993; Redd *et al* 1992). Recovery may occur either after effective treatment or spontaneously with development of sufficient immunity otherwise the

prognosis may involve rapid progression to death. Describing his work in The Gambia, Greenwood *et al.* (1987) estimated between 1-2 annual clinical attacks of P. *falciparum* in children aged 0 to 7 years, and the disease mostly affects children aged between 6 and 24 months. He further suggested that the average duration of morbidity before death was 2.8 days with 52% of deaths in children having a morbid history of less than two days. In a hospital-based surveillance of malaria-related mortality and morbidity in Zaire, Greenberg *et al.*, (1989) reported that 62% of paediatric deaths resulting from malaria occurred in the emergency ward.

P. *falciparum* malaria is a major cause of childhood mortality and currently it has been estimated to account for between one half and two million deaths per year in the sub-Saharan Africa alone (Greenwood, 1990a). In The Gambia, about 1% of children under 5 years of age die each year from malaria (Greenwood *et al.*, 1987), while in Kilifi severe malaria attacks at least 1 in 15 children before their fifth birthday (Snow *et* a/., 1993; 1994a). It is claimed that although infection by *P. falciparum* is extremely common in African children, only "a few of these infections lead to severe and life-threatening illness (Greenwood *et al.*, 1987; 1991). Therefore, the distinction between infection and the manifestation of the disease is extremely important in the study of malaria, since infection of humans with the parasite does not necessarily result in disease and

becoming ill. This was also the findings of Hogh *et al* (1993) when they stated that in individuals living under conditions of intense malaria transmission, infection with *P. falciparum* was not always associated with the clinical conditions of the disease because of the rapid development of immunity. These authors also further found that many infected people in such areas were asymptomatic. In other words, they harboured large number of parasites yet exhibited no symptoms of the disease. However, asymptomatic individuals were found to be major contributors to the transmission of the malaria parasites (Muirhead-Thomson, 1954; Greenwood, 1988). Reports on malaria morbidity have suggested different pathogenic thresholds of *P. falciparum* parasites ranging from 1000 to 15 000 parasites (Trape *et* a/., 1985; Snow *et al,* 1988a; Menon *et al*, 1990; Lyimo *et al*, 1991; Velema *et al*, 1991). These pathogenic thresholds varied, not only with the age of the host but also with the degree of immunity of the human population.

Severe and complicated cases of malaria have been found difficult to diagnose for lack of symptoms that are specific for malaria (Hogh *et al*, 1993). For hospitalised patients, a definition of severe and complicated *falciparum* malaria and the differences between severe malaria in adults and children has been comprehensively considered by the WHO (Warrell *et al*, 1990). There have been no comprehensive clinical descriptions of severe malaria in young

children and therefore, considerable reliance has been placed on description of disease in non-immune adults. However, there are striking differences in the spectrum of severe disease in young African children (Marsh *et al.,* 1991; Marsh *et* a/., 1994) where severe malaria falls into three important groups: cerebral malaria, severe malarial anaemia, and others who fall short of the strict definition of cerebral malaria but who have severe features such as multiple fits, prostration and hyperparasitaemia. In Kilifi, Marsh *et al.* (1994) described the complete spectrum of life-threatening disease presenting to hospital, and pointed out that severe disease fell into two overlapping clinical syndromes: malaria with coma and malaria with respiratory distress.

Oaks *et al.* (1991) emphasised that the most important goal of malaria control is to prevent individuals from becoming severely ill. These authors also pointed out that the highest mortality occurred in the young children and decreased markedly in older children and adults. The concentration of severe malaria morbidity and mortality among the young age groups in areas of high transmission compared to older children in lower transmission areas was explained immunologically. The greatest risk of the severe disease and death from malaria occurred on first exposure to infective mosquito bites, and this risk decreased with repeated exposures until a state of effective anti-disease immunity was reached (Molineaux, 1988). In

high transmission areas, the age-specific peak mortality rose and occurred at a younger age, while this occurred later in life where transmission was low. In areas of extreme challenge, acquired immunity occurred during the first few months of life when passively acquired immunity and other haematological factors offered effective control. Under these conditions it has been hypothesized that there may be very little clinical disease or death from malaria in a population (Greenwood, 1990b; Anon, 1992)

1.2.3 The Malaria Parasite and Disease Severity

The severity of malaria following infection may be influenced by the amount of the dose of the causative organism in the form of sporozoites injected into the host by the mosquito (Greenwood *et al.,* 1991; Marsh, 1992) or by the virulence of the particular strain of the parasite to which the host has been exposed (Marsh 1992; Lines & Armstrong, 1992; Glynn *et al.,* 1994). Studies have demonstrated that the amount of inoculation doses differ from species to species of the transmitting vector but usually less than 20 sporozoites are released at any one time (Rosenbergs *al.,* 1990; Beier & Koros, 1991a; Beier *et al.* 1991a; 1991b; 1991c; 1992; Ponnudurai *et al.,* 1991). Infected mosquitoes probe more than uninfected mosquitoes thereby increasing the chance of infection (Rossignol *et al.,* 1986). Earlier, Rossignol *et al.* (1984) had found that sporozoite-infected *Aedes*

aegypti probe more resulting in an increase in the duration of vector-host contact and thereby improving the chances of parasite transmission. Some field studies have indicated that most exposures to sporozoite-infected mosquitoes do not necessarily lead to the further transmission of malaria (Pull & Grab, 1974; Gazin *et al.,* 1985; Rickman *et al*., 1990). These authors also observed that only 1-20% of the bites from mosquitoes laden with sporozoites in their salivary glands produced infection in the host. However, Vanderberg (1977) using a rodent model, suggested that only ***1%*** of the inoculated sporozoites gave rise to pre-erythrocytic hepatic development. In addition, host skin factors may determine the number of the infective sporozoites reaching the blood (Vanderberg, 1977). In a study by Ponnudurai *et al.* (1991) more sporozoites were injected by feeding mosquitoes intradermally without reaching the capillaries but the sporozoites disappeared from the site of bites within 2 hours of post-feeding.

Greenwood *et al.* (1991) postulated that the severity of malaria depended on the size of the inoculum (the number of sporozoites inoculated in a "unit of time). Hamilton (1947) reported that the time taken to reach detectable parasitaemia levels depended on the amount of the dose estimated from the number of mosquito bites. He further observed that at a given time during this stage of initial multiplication, the parasite load was smaller after a small inocula

than after large ones. Subsequently, Chulay *et al*1986 confirmed that too many bites by infected mosquitoes delivered a large bolus of sporozoites, thereby overwhelming an immune response that would have provided against few bites received in the field. Several authors have shown that five infective bites repeatedly produced 100% infectivity in humans (Ballou *et al.*, 1987; Davis *et al.,* 1989; Rickman *et* a/., 1989). Recently, Hayes *et al.* (1992) highlighted the difficulties of estimating the size of the infecting dose in natural infections. They pointed out that the infecting dose or the number of sporozoites that give rise to an episode of malaria may be influenced by the level of transmission. Trape *et al.* (1993) stated that in low-transmission conditions where infection is accompanied by disease, each episode is likely to have arisen from a single infective bite. Similarly, they also found that in high-transmission conditions, more frequent infective bites were experienced leading to an increase in the size of inocula. They therefore concluded that the frequency of infective bites may affect the inoculum size.

Lines and Armstrong (1992) argued that the inoculum size may be important only if natural immunity to malaria is strain specific. However, Greenwood *et al.* (1991) and Marsh (1992) hypothesized that the severity of malaria depended on the size of the inoculum. These authors further claimed that parasitized erythrocytes adhere physically to endothelial cells that leads to microvascular obstruction.

Further, they showed that uninfected erythrocytes also bind to the surface of erythrocytes of mature forms of parasites, by a mechanism similar to that of cytoadherence to endothelial cells causing rosetting. The evidence they adduced on rosetting and cytoadherence of parasites indicated that these phenomena were important in the clinical outcome of malaria. Carlson *et al.* (1990) working in The Gambia noted that Gambian children with cerebral malaria exhibited much higher degrees of rosetting than those from non-cerebral cases. Marsh (1992) pointed out that all parasite strains exhibited the same pathology as shown during early studies on the treatment of syphilis patients with *falciparum* malaria (James *et al.,* 1932). From such studies, Glynn *et al.* (1994) posed the question whether it was the dose of *falciparum* inoculated or the chance of seeing a rare parasite variant that increased the chance of severe disease.

Trape *et al.* (1993) demonstrated that in areas of high malaria transmission the incidence of malaria attacks was approximately 40 times higher in children less than five years of age than in adults. They further pointed out that under such conditions, clinical malaria was concentrated disproportionately in the youngest age group and the major clinical problem was one of severe malaria anaemia. However, under conditions of lower transmission, Snow *et al.* (1993, 1994b) reported that the burden of disease was more evenly spread among older children, but malaria anaemia remained the

predominant problem in the youngest children, while cerebral malaria emerged in the older children. In their studies in Dakar, Senegal, Trape *et al.* (1993) were concerned with the environmental factors of malaria transmission and observed that the duration of the disease and its presumed severity were similar whether the child lived near or far from the marsh where the *Anopheles* vectors were breeding. However, the incidence of malaria infections sharply decreased with distance from the marsh from one infection per year in children living at a distance of less than 160 metres to one infection every four or five years in those living at adistance of 910 metres away.

1.2.4 Host Factors and Severe Disease

It has been shown by several authors that malaria, like most other infectious diseases, is not homogeneously distributed in human populations, and hence the factors that could plausibly account for the clustering of malaria in individuals or households have been listed as unequal susceptibility to the disease among individuals due to innate or acquired immunity (Armstrong, 1978; Miller & Carter, 1976), unequal risks of contact with vector mosquito (Dye & Hasibeder, 1986; Burkot, 1988), and the likelihood of the parasite to recrudesce or relapse in the individual (Greenwood, 1989). Warrell *et al.* (1990) studied the human host factors that could potentially

influence the outcome of infection and gave them as genetics, physiological, behaviourial and economic. The main genetic factors included haemoglobin genotype AS or AC, cx-thalassaemia, glucose-6-phosphate dehydrogenase (G-6-PD), and immune responsiveness to malaria antigens. Allison (1954) reported that subjects with haemoglobin genotype AS or AC were partially protected against malaria infection, while recent studies done by Flint *et al.* (1987) in Melanesia strongly supported the hypothesis that cx-thalassaemia was also protective.

Most studies on malaria have examined protective effects at the level of infection whereas little or no studies have been conducted on the progression of the developing disease. The possible role of genetically determined immune mechanisms in the susceptibility to malaria has been stimulated by the discovery that the immune response to the repeat antigen of the circumsporozoite protein of *P. falciparum* is strongly genetically determined in mice and probably also in man (Good *et al.*, 1986). An association between clinical malaria and Human Leucocyte Antigen (HLA) phenotypes was reported in the Phillipines by Sy *et al* (1984) while in Tanzania, Osoba *et al.* (1979) found an association between HLA phenotype and malaria antibody levels. More recently, possible HLA associations with the risk of developing severe malaria have been examined in a large case-control study in The Gambia where an HLA class 1 antigen and an HLA

class 2 haplotype were independently associated with protection (Hill *et al.*, 1991).

Boyd *et al.* (1936) and McGregor (1964) reaffirmed that generally, natural immunity has long been recognized as a key factor limiting the temporal "window of mortality" for children living in malaria endemic areas. Deloron & Chougnet (1992) added that such immunity developed early in life under conditions of intense transmission and consequences of its absence were especially pronounced in epidemic situations where nearly everyone was prone to severe disease and death.

Greenwood (1989) observed that socio-economic factors were unlikely to directly influence the severity of disease but acted as effect modifiers. In his review of some of these factors which include maternal education, local beliefs on disease causation, wealth and the efficiency of local transport systems, he pointed out that poor households may not be able to afford mosquito nets or netting on windows, drugs to help prevent and treat malaria illness. For example, he Reported that a mother who cannot recognise the symptoms of malaria early due to her poor education could result in an unacceptable period where no reliable treatment was given; families with low incomes may not be able to afford bednets; and those who have homes far from peripheral health care services may

not be able to use them to alleviate mild disease before it progresses to severe end points. However, he pointed out that it is not known to what extent these factors influence the clinical outcome once infection has occurred.

1.2.5 Vector Factors and Severe Disease

The role of transmission intensity has been reviewed under the section on parasites and severe disease. Coluzzi (1992) stated that transmission intensity or potential is determined by measuring important entomological parameters such as vector density, biting behaviour, infection rates, as well as other vector behaviourial features. Past studies on these aspects have essentially been concerned with answering the question whether more infective bites lead to a greater chance of causing the disease (Boyd & Kitchen, 1937; Jeffery *et al.,* 1959). The study of vector behaviour will offer insights into the role of transmission pressure at a community or individual level and the chance of developing severe disease.

The entomological inoculation rate (EIR) as a marker for malaria transmission intensity has been extensively studied. Trape *et al.* (1993) found that malaria transmission levels in tropical Africa varied considerably being influenced by ecologic conditions, from approximately $10^{'2}$ to 10^{s} infective bites per person per year. Snow

et al. (1994b) reported that people living in the rice growing area of Kilombero District around the town of Ifakara in South Western Tanzania received at least 300 infective bites each year, while Beier *et al.* (1990) recorded that residents of Saridiki and Kisian area of western Kenya received an average of 237 infective bites per year. Children born in such environments were inevitably challenged by multiple *falciparum* infections early in life. In Brazzaville, Congo, Trape *et al.* (1987) described the incidence of pernicious attacks in areas of different levels of transmission. They further found that the incidence of severe clinical forms of malaria and the resulting mortality were similar whatever the level of transmission. For instance, they observed similar results at Mfilou-Ngamaba-Ngangouni, where malaria is holoendemic with over 100 infective bites per person per year and a parasite rate of 81% in school children, and Poto-Poto-Ouenze-Moungali, where malaria is hypoendemic with less than one infective bite per person every three years and a parasite rate of less than 4% in school children.

Variations in the transmission of malaria within small areas have been reported between village populations (Bjorkman *et al.*, 1985; Cattani *et al.,* 1986; Greenwood, 1989; Gamage-Mendis *et al.,* 1991). For example, Mendis *et al.* (1990) reported a marked heterogeneity in the distribution of malaria vectors and their biting habits small areas in Kataragama, Sri Lanka.

Onori and Grab (1980) and Oaks *et al.* (1991) reported that the intensity of malaria parasite transmission by mosquito populations was a key component in the epidemiology of the disease. The two important aspects of malaria transmission they considered were the entomological inoculation rate (EIR) and vectorial capacity (VC). The EIR is a measure of the number of infective bites each person receives per unit time (e.g. per night) and is a direct measure of the risk of human exposure to the bites of infective mosquitoes. Garret-Jones (1964) observed that the EIR varied with time, vector species and parasite species. Further, Dye (1986) pointed out that the vectorial capacity that measures the potential for malaria transmission was based on several key parameters of vector populations.

Greenwood (1989) observed that environmental factors play a key role in malaria parasite transmission. He found that climatic and topographic features determine the ecology of both human and arthropod hosts as well as their contacts. Earlier, Greenwood *et al.* (1987) stated that man-vector contact could affect the frequency of vector feeding on man and thus increase the probability of transmitting the infection they carry. They further, pointed out that ponds and reservoirs in an area were important environmental factors in the malaria transmission as they were the breeding sites for mosquitoes. Many other environmental factors have been found

to influence the level of exposure to mosquitoes of an individual resident in a malaria endemic area. For example, Greenwood (1989) listed some of them as place and type of residence, the use of anti-mosquito measures, and the position of the house within an area. Additionally, Trape *et al.* (1993) reported that many west African villages were surrounded by swamps or irrigated gardens and in such situations those who lived on the outskirts of the village were at greater risk of being afflicted by malaria than those who lived in the centre. Robert *et al.* (1985) worked in irrigation schemes in Burkina Faso and reported that new irrigation schemes and hydroelectric projects were likely to increase the intensity of malaria transmission and may change the seasonal transmission dependent on rainfall into perennial transmission by maintaining a population of the vector anophelines mosquitoes throughout the year. Large numbers of *An. gambiae s.l.* were found in the rice growing areas during the dry season at Ahero (Githeko, 1992) and at the Mwea-Tebere irrigation scheme (Ijumba *et al.*, 1990) maintained by irrigation water.

Further studies on the influence of environmental factors on malaria transmission have been undertaken by many other authors. Schofield and White (1984) demonstrated that the design of the house, as well as its situation, was important in protecting its residents from mosquitoes. Construction of houses on stilts provided some protection against vector mosquitoes which rest close to the

ground. At night mosquitoes entered houses through open doors, window spaces, eaves or holes in the roof. Lindsay *et al.* (1990) reported an increased number of malaria vectors in houses with ceiling than those without ceiling. Fewer mosquitoes were found in houses with closed eaves than in those with open spaces, and children sleeping in rooms with closed eaves had fewer attacks of malaria (Lindsay & Snow, 1988). Differences in house construction reflected differences in the socio-economic status of its inhabitants thus making this status a factor influencing malaria transmission. Gamage-Mendis *et al.* (1991) showed an association between malaria risk, indoor resting mosquito density, and house construction and concluded that house construction was an important determinant of exposure to malaria. They observed that the risk of getting malaria was greater for inhabitants of the poorest type of house construction (incomplete, mud or cajan (palm) walls, and cajan thatched roofs) compared to houses with complete brick and plaster walls and tiled roofs. They further reported a higher number of indoor resting mosquitoes from the poorly constructed houses than from those better constructed houses.

The role of human behaviour in relation to vector contact and the transmission of malaria was recently summarized by Greenwood (1989). He asserted that human behaviour which operates at several different levels depending on the number of others involved and the

social structure within a community could greatly influence malaria transmission. He quantified human behaviour in terms of the methods of avoidance of mosquito bites which included insecticide-treated nets, house screening, mosquito coils, smoky fires, household insecticides, and specialised house construction. In domestic situations, the value of zooprophylaxis, as described by Hess and Hayes (1970), was important in reducing the frequency of mosquito feeding on humans and hence malaria transmission. All these factors contributed to affecting the degree of man-vector contact.

1.2.6 The Distribution of *Anopheles gambiae s.l.* and *An. funestus* in relation to Malaria Transmission

Among the more than 400 described species of *Anopheles,* some 50-60 of them are implicated in the transmission of malaria (White, 1977), the distribution of which could be crucial in determining the presence or absence of malaria in specific geographic areas. White (1975) attempted to analyze the distribution of malaria vectors and found that in tropical Africa the fresh water breeding *An. gambiae s.s.* Giles, *An. aralJiensis* Patton and *An. quadrianulatus* Theobald were found widespread in many parts of Africa. He also found that *An. bwambae* White, was confined only to Bwambwe Forest in Uganda (Davidson and White, 1972; White, 1973). Likewise, *An. merus* Donitz, a salt-water breeder, occurs on the East African coast

(Davidson *et al*1967; Service, 1970; White *et al.*, 1972; Davidson and Hunt, 1973; Service *et al.*, 1978) as does A/?., *melas* Theobold on the West African coast (Ribrands, 1946; Muirhead-Thomson, 1951). Other workers have found that *An. gambiae s.s.* and *An. arabiensis* are the most closely associated with man and are major vectors of malaria with their distribution overlapping extensively and sympatrically in large areas of tropical Africa (Colluzi *et al.,* 1979; High ton *et al..,* 1979). In their studies in Nigeria and Kenya, these authors also showed that the two mosquito species occurred at different frequencies along an ecological gradient with *An. arabiensis* inhabiting the plains and *An. gambiae s.s.* the foothills. Recently, other workers have also confirmed that *An. merus* and *An. melas* are restricted to a narrow coastal range due to strict salt water adaptation (Mosha & Mutero, 1982; Mosha & Subra, 1982; Mosha & Petrarca, 1983). Mosha and Pertraca (1983) worked in a coastal belt village called Jimbo, Kenya, and found that 96% of An. *gambiae s.l.* caught there were *An. merus.*

Coluzzi *et al.* (1979) observed diverse polymorphisms at the intraspeciflc level of *An. gambiae* complex which could enhance the viability of this species in transmitting malaria. For example, they reported *An. gambiae s.s.* as an extremely versatile species in tolerance to a wide variety of micro- and macro-environmental conditions thereby making it more efficient in malaria transmission.

Other workers observed that the genetic plasticity in this species was associated with polymorphisms in chromosome inversions and that individuals carrying different inversions differed in their capacity in transmitting malaria (Coluzzi *et al*1979; Sabatini *et al..,* 1989; Toure *et al,* 1986; Petrarca and Beier, 1992). Colluzi *et al* (1979) further reported that the spatial distribution of karyotypes showed strong association with specific types of habitats and that their frequencies changed seasonally thus affecting malaria transmission.

1.2.7 Vector Dynamics in Kenya in Relation to Malaria Transmission

The three species in the *An. gambiae* complex found in Kenya show considerable variation in their malaria infection rates (Zahar, 1985). In western Kenya, Service *et al,* (1978) reported higher malaria infection rates in *An. gambiae s.s.* than for *An. arabiensis,* while Mosha & Petrarca (1983) found infection rates for malaria sporozoites of 3.3% in the cytogenetically identified *An. merus* sample. Earlier, White *et al* (1972) had reported sporozoite rates of 4.2% in *An. gambiae s.s.* and 0.3% in *An. arabiensis* at Sengera, Tanzania. In the Kisumu area of western Kenya, Highton *et al* (1979) reported sporozoite rates of 5.3% in *An. gambiae s.s.* and 0.3% in *An. arabiensis.* However, Joshi *et al* (1975) reported sporozoite rates of 8% in *An. gambiae s.s.* and 7.8% in *An. arabiensis* at Ahero,

Kano plains near Kisumu, Kenya which were not significantly different. Beier *et al.* (1987) recorded sporozoite rates of 10.9% and 10.2% in *An. gambiae s.l.* and *An. funestus,* respectively, at Saradidi 40 km southwest of Kisumu. Recently, Beier *et al.* (1988) and Petrarca and Beier (1992) observed that about 90% of the transmission in Saradidi was due to species in the *An. gambiae* complex *{An. gambiae s.s.* and *An. arabiensis*) and that *An. funestus* accounted for only about 10%.

Several workers have shown that the biting habits of mosquitoes differ greatly depending on adaptations which could considerably influence the transmission of malaria. Mutero *et al.* (1984) found *An. merus* and *An. arabiensis* biting more often outdoors than indoors, while Highton *et al.* (1979) observed *An. gambiae s.s.* biting more often indoors. These workers asserted that this biting pattern may or may not influence host preference. For instance, in the Kisumu area, Highton *et al.* (1979) reported that 92% of *An. gambiae s.s.* sampled fed on man whereas 59% of *An. arabiensis* fed on cattle. Further, Boreham and Port (1982) observed that some vectors move inside into a shelter after an exophagic meal whereas others move out after an endophagic meal.

There is need to reliably identify the correct sibling species of *An. gambiae* complex caught biting indoors and outdoors, in addition we

need to identify bloodmeals from engorged adults, and determine the sporozoite rates and entomological inoculation rates. There is also the problem of getting unbiased samples of outdoor and indoor resting populations of different sibling species. The following sections below reviews the various methods used.

1.2.8 Importance of Accurate Identifications in Relation Malaria Transmission

Of the importance of discriminating between different species with precision when, as in the case of anopheline mosquitoes, one species may not be a proven vector of malaria and a closely allied one harmless. An erroneous identification might lead to either a loss of life or an expenditure of time, energy and money on superfluous anti-mosquito campaign. Hence several works have developed many methods for identifying the species within the *An. gambiae* complex, including allozyme analysis, polytene chromosome banding patterns, liquid chromatography of cuticular hydrocarbons, and hybridization with DNA probes that are either species-specific or reveal species-specific restriction enzyme fragments (Coluzzi & Sabatini, 1968; Miles 1978; Carlson & Service 1980; Gale & Crampton 1987; Collins *et al.*, 1987).

The cytogenetic method of Coluzzi and Sabatini (1968) which was

modified by Hunt (1973), has been widely used successfully to speciate adult and larval samples of *An. gambiae* complex. However, a problem is that not many field entomologists have acquired the skills to use the method. Although the method has proved powerful in unravelling new genetic entities of epidemiological significance, it is restricted to the analysis of half-gravid females and fourth stage larvae. The isoenzyme typing method of Mahon *et al.* (1976) and Milles (1978) offers greater opportunity for analysis because all adults whether male or females and at whatever stage of gonotrophic cycle, as well as larvae are identifiable. A drawback in this method is that no absolute differences have been found between *An. gambiae s.s.* and *An. arabiensis.* This, according to collins *et al.* (1988) causes uncertainties in the alloenzyme assay.

The need for assays without the above inherent limitations has culminated in the development of new techniques such as DNA probes. Collins *et al.* (1988) speciated species in the An. gambiae complex using intergenic spacer (IGS) regions of their ribosomal DNA (rDNA). They showed that five of the species in the *An. gambiae* complex *{An* gambiae, An. arabiensis, An. merus, An. quadriannulatus,* and *An. melas)* differed sufficiently to be distinguished from each other by species-specific restriction fragment length polymorphisms (RFLP). In addition, Paskewitz and Collins (1990) used the polymerase chain reaction (PCR) of rDNA to identify

two of these species, *An. gambiae s.s.* and *An. arabiensis.* Subsequently, Scott *et al.* (1993) extended this rDNA-PCR approach to include the five most wide-spread members of the complex, except the halophilic member of the complex, *An. bwambae.* Although these techniques are still in developmental stages, they show great promise in sensitivity, specificity and field applicability.

1.2.9 Blood Feeding' Behaviour of Vectors in Relation to Malaria Transmission

Gillies (1972) found that in tropical Africa, 80% of the *Anopheles* species fed on any large mammal that was available. In the Garki project, in Nigeria, the arrival of families in an area and their herds of cattle was accompanied by an increase in the number of *An. arabiensis* (Molineaux & Gramacia, 1980). Working in Tanzania, Gillies (1964), demonstrated that the preference shown by a particular mosquito species for one mammalian host or another was likely to be influenced by environmental conditions. In his studies, the predominant species was the anthropophilic *An. gambiae* which when a calf w&s introduced in a hut where a man also slept, there emerged two strains with specific host preferences. One strain was strictly zoophilic while the other was anthropophilic. In The Gambia, Port *et al.* (1980) also showed that attraction to an individual host was a function of surface area. In the human population the defence

behaviour of adults influences the successful feeding by mosquitoes (Smith, 1955; Port *et al.*, 1980) and hence malaria transmission.

Identification of bloodmeals taken by malaria vectors is important in malaria epidemiology because the degree of human-feeding influences the probability that mosquitoes will come into contact with gametocyte carriers and thus acquire *Plasmodium* infections. Thus, Garrett-Jones *et al.* (1980) working on the feeding habits of mosquitoes, demonstrated that the most successful malaria vectors fed commonly on humans and secondarily on cattle and other domestic animals depending on host availability. In this regard, Washino & Templis (1983) listed several methods that have been designed for blood meal identification and they include precipitin tests, haemaglutination assays, counter-current Immunoelectrophoresis, and immunofluorescence. They pointed out that although not all are field techniques they provide acceptable levels of sensitivity and specificity. Recently, Lombardi and Esposito (1986), Service *et al.* (1986) and Beier *et al.* (1988) developed a variety of enzyme-linked immunosorbent assays (ELISA) to meet the criteria for a fifeld operational kit. Additionally, Savage *et al.* (1991) have developed a dipstick ELISA that allows identification of blood meals in less than two hours.

1.2.10 Sporozoite Determination in Relation to Malaria Transmission

Burkot and Wirtz (1986) asserted that the dissection of salivary glands and detection of sporozoites microscopically has several disadvantages. They reported that when the sporozoite rates are low (e.g. 0.1% or less) large numbers of labour intensive dissections have to be done in order to obtain a statistically significant result. They also mentioned other disadvantages of dissections as the requirement of relatively fresh material, and the problem of identification of the *Plasmodium,* species. In view of this, Zavala *et al.* (1982) had introduced the use of IRMA, while Burkot *et al.* (1984), Beier *et al* (1987), and Wirtz *et al.* (1987) introduced ELISA techniques into the arena of malaria epidemiology in order to overcome most of the above constraints. These authors claimed that these methods are relatively rapid and cannot only distinguish between human and non-human sporozoites, but can identify infectious species. Further, Hoedojo *et al.* (1987) asserted that the greatest advantage these methods offered over the dissection was that samples tould be stored dry or frozen and analyzed at a convenient time, and that the ELISA technique has more scope over the IRMA because it does not rely on radioactive material. Nevertheless, Service (1991) claimed that a few problems with ELISA determination of sporozoite rate still remains.

CHAPTER 2

THE STUDY AREA

2.1 Background to the study

Between 1989 and 1992 the Kenya Medical Research Institute (KEMRI) in collaboration with Oxford University conducted a series of integrated clinical and epidemiological studies on severe, life-threatening malaria. One major focus of work during this period was a case-control study of severe malaria presenting to hospital that hoped to establish host, parasite and environmental risk factors associated with the progression from asymptomatic infection in the community to mild disease as it presents at out-patient hospital departments and eventually to severe disease as found commonly admitted to paediatric wards. This progression can be seen in Figure 2.1 thus:

Figure 2.1 Malaria, the progression from time of infection to death

Uninfected person > Infected (Asymptomatic) —.....>Infected (Symptomatic) .....->Severe illness —>Death

In 1989 the Kilifi District hospital in-patient records were reviewed to establish where the greatest majority of malaria paediatric admissions came from. Over 50% of admissions originated from

seven sub-locations immediately surrounding the hospital at Kilifi town. This became known as the major catchment area for Kilifi District Hospital (KDH) and subsequently referred to as the study area. Not until 1990 did the entomological investigations within the context of the on-going epidemiological studies begin. By then it was possible to characterise the number of cases presenting from different areas of the study area.

Between December 1990 and November 1991, the villages of Sokoke and Kilifi town were selected for longitudinal vector studies because they represented a peri-urban densely populated community close to hospital (Kilifi town) and a rural scattered population area and far away from the hospital (Sokoke). These two communities were selected to evaluate vector sampling techniques and provide a baseline description of vector ecology in the area. These studies provided the basis for vector studies using a case-control approach. The entomological case-control was conducted within the much wider study area between August 1991 and June 1992.

2.2 Description of the Study Area

Kilifi District lies in the Coast Province of Kenya and borders the Indian Ocean to the east. To the south are Mombasa and Kwale districts and to the north is the Taita Taveta District while it borders

Lamu and Tana River Districts to the north-east. The District lies within 3" and 4" latitude (Figure 2.2 and 2.3).

Within the Kilifi District, four agroclimatic zones dictate the resources and potential land use (KDDP,1989). Zone I on the coastal plains is made up of a narrow belt lying below 30 m above sea level, extends to 10 km inland. Zone II, the foot plateau, varies from 60 m to 120 m above sea level and is made up of sandstones and impervious clays. It is characterized by a relatively flat surface, alternated with a number of hills. Zone III, the coastal range lies between 120 and 260 m. This zone has good rainfall and fertile soils whereas Zone IV, the "Nyika" plateau occupies the semi-arid to arid areas of the district. These zones are important with regard to breeding sites of the malaria vectors and hence its transmission.

Agricultural practices could greatly influence the breeding sites and the distribution of the malaria vector mosquito species. Agriculture in Kilifi is mainly based on subsistence crops that include annual crops, mainly, maize, cassava, cowpeas and sorghum. The few cash crops grown irt the district are perrenials and include coconuts, cashewnuts, mangoes, and citrus whose cultivation is unlikely to greatly influence malaria vectors. There are also sisal plantations in the area. Goats and sheep are common and nearly all households have poultry that could provide alternative sources of blood meals for

Figure 2.2

the study area

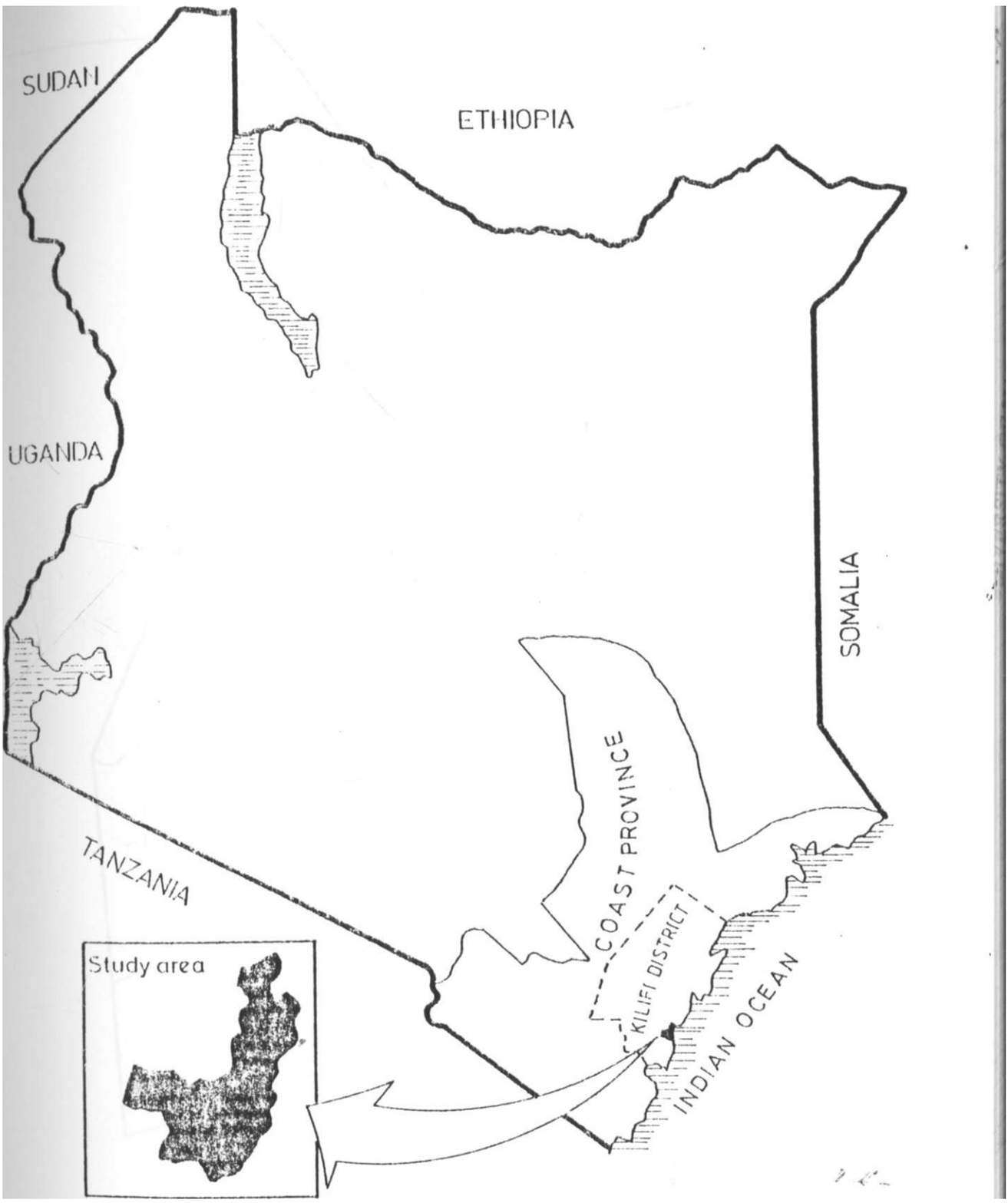


Figure 2.3 Map of the Kilifi District showing the position of th study area

malaria vectors in the area. Agricultural potential and growing periods of crops are largely decided by temperature, annual rainfall, and soil types. The soil types are important in malaria transmission studies because of different capacities to retain, hold and drain rain water where malaria vectors breed.

The local people are predominantly Giriama by tribe, a sub-group of the Mijikenda ethnic group. Their culture and recent migration patterns have been described in details by Parkin (1991). A fraction of resident population are engaged in wage employment in the only industrial factory, the Kenya Cashewnut industry, and in other manual jobs such as quarrying for coral blocks. Others leave their homesteads to work in hotel areas in towns like Mombasa and Malindi. At Kilifi town a larger "white-collar" sector of the community live employed in government services. Kilifi town is the administrative centre for the district. Population movements increases malaria transmission by either introducing nonimmune people into endemic areas or infected people into malaria-free areas.

The use of personal protection measures against insects by the population in this community is minimal. Snow *et al.* (1992b) reported an overall low use of mosquito nets and pyrethrum based mosquito coils in rooms where children sleep. However, the use of local repellents for control of mosquito nuisance was high (32.2%).

The local repellents including Baobab fruits, Mvumbani leaves, Mkilifi leaves, and coconut husks, are burned at night to drive away mosquitoes. Figure 2.4 shows the population pyramid of the resident population of the wider study area. About 50% of the population are below 15 years of age, typical of many developing country populations, indicating the population that is prone to malaria attack. The population pyramid showed a typical trend, the base, which included children aged 0-4 years, made up of 20% of the total population (N = 51,183) implying that more non-immune children area at risk of contracting malaria. Interestingly large populations of economically active males are away from the study, presumably in wage employment elsewhere.

The study area lies in Zone I (Figure 2.3). It extends from the Kilifi creek on the south, borders the Indian Ocean to the east, Arabuko-Sokoke forest to the North, and Mida creek to the north-east (Figure 2.5). Kilifi District Hospital (KDH) is situated about 60 km north of Mombasa, two government dispensaries, and at least 6 private clinics serves the population around it along the coast and inland. KDH provides Maternal and Child Health (MCH), Expanded Programme on Immunisation services (EPI), Out-patient department (OPD) and paediatric in-patient care services for the children of study area. Apart from the District hospital's outpatient service, dispensaries, and private clinics, the population more commonly also seek

population in the study area, W l a y i ^

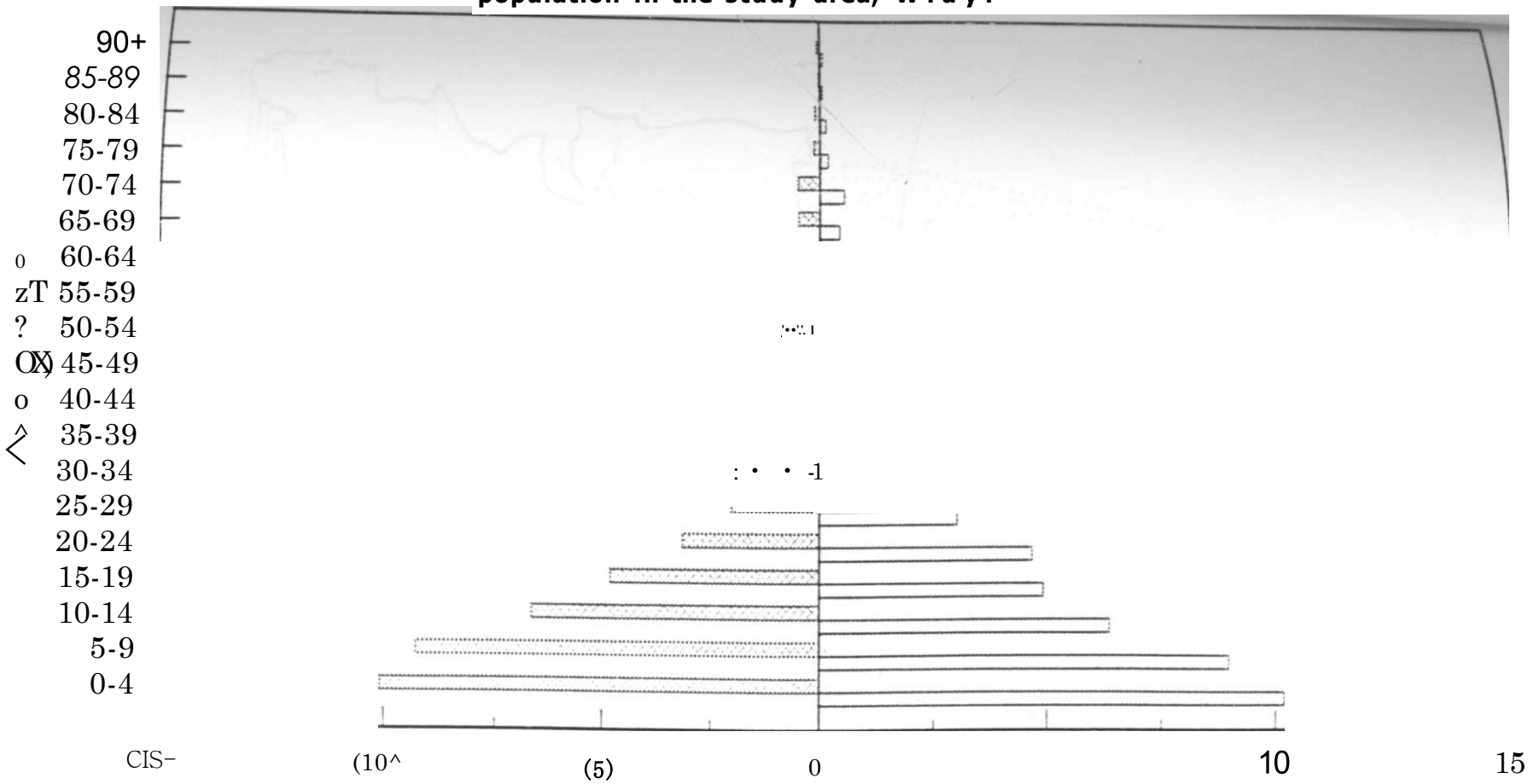
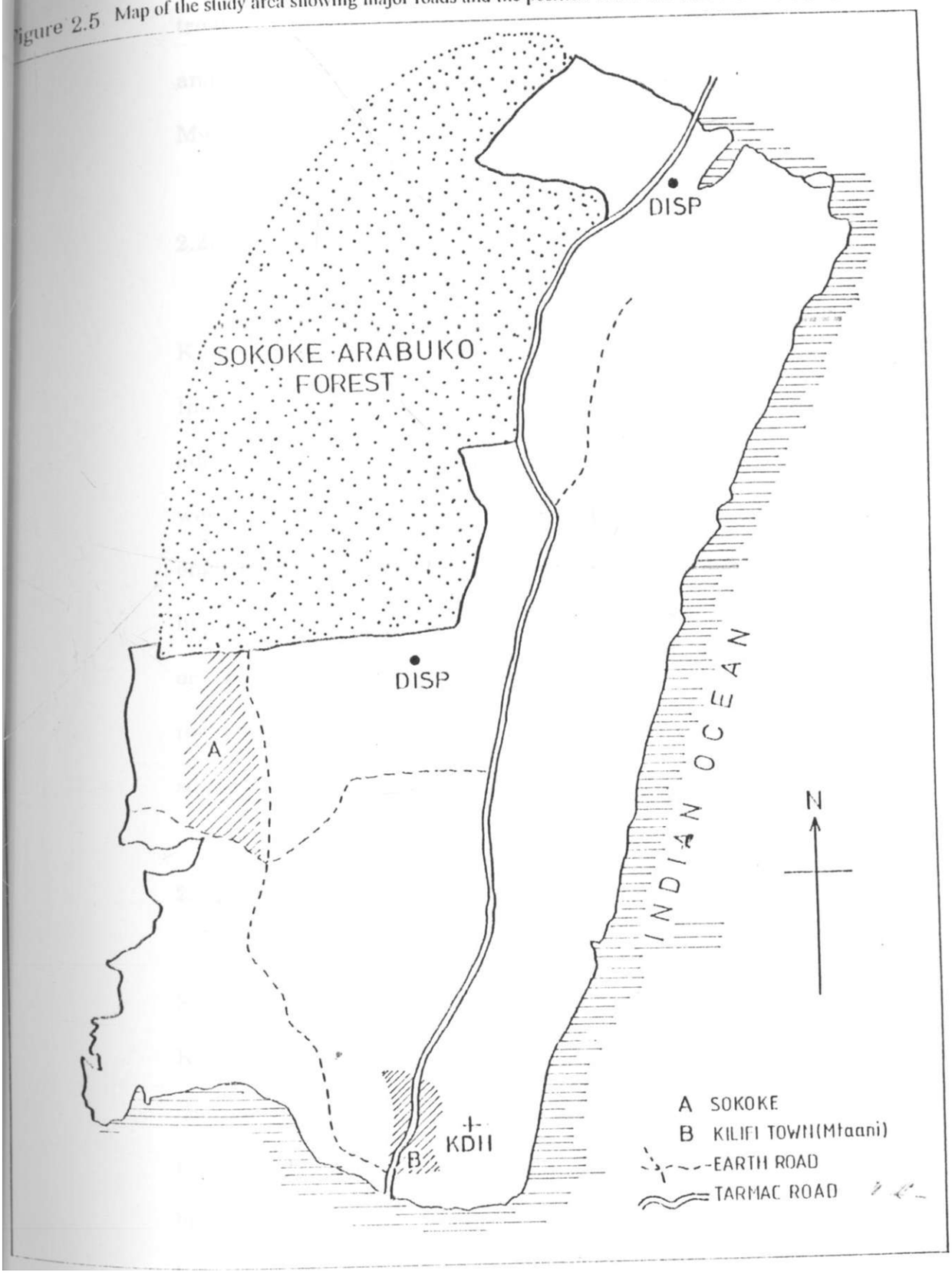


Figure 2.5 Map of the study area showing major roads and the position of the two sites within the area



treatment through home treatment with shop-bought antimalarial and antipyretics or from traditional herbalists (Snow *et al*1992b; Mwenesi, 1993; Mwenesi et al., 1994).

2.2.1 Kilifi Town

Kilifi town is a coastal urban centre lying along the shores of the Indian Ocean (Figure 2.6). The town hosts the district administration headquarters. These studies were conducted in the western half of the town locally called *Mtaani*, where residents are engaged mainly in local government administration and trade. The houses are constructed of mud and stone walls with coconut thatch or metal sheet roofs (Plate 1). The Kilifi town site was 4.0 km" and included 388 adjacent houses with a density of over 800 persons per square kilometre. The resident population in 1989 was 3696 people.

2.2.2 Sokoke

Sokoke is a remote rural settlement scheme located 15 km north of Kilifi town an<t20 km inland, bordering the Arabuko-Sokoke Forest (Figure 2.7). Houses in Sokoke are constructed mainly of mud with thatch roofs (Plate 2). It was common to see houses with incomplete, broken walls or people living in half-finished houses. Most of the houses have large unscreened eaves with large holes in the roofs

; **M.ip of Klllfl town (nitaani) showing household distribution and nu\|or road marks.**

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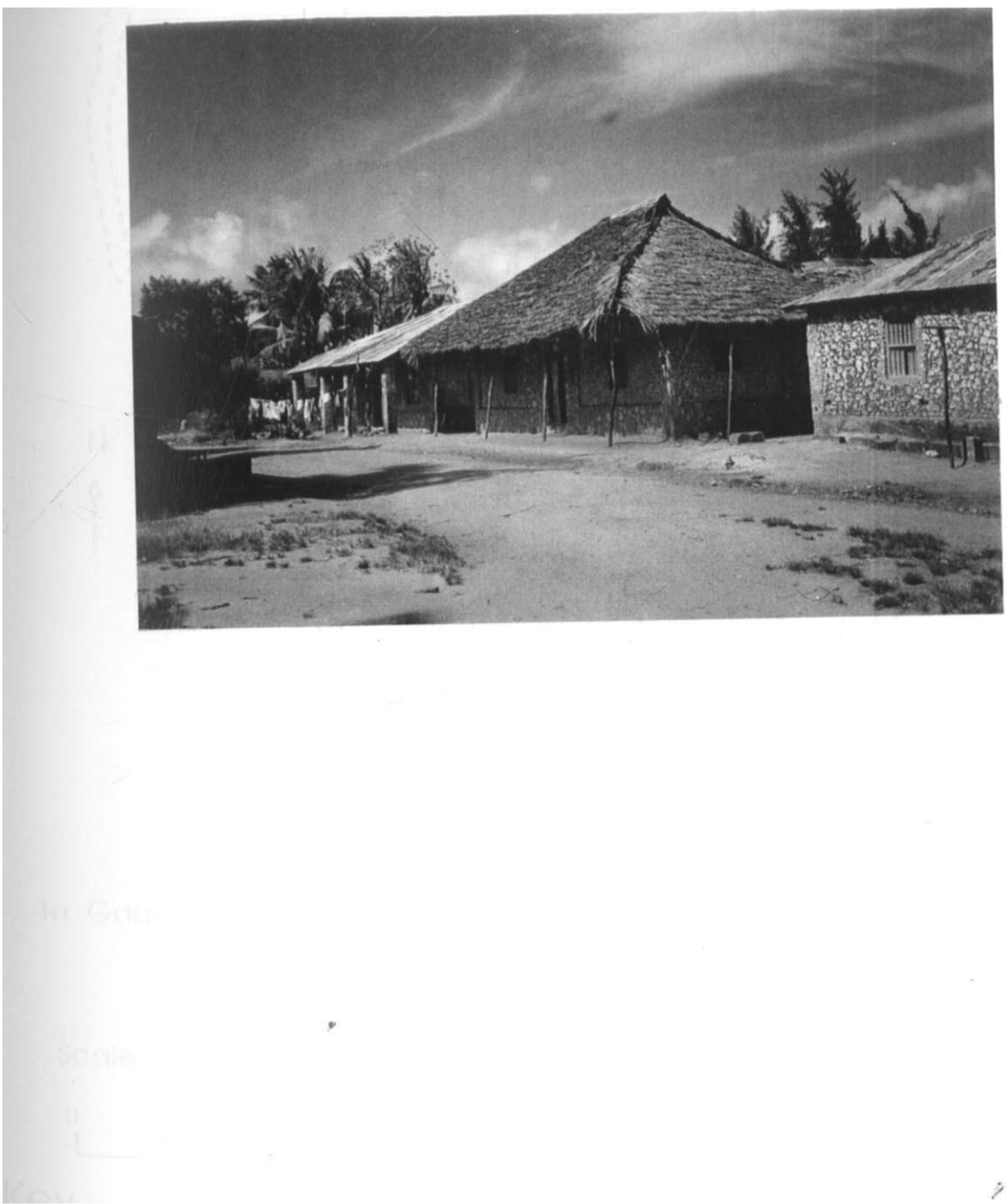
Plate 1 Kilifi town, typical "swahili" type of house from where mosquitoes were sampled



Map of Sokoke area showing the distribution of houses and earth roads transversing the area

0 0.5 1 Km

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Gy . Households

Major Roads

- - - Minor Roads

Plate 2 Sokoke, a typical house with mud and palm thatched roof from which malaria vectors were sampled

which would easily permit entry of malaria vectors. The study site of Sokoke was 7.9 km^2 in size and contained 228 scattered homesteads with an average of 3 houses per homestead. The resident population in 1989 was 6563 people providing a population density of 911 persons per square kilometre.

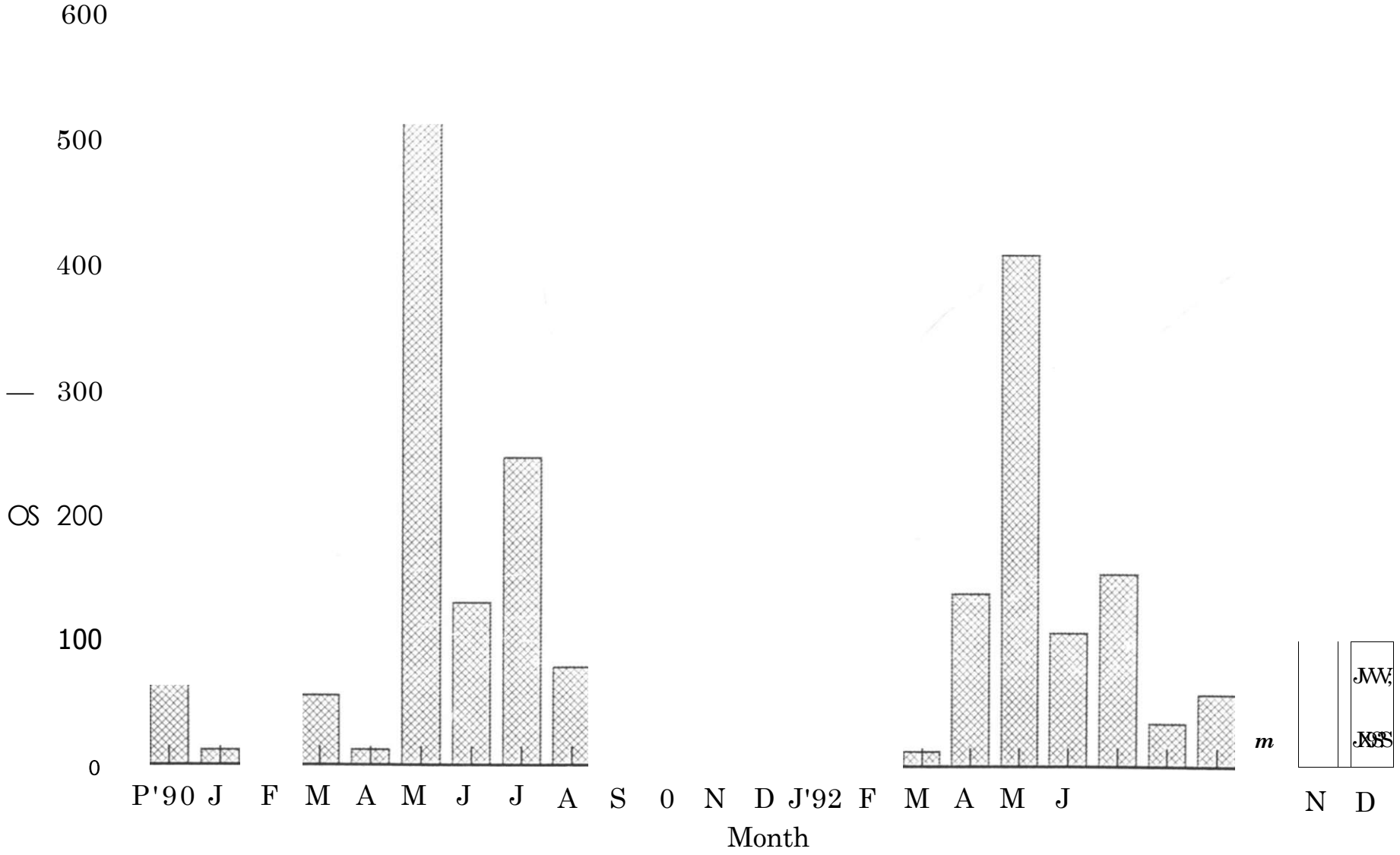
2.3 Climate

Meteorological data was obtained from Mtwapa Research Station 30 km south of the study area. This station had complete records for the full period of investigation and was within the same ecological zone as the study area.

2.3.1 Rainfall

Many insect vectors, including those that transmit malaria depend on surface water for breeding and therefore the rainfall regime of the study area was to be understood. The mean monthly precipitation was 99.9 ± 161.7 mm. The highest rainfall was recorded in May 1991 (563.2 mm) while the lowest was in January 1992 (0.3 mm) (Figure 2.8). The long rains fall between April and July with a peak in May, while the short rains fall between October and December although it rains almost every month of the year. The mean annual precipitation was 1,167.1 ± 45.5 mm (1991- 1992). A total of 1199.2

Figure 2.8 Monthly rainfall in milimetres, December 1990 to December 1992

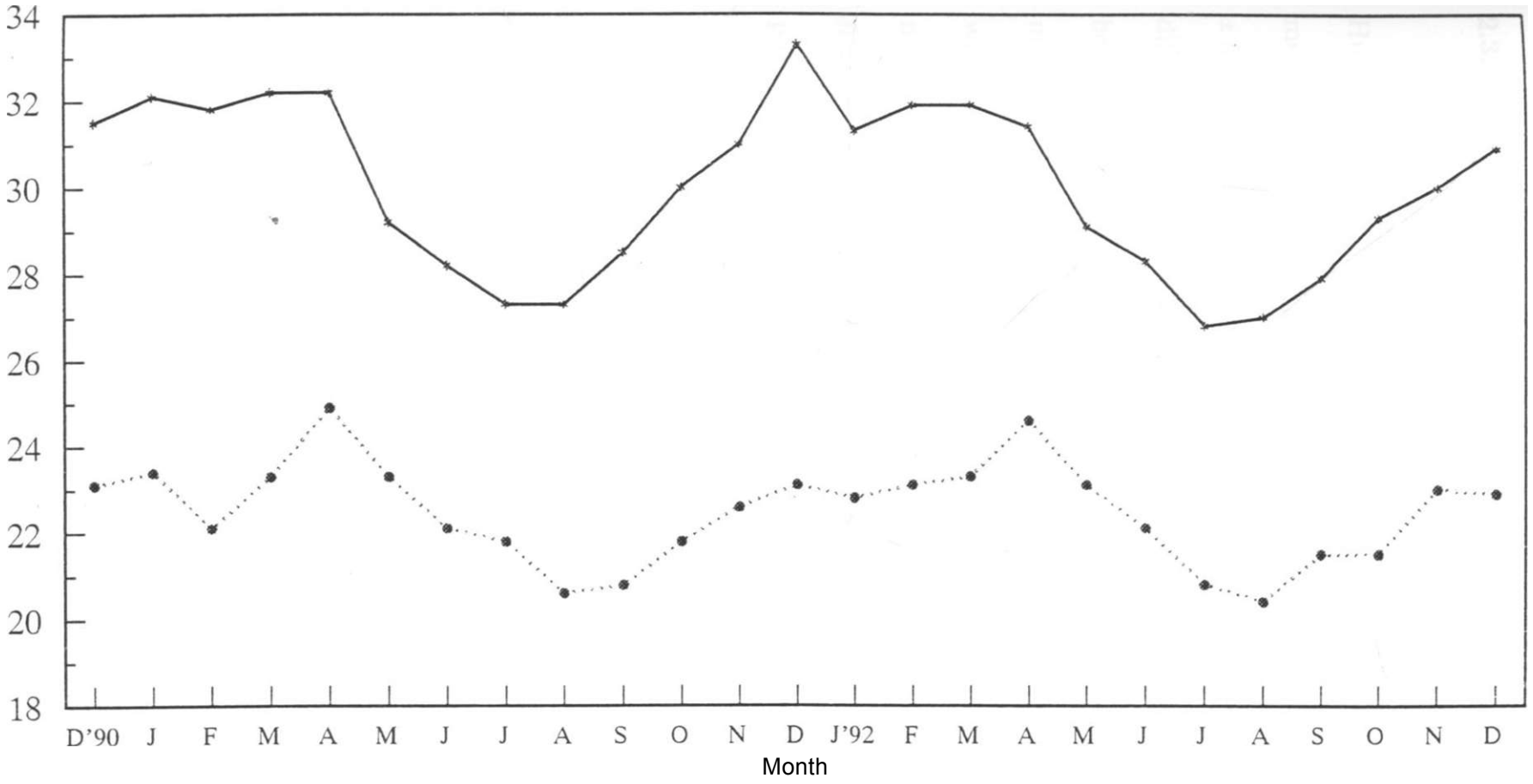


mm and 1134.9 mm of rainfall was recorded in 1991 and 1992, respectively. Monthly rainfall varied substantially from year to year as can be seen in Figure 2.8.

2.3.2 Temperature

An increasing temperature increases the growth of vector populations by shortening the intervals from oviposition to emergence and vice versa. Higher temperatures would also accelerate the evaporation rate thereby causing many mosquito vector breeding sites to dry up. During the study period the mean maximum and minimum monthly temperatures were 30.3 ± 2.1ⁿC and 22.5 ± 1.2"C, respectively. The hottest months were March 1991 (32.2°C) and December 1991 (33.3°C), while the coolest month was July 1992 (26.8"C). The mean annual difference of the maximum and minimum mean monthly temperatures was 7.8 ± 1.5"C with December 1991 having the largest difference of 10.4°C and July 1991 having the least (5.5"C). Figure 2.9 shows the mean maximum and minimum temperatures. The optimal range of temperature for most malaria vectors is 20-30"C (Wernsdorf & McGregor, 1986).

Figure 2.9 Monthly mean maximum and minimum temperatures at Kilifi, December 1990 to December 1992

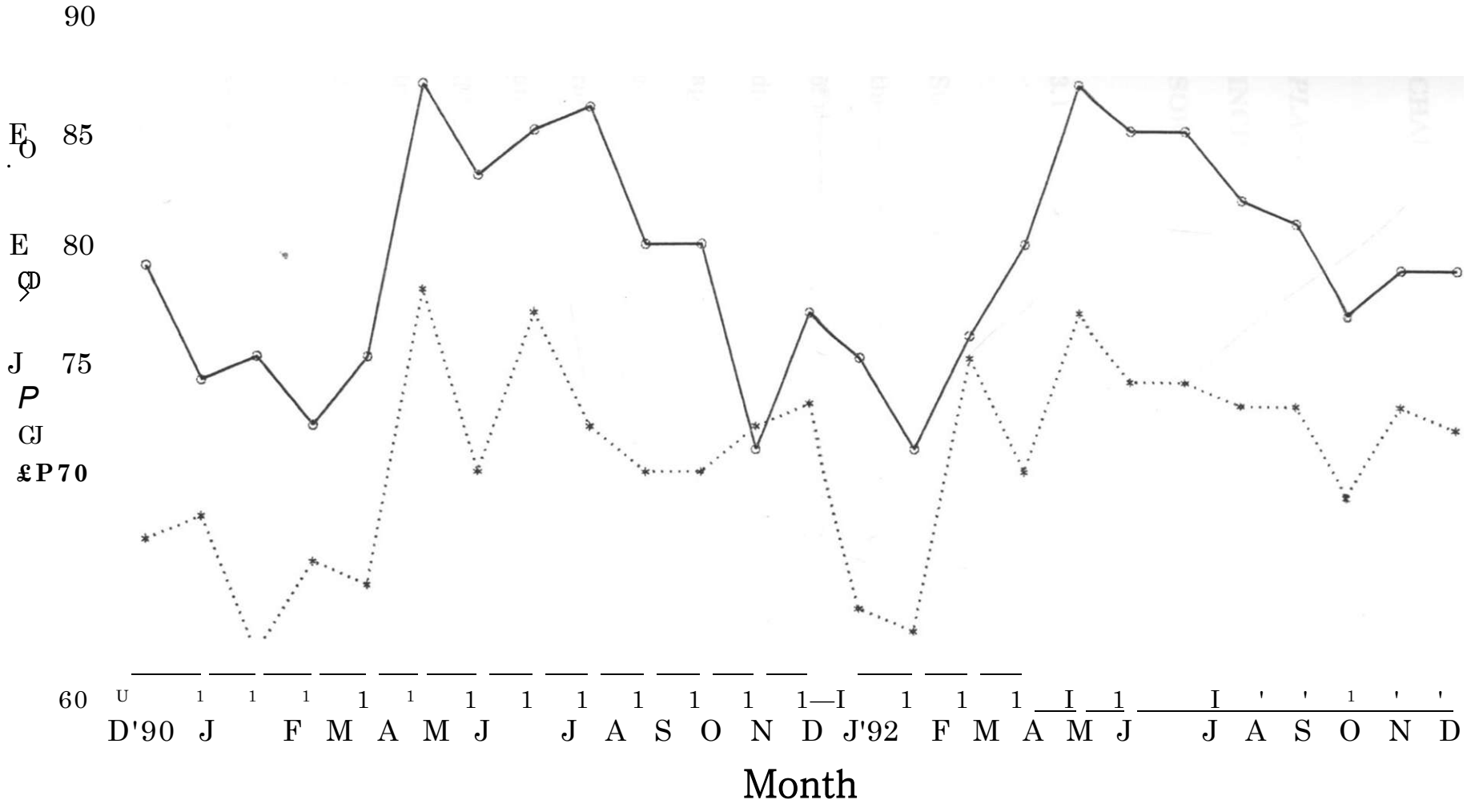


v

2.3.3 Relative Humidity

Humidity data were obtained daily at 0600 hour and 1200 hour. The mean maximum and minimum monthly relative humidity was 78.8 ± 5.6% and 70.3 ± 4.7%, respectively (Figure 2.10). The mean annual difference of the maximum and minimum monthly relative humidity between 0600 h and 1200 h was 8.2 ± 3.7% (range 1% -14%). The most humid month was in May 1991 (86.0% RH) and November 1991 was the least humid (71.0%). The optimal relative humidity for malaria vectors range from 70-80%. This type of relative humidity favours the breeding of mosquitoes and the development of the parasites (Wernsdorf & McGregor, 1986).

Figure 2.10 Mean monthly relative humidity at 0600 h and at 12.00 hours at Kilifi



CHAPTER 3

PLASMODIUM FALCIPARUM TRANSMISSION AND THE INCIDENCE OF SEVERE MALARIA INFECTIONS AT SOKOKE AND KILIFI TOWN ON THE KENYAN COAST

3.1 Introduction

Studies on the transmission of the malaria parasite *P. falciparum*, by the vector mosquito species are essential for the better understanding of the epidemiology or the factors that determine the frequency and distribution of malaria in human populations. Traditional approaches to the study of malaria epidemiology have tended to concentrate on relationships between the entomological inoculation rate, defined as the product of man-biting rate and sporozoite rate, and the dynamics of parasitization in the human population (Molineaux *et al.,* 1988). These investigations hope to elucidate the traditional malaria epidemiology with reference to malaria as a disease that can lead to death.

»

Most infected children living in malaria endemic areas are parasitized asymptomatically, that is, without showing signs of the disease and only a minority of the infections progress through symptomatic to become severe and life-threatening (Greenwood *et al.,*

1991; Marsh, 1992). This chapter will describe studies aimed at defining the principal vectors of malaria in this area of Kenya. Although malaria vector populations have been extensively studied around Lake Victoria in the western part of Kenya (Roberts, 1974; Beier *et al.,* 1990) and in the Mwea-Tebere irrigation scheme in the Central Province (Ijumba *et al.,* 1990), there have been no longitudinal studies of malaria parasite transmission on the coast since the studies of Wiseman *et al.* (1939). The main objectives of this part of the studies were to (a) define components of the entomological inoculation rate (EIR) in two areas of the Kilifi District and (b) relate the EIRs to rates of severe malaria in the two communities studied.

3.2 Materials and Methods

3.2.1 Vector Sampling"

Twenty houses were selected at Sokoke and at Kilifi town each for mosquito sampling. These study sites have been described in Chapter 2. The houses were chosen if the house contained at least one child less than 5 years of age, the target group. Mosquitoes were sampled in all the houses, once a week by aspiration, twice a week by elevated bednet collections, and inside 12 houses per site by the all-night human-bait collections for one complete year, from

December 1990 to November 1991. A total of 12 mosquito collectors were used. All were recruited from the study area and trained for 4 consecutive days.

3.2.1.1 Day Resting Indoor Collections

Day-resting indoor (DRI) collections were made for a standard time of 15 minutes in each house by 2 collectors from 0630 h to 1000 h by oral aspiration. A torch was used to search for mosquitoes from all the walls, under the beds, rafters and all other accessible places inside the house. Mosquitoes found were orally aspirated with an aspirator.

3.2.1.2 Elevated Bednet Collections

Elevated bednet (EB) sampling was conducted in the 20 houses per site two nights per week, on the same nights as biting collections. A bednet was suspended from the ceiling, with the bottom of the net elevated about 30 cm, and a volunteer from the household was asked to sleep under the net throughout the night. The placement and use of all nets was "inspected by field supervisors from 2000-2200 h. At 0600 h, the volunteer was asked to lower the net to touch the ground until the inside of the net was searched for resting mosquitoes by one of the team collectors before 0700 h.

3.2.1.3 All-night Human-bait Collections

This method that was intended to collect indoor biting mosquitoes involved one mosquito collector sitting inside a house with legs exposed to his knees and caught all landing mosquitoes from himself with an aspirator after locating the mosquito with flashlight. Captured mosquitoes were placed in paper cups and provided with sugar solution soaked in cotton wool. Collections were made for 30 minutes each hour from 1830 h to 0600 h, allowing the collectors to rest for the next 30 minutes. This duration was selected to ensure that any mosquito that landed to bite at whatever its active time of night was collected. The mosquito collectors rotated among all the 12 houses such that it took 12 catching nights for the same mosquito catcher to return to the same house. This was done in order to avoid biased catches from the same house by the same collector. Strict supervision was maintained throughout the night by a team of two technicians to make sure that the mosquito collectors did not sleep and a driver was available to transport those moving to houses far apart.

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3.2.2 Mosquito Identification and Processing

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All mosquitoes collected indoors by all the three sampling methods above were placed in cool boxes and transported to the Kilifi

Research Unit. In the laboratory, all mosquitoes were morphologically identified to species and classified by abdominal stages, and then prepared for sporozoite testing. Mosquitoes for species identification were collected from the two sites and from the much wider study area. Individual mosquito species were then placed in cryogenic vials, labelled with the house number, site, date and method of collection. The cryogenic vials were then placed in driarite for at least four days at room temperature to ensure that all the mosquito specimens were completely dry. The abdomen and other mosquito parts such as legs and wings were placed in 1.5 ml microfuge tubes and kept until tested by PCR. The head and thorax of the dried mosquitoes were left at room temperature until they were tested for sporozoites by ELISA method.

For half gravid females, ovaries were removed and placed in Carnoy's fluid (3:1, ethanol: acetic acid) and left to fix at room temperature for 24 hours before being placed at -20"C in a freezer. The samples were then air freighted to Rome to Professor Petrarca's laboratory for species identification. Many problems were encountered with preserved ovaries, including the unreadability of chromosomes for most of the specimens. It was therefore decided to use a few of the preserved specimens for PCR identification at Nairobi in order to give additional information to results done by cytogenetics in Rome by Petrarca.

3.2.3 Processing" of Individual Mosquitoes and DNA Extraction

Each mosquito was placed individually in a 1.5 ml eppendorf microfuge tube, and the sample incubated on ice for 20 minutes. One hundred microlitres (100 /jl\) of grinding buffer and the mosquito were homogenized in this solution with a grinder. The sample was then incubated at 65"C for 30 minutes. After cooling the tube on ice, 14 /xl of 8 M potassium acetate were added and the sample incubated on ice for 30 minutes. The contaminating proteins were removed by dentrifugation for 10 minutes at high speed. Two hundred microlitres of 95% ethanol were added to the supernatant to precipitate the nucleic acids and then incubated in freezer for 40 minutes. The precipitate was then collected by centrifugation for 20 minutes at high speed. The DNA pellet was washed two times in 70% (v/v) ethanol and 95% (v/v) ethanol, dried in the oven, resuspended in 100 /x 1 sterile water and stored at -80"C until polymerase chain reaction (PCR) was done.

The PCR was carried out in a total volume of 25 /xl, which contained 1 *ijl*\ of the DNA sample extracted as described above, primer at (in 2.5 fi\ lOx buffer, 0.1 /xl Mgcl,, 0.25 /xl .lxtrinton X, 8.7 /xl dTTP, 8.7 Ml dCTP, 8.7 pi dATP, 8.7 /xl dGTP) and 0.2 /x 1 of Taq polymerase (Perkin Elmer Cetus). The reactions were allowed to progress for 30

cycles, each consisting of DNA denaturation (94"C, 1 min), annealing of primer to sample DNA (60"C, 1 min) and chain extension from the primer (74"C, 1 min) in an automated programmable Thermal controller (MJ Research, Inc). The reactions were overlaid with 5 drops of mineral oil to prevent evaporation.

After the PCR was complete, the entire 25-/xl reaction volume was removed from under the mineral oil, mixed with a standard agarose gel loading buffer containing a small amount of bromophenol blue and electrophoresed through a 2.5% agarose-Tris-boric acid-EDTA gel containing ethidium bromide. The amplified fragments were visualized by illumination with short wave utra-violet light.

3.2.4 Sporozoite ELISA Testing

The method by Beier *et al.* (1987) for processing the malaria vector mosquitoes for sporozoite testing was employed. Individual mosquitoes were cut transversely at the thorax between the first and third pair of legs. The anterior portion was placed in a 1.5 ml microfuge tube'containing 50 ⇟\ boiled casein blocking buffer (BB) [1 litre of blocking buffer contains: 5.0 g, casein in 100 ml 0.1 N sodium hydroxide: 0.1 g, thimerosal: 0.01 g phenol red: 900 ml phosphate buffered saline powder (PBS), pH 7.4] with Nonidet P-40 (NP-40)(5 M1 NP40/1 ml BB). The samples were triturated manually with

plastic tissue grinders. After triturating, 200 of blocking buffer was added to each sample to bring the final volume to 250 n\ per mosquito sample. Samples of the mosquito triturates were stored at -20"C until they were tested.

All wells of a 96-well polyvinyl microtitre plate were coated with 0.1 fig *P. falciparum* capture monoclonal antibody diluted in 50 /jl\ PBS/well and incubated for 30 minutes at room temperature in subdued light. Before testing process, the triturates were removed from the freezer and left to thaw at room temperature. After 30 minutes, well contents were then aspirated and the wells filled with 200 /xl of blocking buffer to block the remaining active sites. After one hour, the blocking buffer was aspirated and 50 /jl\ aliquots of each homogenized mosquito triturate added to each well, leaving four wells for negative and two for positive controls. In the first well of the microtitre plate an aliquot containing 100 picograms (pg) of recombinant *P. falciparum* positive control CS protein ($R32tet_{32}$) in 50 ⁄j,\ blocking buffer was added and in the second well 10 pg CS protein/50 ⁄jl\ blocking buffer was also added as positive controls (Wirtz *et al.*, 1987, 1989). In the next four wells 50 *n* \ triturates of wild-caught males of *An. gambiae 8.1.* or *An. funestus* in blocking buffer was added as negative controls. After 2 hours of incubation, the mosquito triturate was aspirated and the wells washed two times with PBS-Tween 20 solution. The plates were shaken dry and 50 jA

of peroxidase conjugated monoclonal antibody added to each well. After one hour, the solution was aspirated and the plates washed three times with PBS-Tween 20 solution. 100 ^ul of peroxidase substrate was added to each well and the plates placed in the dark for 30 minutes after which samples were assessed visually for positivity (Beier & Koros, 1991b). Sporozoite ELISA solutions and step-by-step detailed procedures are shown in Appendix 2.

3.2.5 Demographic Data

Since these studies focused on children aged between 1 to 4 years of age, the population of these children in the two areas of study, Sokoke and Kilifi town, for 1991 was computed using data collected by the Population studies staff of the Kilifi Research Unit as part of on-going malaria study project at Kilifi and those from the Central Bureau of Statistics (CBS) from the 1989 National Census (CBS, 1994). Site specific census data on the 1 to 4 year old populations in 1989 from each of these two sites were amalgamated and corrected using the estimated growth rate of 3.92% per annum to provide denominators for the childhood populations in 1991. Using the estimated growth rate, the childhood populations for 1991 was estimated to be 456 and 479 children aged 1 to 4 years old in Sokoke and Kilifi town, respectively, representing the childhood populations at risk of developing severe malaria.

3.2.6 Clinical Classification of Malaria and Surveillance Methods

Malaria infections in children aged 1 to 4 years from the two study sites were detected and categorised as follows. Children infected with *P. falciparum* were identified through community-based malaria prevalence surveys in April and in October 1991. Blood smears were taken from children aged 1 to 4 years and stained with 3% Giemsa. Each smear was examined for 200 high-power fields per Giemsa stained thick blood film. Records were made of all asexual *Plasmodium* species seen and sexual stages. The second category of children were selected from the out-patient at the Kilifi District Hospital with primary diagnosis of malaria and who had parasitaemia >5,000 per jiil. All had to be resident of the two study areas. The third category consisted of children suffering from severe malaria. Such cases were detected by systematic screening of all admissions to the paediatric ward of Kilifi District Hospital. All the children admitted to the paediatric ward during the time of this study were examined for malaria parasites and haematologic analysis from a finger prick blood sample. Malaria was diagnosed following a review of clinical, parasitologic^, haematologic, and other laboratory tests. Cases of severe malaria were defined as a child who had a primary diagnosis of malaria with at least one of the following signs: coma (unable to localise to pain), haemoglobin less than 5.1

g/dl with a peripheral parasitaemia of greater than 10,000 /j\\ prostrated (unable to sit or stand unsupported or take oral medication); 2 or more generalized convulsions within 24 hours prior to admission; hyperparasitaemia (20% or more infected red cells); or death confirmed as being due to malaria without any of the aforementioned complications.

3.2.7 Indices of Malaria Parasite Transmission

The intensity of malaria parasite transmission was determined as the product of the "human-biting rate" and the "sporozoite rate" which has already been defined above as the entomological inoculation rate (EIR) (Garrett-Jones & Shidrawi, 1969) was adopted in these studies. Human-biting rates *(ma)* is the number of mosquitoes per human-night determined through all-night human-bait catches. Sporozoite rates (s) is the proportion of anophelines testing positive for circumsporozoite protein based on ELISA testing of mosquitoes obtained from all collection methods. Thus; EIR = *mas.* The EIR, a standard measure of transmission intensity is expressed as number of infective bitgs each person receives per night, and is also a direct measure of the risk of human exposure to the bites of infective mosquitoes (Molineaux *et* a/., 1988).

3.2.8 Relationships between Sporozoite Inoculations and Symptomatic Disease

Another index used to describe the intensity of malaria parasite transmission was the inoculation to disease ratio. It was derived as the average number of sporozoite inoculations per disease event calculated as the yearly EIR times the children population size, divided by the number of children per infection category. For example, an inoculation to disease ratio of 10:1 indicates that there were an average of 10 sporozoite inoculations for each disease event. This ratio explains the relationship between the number of infective bites or biting intensity producing infection, illness, and the severity of the disease.

3.3 Results

The monthly mosquito collections which lasted for 12 months from Sokoke and Kilifi town using various sampling methods yielded 701 anopheline specimens which comprised five species, namely, *An. gambiae s.s., An. arabiensis, An. merus, An. funestus* and *An. coustani.* However, in analyzing the role of these species in the transmission of malaria, the first three species were lumped together and treated as a single group of *An. gambiae s.l.*

Table 3.1 shows that the *An. gambiae* complex which was the most predominant in the all-night human-biting collections representing 88% and 99% at Sokoke and Kilifi town, respectively, of the total anopheline collected, was the only one which yielded malaria parasites (P. *falciparum)* infections at the rate of 4.1% and 2.2% at Sokoke and Kilifi town, respectively. Neither, the *An. funestus* nor the *An. coustcini* species contained any malaria infections. These findings led to conclusion that the species in the *An. gambiae* complex were the most likely ones to be involved in the transmission of malaria at the coast where they occur in large numbers for the greater part of the year as shown in Figures 3.1a and 3.1b.

The polytene chromosomal analysis yielded 7 (58%) *An. gambiae s.s.,* 3 (25%) *An. arabiensis,* 2 (17%) *An. merus*, thus revealing the number, kind and the predominance of the species comprising the *An. gambiae* complex found in the two study sites. The following polymorphisms were scored for *An. gambiae s.s.;* 2R +/+, 2L +/+, 2L +/a, and 2L a/a, while *An. arabiensis* had 2R +/+, 2R +/b, 2R b/b, and 3R +/+. *Anopheles merus* had standard polymorphism. The PCR yielded 51% (3V) *An. gambiae s.s.,* 45% (33) *An. arabiensis,* 1.4% (1) *An. merus* and 2 were hybrids.

Table 3.2 shows that the human-biting rates of An. *gambiae s.l.,* occurs throughout the year, and that *P. falciparum* infection rates

Table 3.1 Summary of anopheline mosquito collections and *Plasmodium falciparum* sporozoite enzyme-linked immunosorbent assay results for Sokoke and Kilifi town (December 1990 to November 1991)

Site	Species	No. of mosquitoes per sampling technique*			*P. falciparum* infection	
		NBC	EB	DRI	No. tested	% positive
Sokoke	*An. gambiae s.l.*	286	111	94	491	4.1
	An. funestus	41	11	5	57	0.0
	An. coustani	12	0	0	12	0.0
Kilifi	*An. gambiae s.l.*	98	24	16	138	2.2
	An. funestus	1	1	0	2	0.0
	An. coustani	1	0	0	1	0.0

* NBC = night biting collections, 1,030 human nights; EB = elevated bed nets, 3,814 trap nights; DRI = day resting collections inside houses, 1,880 collections.

Table 3.2 *Monthly* **summary** *of Anopheles gambiae s.l.* **human biting rates,** *Plasmodium falciparum* **sporozoite rates, and the number of severe malaria cases admitted to Kilifi District Hospital from December 1990 to November 1991,** for the study sites of Sokoke (S) and Kilifi town (K)

	No. of human nights		Daily human biting rate		*P. falciparum* sporozoite rate (N)		No. of severe cases*	
Month	S	K	s	K	S	K	S	K
Dec	30.0	29.5	0.01	0.20	11.1 (9)	0(8)	2	1
Jan	52.5 '	54.0	0.15	0.19	18.2 (11)	5.6 (17)	1	0
Feb	36.0	35.5	0.03	0.03	0 (1)	0 (1)	0	0
Mar	47.5	42.0	0.00	0.00	0 (0)	0 (0)	0	0
Apr	47.0	38.0	0.09	0.03	0 (5)	0 (2)	0	0
May	53.5	51.0	0.52	0.16	0 (33)	0(10)	0	0
Jun	47.0	42.0	1.96	0.41	4.5 (177)	0 (25)	2	0
Jul	37.5	43.5	0.75	0.41	8.3 (36)	5.9 (28)	3	0
Aug	48.5	45.0	1.88	0.53	2.5 (162)	0 (31)	1	0
Sep	42.5	49.5	0.64	0.14	3.9 (51)	11.1 (9)	1	1
Oct	36.0	43.5	0.06	0.00	0 (3)	0(3)	0	0
Nov	40.5	37.5	0.05	0.16	0 (3)	0(7)	1	0

' From the population of children 1 to 4 years of age, in Sokoke (n = 456) and Kilifi town (n = 479)

Figure 3.1a Monthly distribution and intensities of human-biting rates for An. gambiae s.l. and An. funestus at Sokoke

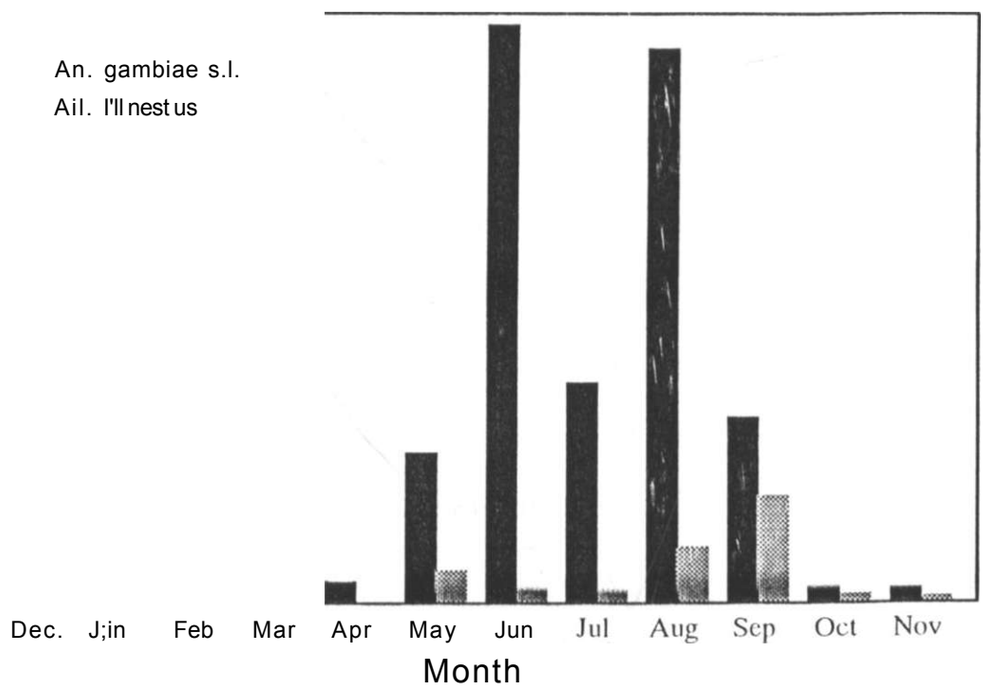
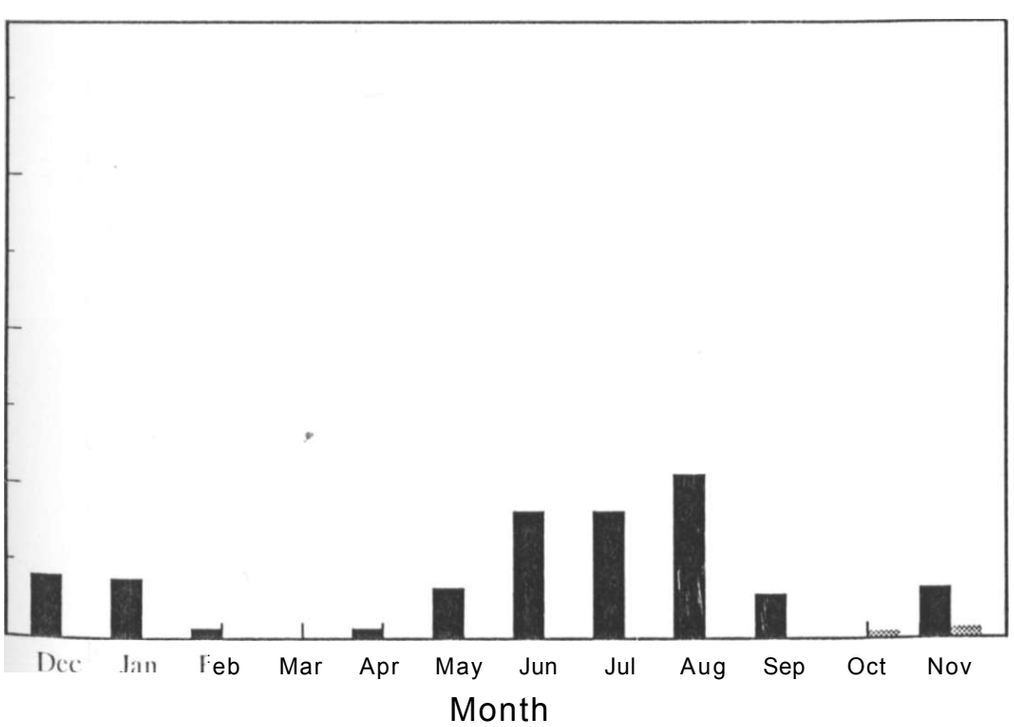


Figure 3.1b Monthly distribution and intensities of human-biting rates for An. gambiae s.l. and An. funestus at Kilifi town



and the number of severe *P. falciparum,* infections were detected during six months in Sokoke and during three months in Kilifi town, indicating an association between biting rates and *P. falciparum* infection rates. The all-night human-biting collections shown in Figures 3.1a and 3.1b indicated that the predominant vector *An. gambiae* complex was present during all months except March in Sokoke, and in all months except March and October in Kilifi town, leading to the conclusion that this group of *An. gambiae* complex is important in malaria parasite transmission.

Figure 3.2a and 3.2b shows that Sokoke residents received 0.01 to 0.09, an average of 0.05 infective bites by the *An gambiae* complex per night, during the 6 months when transmission was detectable. The period between June and September had the highest inoculation rates, thus being the most dangerous part of the year for malaria attacks. The children born in Sokoke environment were thus inevitably challenged by multiple *P. falciparum* infections that caused a yearly rate of severe malaria of 24.1 per 1,000 children (Figure 3.2b). Seasonal increases in the incidence of severe cases corresponded ctosely with increases in vector densities and associated EIR's during the period of June to September.

Figure 3.3a and 3.3b shows that the residents of Kilifi town received 0.01 to 0.02, an average of 0.015 infective bites per human per night

Figure 3.2a Monthly entomological inoculation rates (EIR) for Sokoke

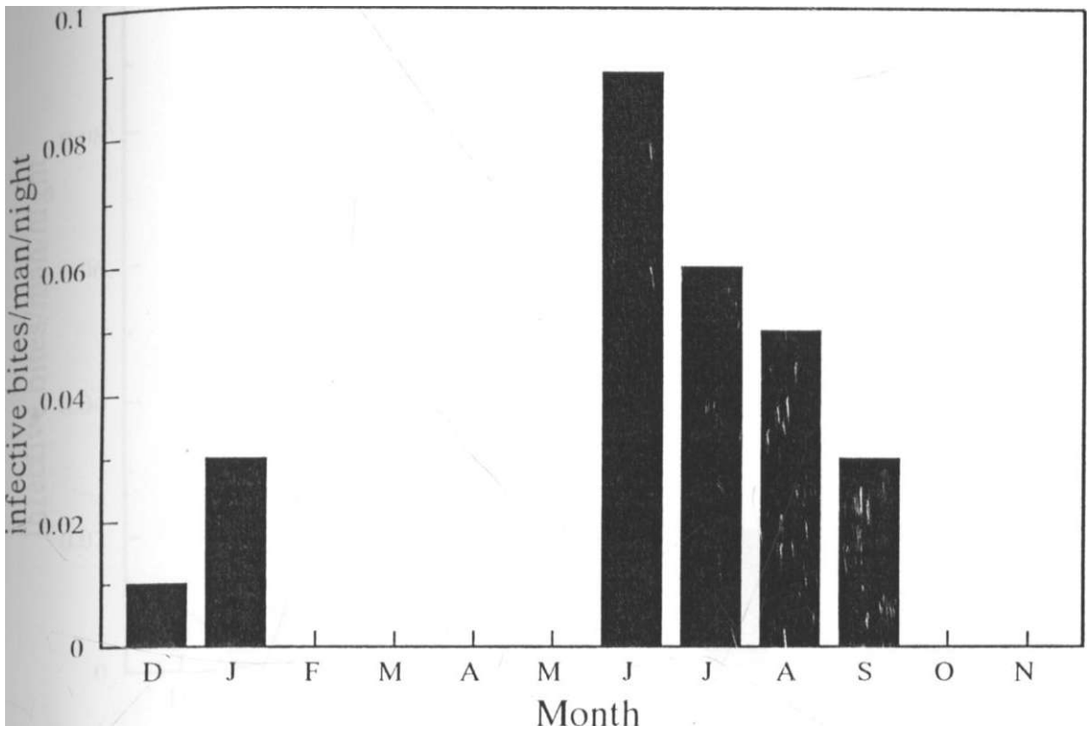


Figure 3.2b Monthly incidence rates of severe malaria infections in children 1-4 years of age at Sokoke

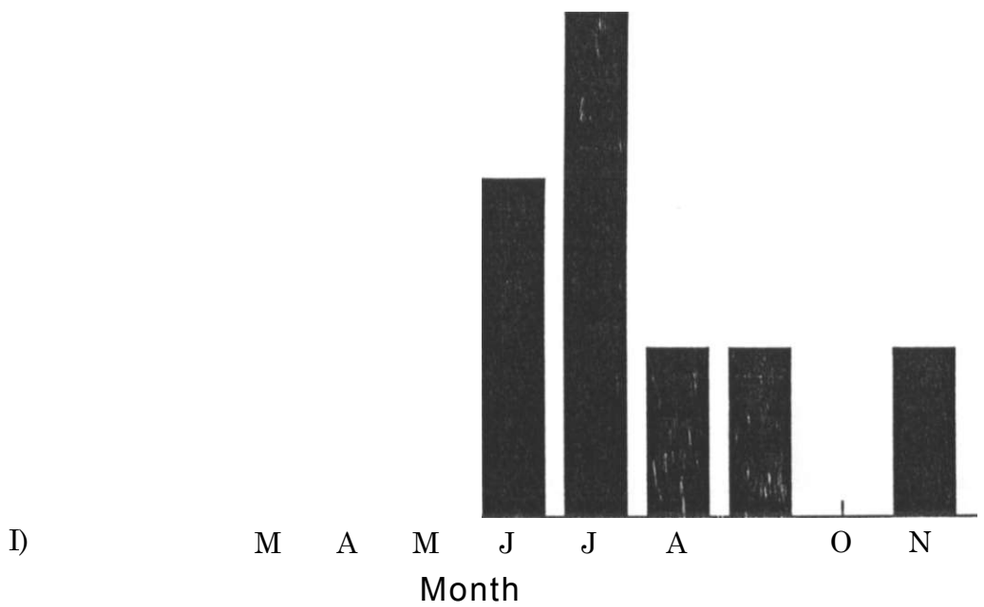


Figure 3.3a Monthly entomological inoculation rates (EIR) at Kilifi town

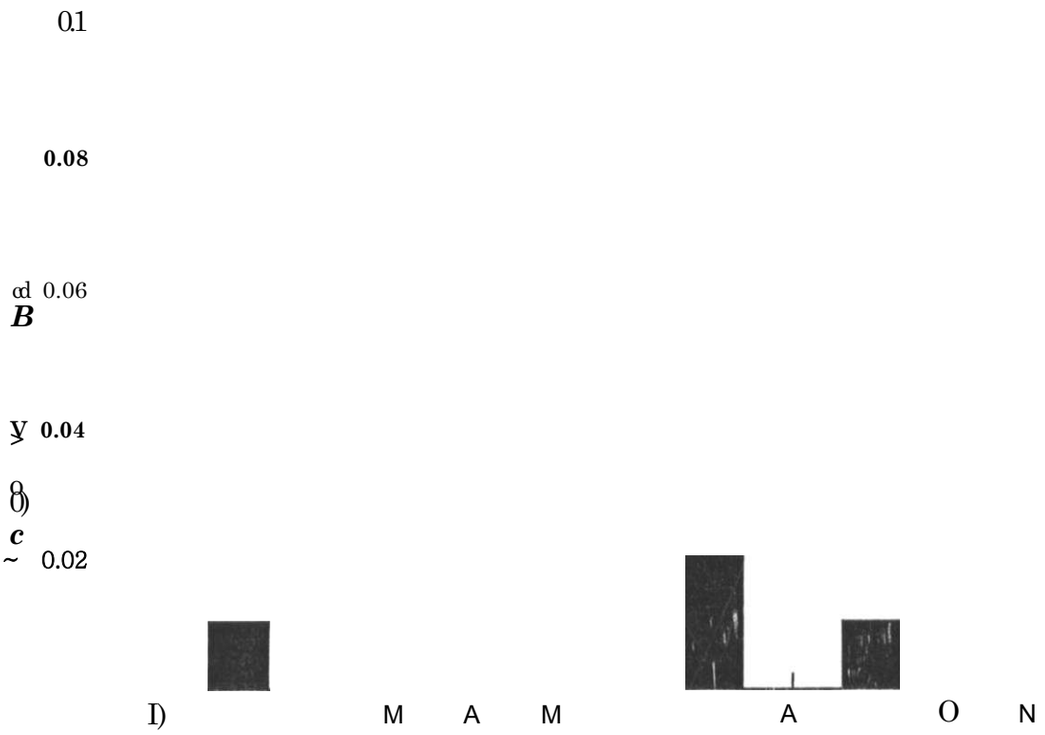
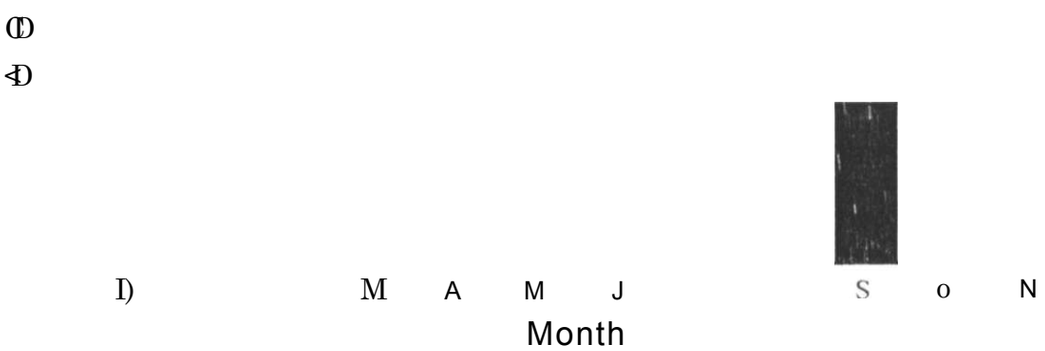


Figure 3.3b Monthly incidence rates of severe malaria infections in children 1-4 years of age at Kilifi town



during the 3 months when transmission was detectable. The Lower inoculation rates indicates that children residents of Kilifi town were less challenged by *P. falciparum* infections that caused an annual rate of severe malaria of 4.2 per 1,000 children (Figure 3.3b).

All children were considered at risk of developing infections because the average number of infective bites per person per year was 8.0 in Sokoke and 1.5 in Kilifi town. Table 3.3 shows that asymptomatic *P. falciparum* infections were detected in 44.6% (25/56) and 17.1% (6/35) of the children aged 1 to 4 years in Sokoke and Kilifi town, respectively. Interestingly, 32% and 16.7% of the infected children in Sokoke and Kilifi, respectively, had parasitaemias >5,000/ju,l but no apparent clinical symptoms. During the study period, 31 of 88 recruited children in Sokoke reported to the District Hospital with febrile illness and high parasitaemias, compared with 15 of 81 children in Kilifi town. Severe malaria infections were detected in 11 of 456 (24.1 per 1,000) children in Sokoke and in 2 of 479 (4.2 per 1,000) in Kilifi town. Inoculation to disease ratios indicated that one in every 23 and one in every eight sporozoite inoculations produced febrile illness associated with high parasitaemias (mild disease) in children residing in Sokoke and Kilifi town, respectively (Table 3.3). However, only 1 in 332 inoculations in Sokoke and 1 in 359 inoculations in Kilifi town produced severe disease.

Table 3.3: *Plasmodium falciparum* infections, clinical illness, and inoculation to disease ratios for children, 1-4 years of age, in Sokoke and Kilifi town, Kilifi District, Kenya (December 1990 to November 1991).

Malaria classification	Study site		Inoculation to disease ratio	
	Sokoke	Kilifi town	Sokoke	Kilifi town
P. falciparum prevalence*				
April 1991	21.0% (13/62)	2.0% (1/51)	ND	ND
October 1991	26.8% (15/56)	17.1% (6/35)	ND	ND
Either survey	44.6% (25/56)	17.1 (6/35)	ND	ND
Clinical illness				
infected, symptomatic, parasitaemia s>5,000/^f'	352 per 1,000	185 per 1,000	23:1	8:1
Severe malaria^	24 per 1,000	4 per 1,000	332:1	359:1

' Infections detected in cohorts of children sampled in April and October 1991. The category "Either survey" shows the overall percentages of children with infections during the two prevalence surveys.

" Infections in children reporting to the outpatient clinic of the Kilifi District Hospital.

? Children with severe malaria, as defined in methods, admitted to the Kilifi District Hospital.

3.4 Discussion

This study demonstrates surprisingly low levels of *P. falciparum* transmission at two ecologically distinct sites in Kilifi District, Kenya, an area usually considered to be typical of high, stable endemicity (Roberts, 1974). The predominant vectors in the *An. gambiae* complex, occur throughout the year, with peaks of population abundance coinciding with seasonal rains. Findings that residents are exposed to fewer than 10 infective bites per year emphasize that transmission intensity on the Kenyan Coast is certainly much lower than in the other main areas of stable endemicity in Kenya, such as western Kenya where up to 300 infective bites per year have been documented (Beier *et al..,* 1990). In the Kilifi sites, the rate of parasite transmission during most of the year was below a threshold where even intensified mosquito sampling was not sufficient to adequately measure transmission. Clearly it is obvious that the reliability of sporozoite rates depends on sample size and hence EIRs in areas of low vector abundance needs to be taken with caution.

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The polymorphisms of the *An. gambiae* resembled very much those observed in the Comoro Islands, on the northern coast of Mozambique and on the west and east coasts of Madagascar (V. Petrarca, personal communication). However, this differed with

those from the Kisumu area as there were no polymorphisms on the arm 2R, otherwise everything was standard.

Inversion 2La is associated with higher *P. falciparum* infection rates (Petrarca & Beier, 1992) and this inversion is also associated with climate, biting and resting behaviour (Coluzzi *et al.*, 1977), seasonality (Rishikesh *et al.*, 1985) and morphometric parameters (Petrarca *et al.*, 1990). In Kilifi, the absence of inversion 2Rb in *An. gambiae* is remarkable in that it would be possible to understand more precisely the influence of inversion 2La on malariometric parameters. These results indicate that the inversion polymorphism of the *An. gambiae s.s.* and *An. arabiensis* is more "simplified" than that observed in the Kisumu area by Petrarca & Beier (1992). However, the presence of *An. merus* in the complex is remarkable and it would be interesting to know the participation of this species to the vectorial system, especially in sites where animals are few and scattered.

Nevertheless, spatial and temporal patterns of severe disease were associated witlT intensities of parasite transmission. The EIR was 5 times greater in the rural area of Sokoke than in the urban area of Kilifi town, and this corresponded to a 6-fold increase in the presentation of severe disease at Kilifi District Hospital. Clearly, not all severe disease events were identified through hospital

surveillance (i.e., true rates can only be higher). Access and utilisation patterns will influence this measure of severe disease rates in a community. The only true marker of community-based severe disease is malaria-specific mortality. Verbal Autopsy (VA's) have been used in the past (Gray *et al.,* 1990) to describe this but they have recently been shown to be inefficient at uniquely defining malaria mortality (Snow *et al.,* 1992a). Even though residents of Sokoke had to travel 20 km or more to the District hospital, rates of severe malaria in Sokoke were still higher than in Kilifi town due to poor access to hospital.

Despite the limitations discussed above this was one of the first attempts to evaluate the incidence of severe malaria in relation to standard indices of parasite transmission by vector populations. In Africa, the only comparable data are those from The Gambia, where transmission is also low but markedly seasonal. The incidence of clinical disease (i.e., defined as fevers associated with parasitaemias £5,000 per in children 0-9 years old) was 3 per 1,000 in an urban area where annual EIR's were less than 1 (Lindsay *et al.,* 1990). Corresponding studies in rural areas west of Farafenni detected fever and high parasitaemia incidence of 175 per 1,000 (Snow *et al.,* 1988b), where site-specific EIR's ranged from 4 to 24 infective bites

> *4'.*

during the transmission season (Lindsay *et al.,* 1989). As in Kilifi, these data from The Gambia indicate that even slight increases in

EIR's can be associated with pronounced increases in mild clinical disease.

Unlike mild disease the rates of severe malaria between the two sites were not as disparate. Infact very similar numbers of inocula resulted in one case of severe disease (Table 3.3). There are difficulties in interpreting this data as already discussed and the confidence intervals on these rare events are large. Nevertheless, assuming that severe disease rates were similar, raises the possibility that above a certain threshold of challenge which must be low, increases in EIR are associated with bigger increases in mild rather than severe disease. This would suggest that factors other than transmission intensity *per se* are related to rates of severe malaria.

The as yet undefined relation between transmission intensity and severe malaria must be influenced by natural immunity. In this study area, there is minimal severe-related disease in children greater than 5 or 6 years of age (Snow *et al.*, 1993) or in adults (KM, unpubl. data). Thus, even EIRs less than 10 per year are sufficient to produce the characteristic picture of natural immunity against mild disease. In many respects, the malaria situation in Kilifi should be considered unstable with such low EIRs. Thus, the situation in Kilifi is very similar to that reported in The Gambia and both

illustrate how natural immunity can develop and operate even under conditions of extremely low transmission.

Under conditions of low-level transmission, even limited numbers of sporozoite inoculations can produce illness in children that requires outpatient care. For example, as few as one in eight sporozoite inoculations produced febrile illness and high parasitaemias in children residing in Kilifi town. Comparable estimates in the rural area of Sokoke were higher (1 in 23), probably because it was more difficult for families in rural areas to bring affected children to the out-patient clinic and many children are probably treated at home or they have a greater degree of acquired immunity being under areas of higher transmission.

Evidence that only 1 in 300 or more infective bites leads to severe infections certainly highlights the possible role of host- and parasite-related determinants of severe disease (Greenwood *et al.*, 1991; Marsh, 1992), but it also raises further questions regarding variation in both the quantity and quality of sporozoites inoculated by blood-feeding mosquitoes (Beier *et al.*, 1992). Variations in sporozoite dose, a possible contributing factor for severe disease (Greenwood *et al.*, 1991; Marsh, 1992), are difficult to evaluate even by *in vitro* methods (Beier *et al.*, 1991c) because sporozoite rates are less than 5% in Kilifi. At this point, there is no evidence that sporozoite loads of

naturally infected *An. gambiae* s.l and *An. funestus* in Kilifi (EWK, unpul. data) are any higher than those reported from vectors in Kisumu, western Kenya (Beier *et al*., 1991b).

The current basis for vector control in Africa is that malaria specific mortality can be reduced by effective use of residual insecticides (Payne *et al,* 1976) or insecticide-impregnated bednets (Alonso *et al,* 1991). Reducing levels of parasite transmission by vector populations through control efforts should yield corresponding decreases in disease. Interestingly, this study in Kilifi provides an example of how high rates of severe disease can be associated with even low-level transmission. In perspective, a yearly incidence of severe disease of approximately 25 per 1,000, as in the rural site of Sokoke, would mean that at least 10% of the children will experience severe malaria infections by the age of 5 years. This situation in Kilifi exists even though individuals receive an average of less than 10 infective bites per year, extremely low-level transmission by African standards. For endemic conditions of stable malaria like this in Africa, it will be important to determine the long-term impact of carefully executed vector control -r efforts on the incidence of severe disease and associated mortality.

CHAPTER 4

ENTOMOLOGICAL RISK FACTORS FOR CAUSING SEVERE MALARIA.

4.1 Introduction

Since in endemic areas most malaria infections are asymptomatic, the major question has been "why is it that only a few of the infected persons develop severe and life-threatening malaria?" (Greenwood *et al,* 1991; Marsh, 1992). To answer the question, it has to be borne in mind that relative to the control of the infection, individuals with severe malaria require extensive supportive health care. This is why nearly half of those who develop the severe disease in the rural areas of Africa die due to lack of well equipped health services to deal with the accompanying complications (Greenwood *et al,* 1987; Greenberg *et al,* 1989; Snow *et al,* 1994a). Factors that may cause the onset of the clinical phase of malaria may, as already stated be related to the human host, the parasite, the vector, or other unknown environmental parameters. However, the relative importance of any of these factor^ has so far been unclear.

In this Chapter, investigations were conducted on the vector factor ***' 4'.*** through a case-control study design to evaluate the role of the anopheline mosquitoes in the occurrence of clinically severe malaria

in children residing in Kilifi District, Kenya. Secondly, determining how children with severe disease differed in their exposure history compared with infected children who did not develop severe disease (symptomatic) provided a basis for evaluating the human host factor and environmental parameters of the households to identify and quantify exposure risk, thereby evaluating the epidemiology of severe malaria at the household level. It was decided that comparing uninfected or infected "well" children in the community with severe disease cases would not be of interest as clearly the development of disease will be related to some exposure to infection. A far more important question was whether disease outcome varies with exposure levels. To achieve these objectives mosquito sampling was done from wider study area where all the severe malaria cases originated.

4.2 Materials and Methods

4.2.1 Study Population

From recent retords of past studies, the human population in the study area was estimated at 63,834 people, of which 12,000 were children less than five years of age (Snow *et al.*, 1993). Approximately 200 cases of severe malaria were annually admitted to the Kilifi District Hospital (KDH).

The study population consisted of all children less than five years of age with a primary diagnosis of severe malaria admitted to KDH from August 1991 through June 1992. It was arranged with the Hospital authorities so that each admission to the paediatric ward at Kilifi District Hospital underwent a full clinical, parasitological and haematological examination and all primary diagnoses of *falciparum* malaria were further defined as severe malaria as described above in Chapter 3. The control group was selected from children less than five years of age who attended the outpatient clinic at KDH and who had a diagnosis of malaria with peripheral parasitaemia ^5,000 parasites per ^ul blood, indicating the presence of significant infection but the patient could easily be managed as an out-patient.

Identified severe malaria cases were matched with controls by age (±6 months) and time of admission/treatment (within 2 weeks). Home addresses were obtained from all individuals enroled in the study, and interviews and entomological surveys were conducted at the household within one week of identification. The procedures were as follows: Clinical teams working on the KEMRI unit and paediatric wards notified the epidemiological team who recorded all the basic demographic details. These details were then forwarded to the entomological team who visited the homes of these children and recruited the houses for mosquito sampling.

4.2.2 Anopheline Sampling Methods

Mosquitoes were collected from households of the children who had been monitored at the Hospital within one week of their enrolment. Mosquito collections were made by three techniques: human-biting catches and day-resting indoor collections as already described above, and CDC light traps as described below. On four successive days, each house was sampled for mosquitoes by two all-night light trap collections, one all night human-bait collection, and a day indoor resting collection to assess mosquito numbers and sporozoite rates. A study was also undertaken to ascertain the extent to which mosquito catches over a 4-day period could be used to infer the mosquito density at the time of infection (i.e. approximately 2 weeks previously). In order to do this, a second set of collections was made 2 weeks after the first and the numbers of *An. gambiae s.l.* in the two sets of collections compared.

Anopheline mosquitoes are easily collected in light traps placed in or very near their flight paths, such as inside huts or under the eaves, in animal shelters or cattle corals, or near other aggregations of hosts (Service, 1976). There are many different designs of light traps but the one used in these studies is the CDC trap. It employs a small torch bulb which operates from a 6-volt dry cell battery or a motor cycle battery. Since it does not produce a bright light, the CDC trap

can be placed at night in houses to catch mosquitoes without disturbing the occupants.

A CDC light trap (Model 512, John W. Hock Company, Gainesville, FL) with a lid was hung inside houses where children sleep, about 1.5 m from the floor and about 50 cm from the child's bednet. All the occupants of these bedrooms were given untreated mosquito nets which were provided specifically for this study. Light traps were set at 1830 h and collected the following morning at 0700 h. Enquiries were made as to whether the trap fan and light had both worked all night and catches from faulty traps were discounted. All anopheline mosquitoes were identified, and tested by ELISA for the presence of *P. falciparum* circumsporozoite protein (Wirtz *et al..,* 1987).

4.2.3 Household and Environmental Parameters

A questionnaire (Appendix 1) to characterize the demography, house construction, animal husbandry practices and environmental surroundings was filled in for each house by a team of trained interviewers. Demographic data included information on family size and composition, and activities or behaviours that might affect exposure to vectors such as the use of mosquito repellents and bednets or the patterns of nocturnal activity. Information on housing construction focused on factors potentially affecting mosquito entry

in the houses, including the type of construction materials of the walls and roof, eaves, sleeping arrangements within the households, and the presence of impediments to mosquito movements such as doors or window screens. Data on numbers and kinds of various domestic animals were obtained to assess the potential roles of various animals as risk factors for vector exposure and as alternative hosts. Environmental factors included such variables as the location of the house relative to woodland edge, the presence of various crops, temporary pools and permanent water sources, and materials that might serve as breeding or resting sites for mosquitoes.

4.2.4 Data Analysis

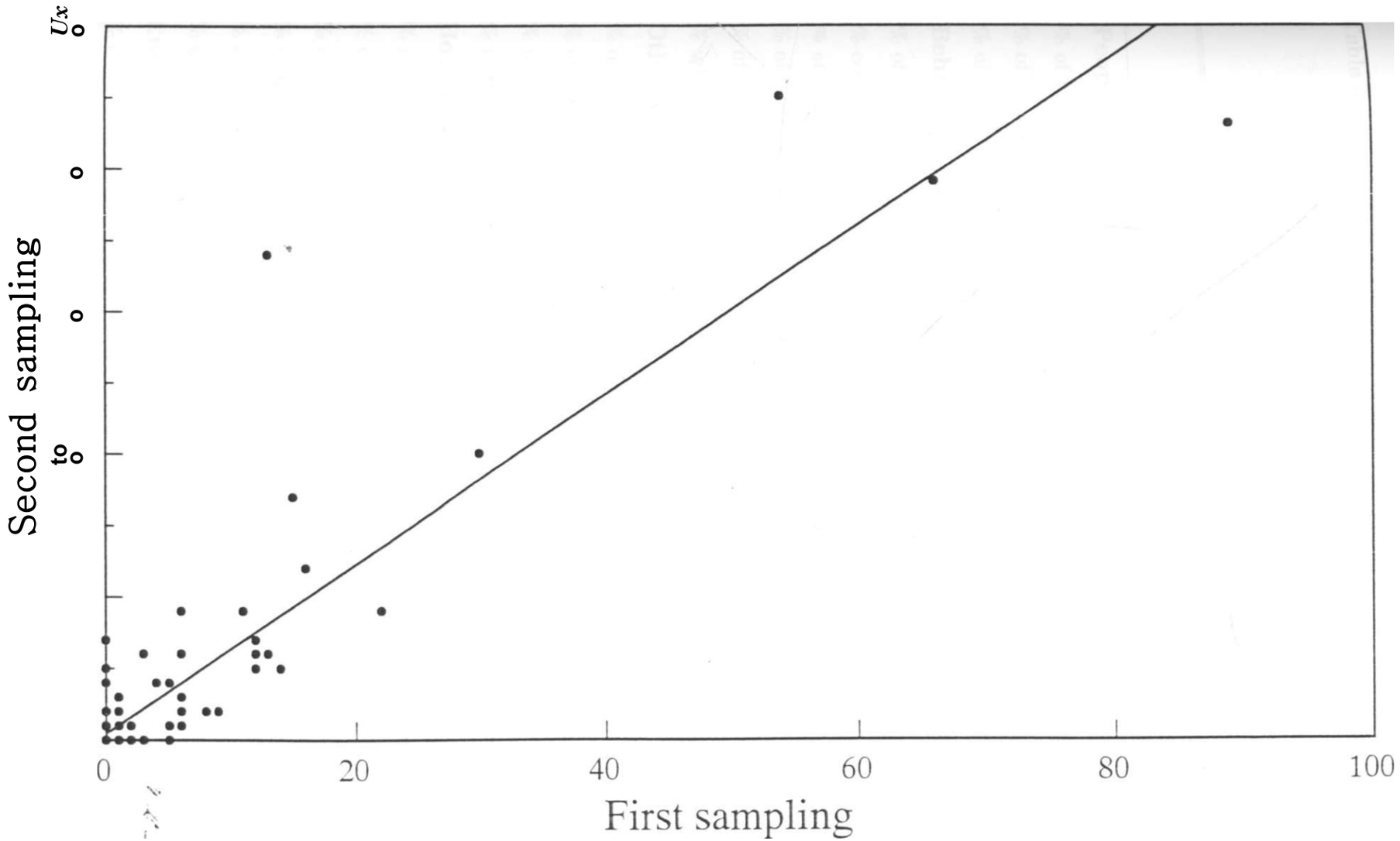
Differences in the abundance of the anopheline mosquito species between case and control houses were determined by paired Student's t-test. For qualitative variables, which include the presence or absence of particular factors, the percentage with the attribute was calculated for each of the groups. Quantitative variables were categorized into quartiles in order to derive other variables. Odds ratios were calculated for matched case-control data to test for associations between the study variables and the occurrence of severe malaria. Conditional logistic regression was used to control for confounding variables. Variables that were statistically significant in the matched analyses were included in the

initial regression model and their contributions tested by partial F-tests. The remaining variables then were added and retained if they contributed significantly to the model. After selection of the final model, all possible interactions between variables were assessed and retained if statistically significant ($P<0.05$). Analyses were performed using EGRET Program (Egret, 1991). Patterns of mosquito abundance and infection rates were examined in case and control households for variables found to be significant in the regression model. Trends in the prevalence of mosquitoes abundance in case and control households were tested by x~ analysis.

4.3 Results

Figure 4.1 shows a good correlation between the first and second sampling over a 2-week period indicating that the relative abundance of anopheline vectors in the houses was stable. Age matched pairs with completed household surveys were available for 119 pairs (87.5%) of households.

Table 4.1 reveals entomological risk factors for malaria in the houses of children with severe malaria and their controls. It shows that the use of mosquito nets or other protective devices were minimal in both groups, 2.5% and 5.9% in case and control households, respectively. The proportion of houses with open eaves and without ceiling was


Second sampling
0
20
40
60
80
100
First sampling

fable 4.1 Summary results of questionnaires characterising the demography, house construction structure, animal presence and general environmental setting carried out in 238 houses within the study area.

Variable	Case (n=119)	Control (n=119)
People in house		
% of Children < 7yrs	47.9	62.2
% of population 7-20 years old	77.3	74.8
% of population > 20 years old	68.9	71.4
Behaviour/activity		
% of people sitting outside regularly	91.6	87.4
% cooking inside houses	41.2	40.3
% of pooplo vising mosquito coils rogrulnrly	23.n	24.4
% of population using insect sprays	1.7	0.8
% of population protected by mosquito nets	2.5	5.9
% who go to bed before 2100 hours	74.8	76.5
Other hosts available		
% of houses with chicken	85.7	80.7
% of houses with cows	7.6	10.1
% of houses with goats	65.5	63.0
% of houses with sheep	11.8	7.6
House construction		
% of houses roofed with makuti	95.0	94.1
% of houses roofed with ironsheets	5.0	5.1
% of houses with ceiling	6.0	7.6
% of houses with windows	36.1	34.5
% of houses with eaves	83.2	79.0
% of house with doors that close	80.7	84.0
Environment		<' -
% of houses near farmed fields	78.2	81.5
% of houses near standing water	1.7	7.6
% of houses near burrow pits	54.2	50.0

similar for children who had severe malaria and for those who had mild malaria. About 76% of householders woke up before 06.00 hours and go to bed before 21.00 hours.

Table 4.2 shows that overall, abundance of anopheline mosquitoes was similar in case and control houses (Paired Student's t-test, t=-0.85; df=103; P=0.399) indicating that there was no association between abundance of either species of mosquito (none, 1-7, > 7 mosquitoes) and severe disease (x^J for trend, P > 0.05 for all comparisons). Malaria vectors of either species were found in 38.2% (91) of the 238 houses comprising of 1,637 (96.2%) *An. gambiae s.l.* and 65 (3.8%) *An. funestus* indicating that the former species were the main vectors involved in malaria transmission in the areas.

Table 4.3 reveals that the proportions of anopheline mosquitoes infected with P. *falciparum* were also similar between the households of children who had experienced severe malaria (cases) and those of the controls *(An. gambiae s.l.* \ x~ = 0.56, P = 0.45; *An. funestus*; x~ = 0.41, P =0.52; Fisher's exact test). Of the 49 case houses with at least one mosquito collected, 16 (33.3%) yielded P. *falciparum* sporozoite positive mosquitoes compared to 13 of 43 (30.2%) in control houses. The mean sporozoite rate per house was similar in case and control houses (OR = 0.96; CI: 0.39, 2.33).

Table 4.2: Arithmetic means for *An. gambiae s.l.* and *An. funestus* caught in case and control houses by three trapping techniques in Kilifi District, Kenya, (August 1991 to June 1992) to show overall abundance of anopheline mosquitoes.

Trapping technique"	Mean number of anopheline mosquitoes			
	case	control	t	P
HBI	4.9	3.9	-0.55	P=0.59
LT	3.4	5.3	0.74	P=0.46
DRI	0.4	0.4	0.15	P=0.89
Total	9.1	7.2	-0.85	P=0.40

' HBI =human-biting indoor catches; LT= CDC light traps; DRI= day-resting indoor collections.

Table 4.3: *Plasmodium falciparum* infection rates for *An. gambiae s:l.* and *An. funestus* caught in case and control houses in Kilifi, Kenya.

	% of mosquitoes positive by ELISA (N)			
Species	Case	Control	x^2	P
An. gambiae s.l.	4.9 (906)	4.0 (731)	0.56	0.45
An. funestus	11.4 (35)	3.7 (27)	0.41	0.52
Total	5.1 (941)	4.0 (758)	1.01	0.32

In matched analyses, 11 of 75 derived environmental and demographic variables were associated ($P<0.05$) with severe malaria (Table 4.4). The risk of severe disease decreased in the households with more than three adults, less than two children <7 years of age. Similarly, Table 4.4 also shows that this risk decreased in houses with two bedrooms, houses with more than one dog, and houses with small temporary pools of water. The risk of severe disease increased in households with less than three adults, households with more than six individuals who did not use bednets, households with more than two children less than seven years of age and aged between 7-20 years. Houses that had at least two or more windows or doors that could not be sealed or closed, and households with more than two sheep were associated with severe disease (Table 4.4).

Seven variables met the criterion for inclusion in the final conditional logistic regression model (Table 4.4). There were no significant interactions among the variables. Despite the absence of an overall difference in mosquito abundance between case and control houses, there were no significant differences in the patterns of mosquito presence between case and control houses for variables in the final regression model.

Table 4.4. Odds ratio, 95% confidence interval and conditional logistic regression for some variables associated with malaria disease in Kilifi, Kenya.

Variable	Matched analysis		Conditional logistic regression analysis	
	Odds ratio	95% CI	Odds ratio	95% CI
People in the house				
is3 people >20 years old	2.18	(1.07, 4.45)		
>3 people >20 years old	0.38	(0.15, 0.96)	0.08	(0.02, 0.35)
>6 people exposed	4.55	(1.16, 3.94)	6.27	(2.28, 17.27)
£2 children <7 years old	0.63	(0.36, 1.12)	0.45	(022, 0.94)
>2 children <7 years old				
>2 children 7-20 years old	2.18	(1.07, 4.45)		
Presence of animals				
>2 sheep	11.00	(1.42, 85.20)	11.35	(1.33, 96.96)
>1 dog	0.43	(0.20, 0.93)	0.28	(0.09, 0.80)
Opening (doors + windows)	1.22	(1.01, 1.47)		
2 bedrooms	0.53	(0.31, 0.89)	0.34	(0.16, 0.69)
Temporary pools	0.12	(0.02, 0.99)	0.08	(0.01, 0.88)

4.4 Discussion

This study demonstrates that on the coast of Kenya, malaria parasite transmission intensity as measured crudely through retrospective case-control studies is not associated with the risk of severe disease. Under conditions of low vector densities and transmission intensity, the risk of severe malaria may be multi-factorial and not strictly associated with transmission intensity. This further highlights the possible role of human host- and parasite-related determinants of severe malaria infections compared to mild disease as already stated above in Chapter 3. The low sporozoite inoculation rate coupled with high degree of human feeding by vector populations, may be contributing to the efficiency of parasite transmission even at extremely low vector densities.

However, modifiers of vector abundance were associated with the development of severe malaria. For instance, the risk of severe disease increased in houses with more than 6 people who did not use bednets and in presence of more than two sheep in a house. Whereas this emphasizes the need for personal protection against biting vector mosquitoes, in order to reduce human-vector contact, the evidence that the presence of domestic animals in the compound is a risk factor was unexpected, and therefore, this association may have arisen by chance. Such analysis need to be treated with extreme

caution. It is possible that household features are associated with socio-economic status, itself very hard to measure, which in-turn affects educational levels of mothers and their treatment seeking behaviours. The fact that it was not possible to demonstrate any effects of the presence of other animals suggest that this finding is either a marker for something else or simply a spurious result given the number of cross-tabulations performed in the final analysis. Other authors have shown marked differences in households with eaves (Snow *et al*., 1988a; 1988b; Lindsay *et* a/., 1991) and vector abundance, severe disease and no disease (Adiamah *et al*., 1993).

However, this has to be carefully evaluated at household level since there are marked environmental heterogeneities among houses. The existence of severe disease in houses with undetectable vector mosquitoes, coupled with the lack of association among more than 60 house and environmental parameters suggests that severe disease can occur in all environments despite interesting time-space clustering of disease patterns (Snow *et al.,* 1993). Overall, these results indicate that children who develop severe malaria were exposed to a siriiilar number of infected mosquitoes as children who developed mild malaria at the time of presentation at hospital and that this was probably also the case at the time of their infection.

> 4'*

This data further demonstrates the difficulties involved in providing

reliable estimates of vector activity and behaviour in areas of low vector abundance. However, it needs to be concluded that despite these limitations exceptionally low vector abundance can still lead to severe disease and that these situations equally prevail among households where mild disease occurs. Within this geographical area of Kilifi, increases in transmission intensity are probably not associated with development of progressing from mild to severe and life-threatening disease.

In conclusion, evidence is presented that the risk of severe malaria infections in children on the coast of Kenya is not associated with vector abundance and environmental heterogeneity. As regards vector control, it is unclear whether reduction in abundance of vectors can correspondingly reduce the risk of severe malaria for endemic areas with stable, low level exposure to infected mosquitoes. It would thus appear that along the coast of Kenya, it may not be possible to target vector control to specific areas because even barely detectable levels of transmission are associated with a high risk severe disease.

CHAPTER 5

BLOOD-FEEDING AND BITING BEHAVIOUR OF *AN. GAMBIAE S.L.* AND *AN. FUNESTUS*

5.1 Introduction

The blood-feeding and biting behaviour of anopheline vectors of malaria are an important parameter in malaria epidemiology. The degree of human-feeding influences the probability that mosquitoes will come in contact with gametocyte carriers and thus acquire *Plasmodium* infections. The most successful malaria vectors feed commonly on humans and secondarily on cattle and other domestic animals depending on host availability (Garrett-Jones *et. al.*, 1980).

It is well known that *An. gambiae s.l.* and *An. funestus* are highly endophilic and endophagic, but their potential for transmitting malaria outdoors is less understood. The human biting rate is an important component of entomological inoculation rate and is also a parameter that is useful for comparing malaria transmission in different sites and at different times, and also that is reasonably easy to measure. The measurements of the biting rhythm of anophelines is important in malaria control programs.

Malaria control methods, such as those using bednets and eave

curtains, designed to reduce the degree of man-vector contact require baseline data on human biting rates, such data will be useful in modelling the critical man-biting levels required for significant reductions in malaria transmission in control projects. These data have not been available for Kilifi area where malaria transmission is low.

In Kenya, mosquito feeding behaviour has been studied extensively in the Kisumu area western Kenya (Joshi *et al.*, 1973, 1975; Service *et al.,* 1978; Highton *et al.,* 1979; Beier *et al.,* 1988; Petrarca *et al.,* 1990), but there is limited information on anopheline feeding behaviour on the coast (Mutero *et al.,* 1984). This sub-study examines the host feeding patterns and biting rhythm of malaria vectors on the Kenyan coast relative to host availability.

5.2 Materials and Methods

5.2.1 Anopheline Collection Methods

For bloodmeal analysis mosquitoes were collected in 25 sites of the much wider study area in Kilifi District from June 1991 to August 1992 using day-resting collections (DRI), Pyrethrum spray catch (PSC), and CDC light traps (LT) as already described. The area for these studies covered a much wider area than the study sites

previously mentioned because it was noted few blood-fed mosquitoes were collected from these sites. The presence or absence of cattle and other domestic animals around households was determined by on-site inspections during mosquito collections and was recorded for 95 houses out of 101 sampled.

Blood-fed mosquitoes from each collection were identified, placed in vials, and then air-dried at room temperature for up to 4 days. They were cut transversely between the thorax and abdomen, and posterior portions containing the bloodmeal were placed individually in labelled vials. Each mosquito was ground in 50 PBS, with 950 ix\ PBS added after grinding; these were stored at -20"C until testing.

5.2.2 Hourly Biting Catches

For biting rhythm behaviour studies, all night human-bait collections were carried out both indoors and outdoors as already described at Mtondia area between June and August 1993, during periods of high vector abundance. Catches were carried out for two nights each week between 1800 h and 0600 h. Hourly catches were undertaken in order to establish the biting pattern of vector species with regard to time. The caught mosquitoes were transported to the laboratory, identified to species and counted, keeping indoor and outdoor samples separate.

5.2.3 Blood Meal ELISA Procedure

Bloodmeals in the mosquitoes were identified by direct enzyme-linked immunosorbent assay (ELISA) using anti-host (IgG) conjugates against human and cow, then non-reacting samples were tested for goat (Beier *et al..,* 1988). Mosquito triturates (50 /JL\) were added to wells of polyvinylchloride, U-shaped, 96-well microtitre plates, which were covered and incubated overnight at room temperature. Each well was then washed twice with PBS containing 0.5% Tween 20 (PBS-TW 20). This was followed by the addition of 50 fi\ host specific conjugate (antihuman IgG, H&L) diluted 1:2,000 (or 1:250 for bovine) in 0.5% boiled casein containing 0.025% Tween 20. The boiled casein was prepared by dissolving 5 g casein in 100 ml 0.1 N sodium hydroxide by boiling, adding 900 ml PBS, adjusting pH to 7.4, adding O.lg Thimerosal (sodium ethylmercurithiosalicylate) and 0.02 g phenol red, and storing at 4nC. After 1 hour, wells were washed three times with PBS-Tween 20, and 100 of ABTS (2,2'-azino-di-[3-ethylbenzthiazoline sulfonate]) peroxidase substrate was added to each well. The dark green positive reactions for peroxidase, or dark yellow reactions for phosphates assessed visually after 30 minutes. A second host source was determined in the same microtitre plate where mosquitoes were screened for human blood. The second conjugate, phosphatase-labelled anti-bovine IgG (1:250 dilution of 0.5 mg/ml stock solution) was added to the peroxidase-labelled

antihuman IgG solution. Bloodmeals were screened first for human IgG by the addition of peroxidase substrate according to the methods described above. After reading the plates at 30 minutes, the wells were washed 3 times with PBS-Tween 20, and 100 /JL\ phosphatase substrate was added to each well. Plates were read after 1 hour to determine positive cow reactions.

Non-reacting samples were then tested for goat. For each test, 1:500 dilutions of human, cow, goat, dog, cat and chicken serum were added to the conjugate solution to reduce background absorbance. Each plate contained control serum samples (1:500 dilution in PBS) of human, cow, goat, dog, cat and chicken and four field-collected male *Anopheles* ground in PBS at the same dilution as test samples. Blood meal ELISA solutions and step-by-step procedures are described in details in Appendix 3.

5.3 Results

Human IgG was detected in samples from 94.4% of 534 *An. gambiae s.l.* and 90.8% 'of 76 *An. funestus* (Table 5.1). No positive reactions were detected for cow and only 4 samples of *An. gambiae s.l* were positive for goat. Overall, positive reactions were detected in 95.1% of the *An. gambiae s.l* and 90.8% of the *An. funestus.*

Table 5.1: Bloodmeal sources for *An. gambiae s.l.* and *An. funestus* collected by three tra Kenya.

Species	Trapping technique*	No. tested	human	cow
An. gambiae s.l.	DRI	333	96.7	0.0
	LT	61	91.8	0.0
	PSC	140	90.0	0.0
	Total	534	94.4	0.0
An. funestus	DRI	64	89.1	0.0
	LT	5	100.0	0.0
	PSC	7	100.0	0.0
	Total	76	90.8	0.0

" DRI, day$_c$.resting indoor collections; LT, CDC light trap indoors; PSC, pyrethrum spray

Table 5.2 shows the percentage of human blood meals identified for *An. gambiae s.l* and *An. funestus* according to 25 collection sites throughout Kilifi District, Kenya. In all sites, both species fed predominantly on humans.

Table 5.3 shows that the human blood index (HBI) was high irrespective of host availability. It also shows that 75 % of houses had goats, 11% had cows with goats, but none of the houses had cows without goats indicating that the presence of animals did not deflect mosquitoes from feeding on humans.

The hourly biting activity of female *An. gambiae s.l.* is shown in Figure 5.1. Female *An. gambiae s.l.* started biting both indoors and outdoors at 18.00 hours, and this biting activity continued throughout the night. The indoor biting activity reached a peak at between 23.00 and 01.00 hours. The numbers decreased towards dawn but increased slightly at 04.00 hours and about 7% of the vector population was still biting at 06.00 hours. About 30% of the outdoor biting took place between 19.00 and 23.00 hours with a peak at 01.00 hours, whereas'during the same period 42% of the indoor population was biting. No *An. funestus* was caught biting either indoors and outdoors.

»rable 5.2: Percentage of human blood meals identified for ***An. gambiae s.l.*** ₙd ***An. funestus*** from 25 sites in Kilifi District, Kenya.

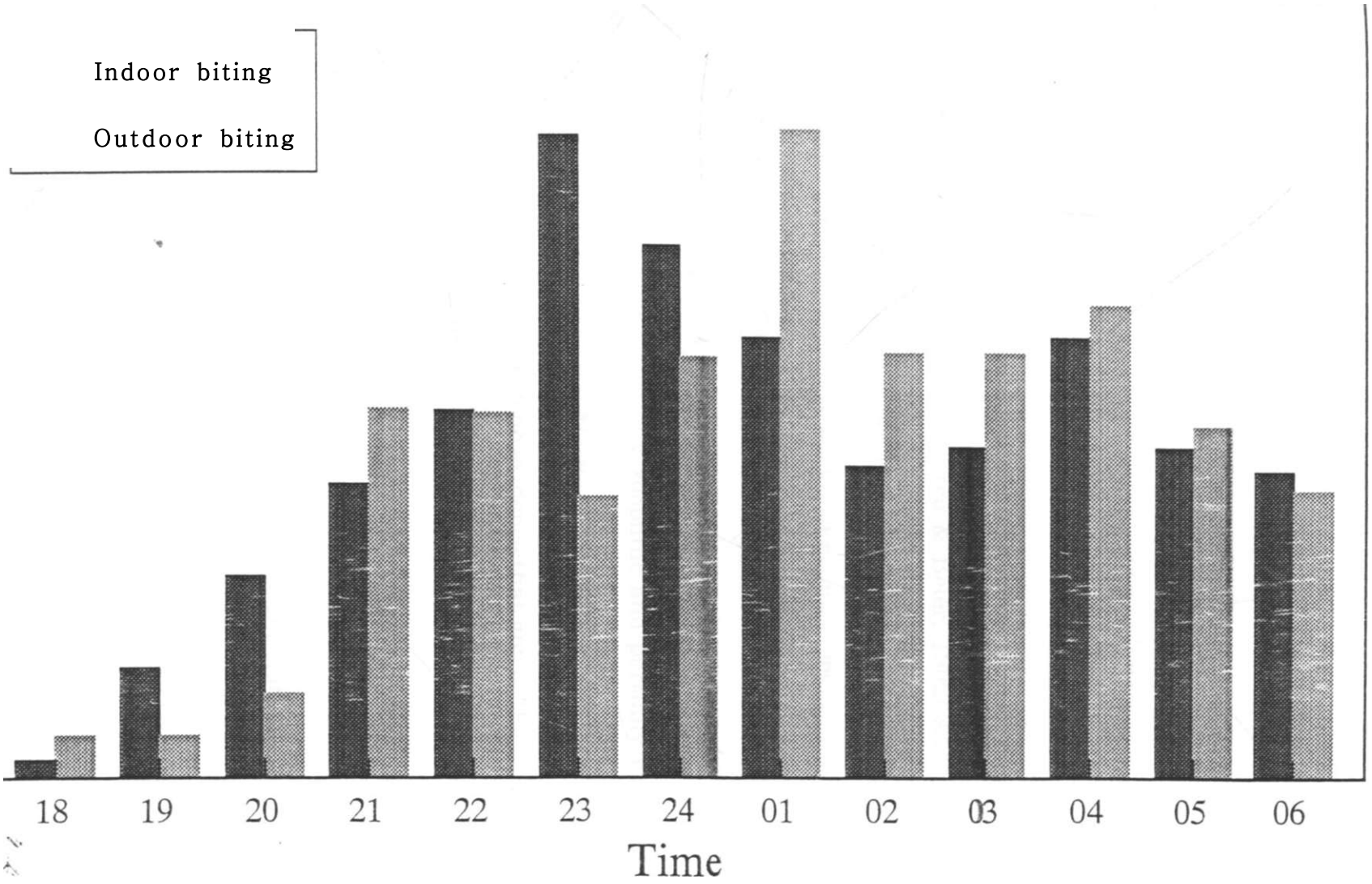
Site	*An. gambiae s.l.*		*An. funestus*	
	No. tested	% human	No. tested	% hums
Bofa	1	100.0	0	0.0
Chumani	10	90.0	0	0.0
Chokwe	1	100.0	0	0.0
Dera	32	96.9	11	100.0
Fumbini	13	92.3	1	100.0
Gongoni	0	0.0	1	100.0
Kibarani	60	93.3	3	100.0
Kiwandani	1	100.0	0	0.0
Konjora	28	96.4	18	94.4
Kitengwani	41	97.6	11	81.8
Kilifi town	9	100.0	1	100.0
Majajani	18	94.4	0	0.0
Majaoni	37	94.6	0	0.0
Mikingirini	1	100.0	0	0.0
Matsangoni	11	100.0	1	100.0
Mtondia	9	100.0	0	0.0
Mwandoni	1	100.0	0	0.0
Mdzongoloni	38	92.1	4	100.0
Ngerenya	13	100.0	0	0.0
Roka	8	100.0	0	0.0
Sokoke	* 116	96.6	1	0.0
Shauri Moyo	3	100.0	0	0.0
Tandia	52	90.4	8	100.0
Tezo	1	100.0	1	100.0
Vipingo	30	80.0	15	80.0
Total	534	94.4	76	90.8

Table 5.3: Human blood index relative to host availability for ***An. gambiae s.l.*** and ***An. funestus*** in Kilifi District, Kenya.

Host availability	No. houses	Human blood index (no. samples)'	
		An. gambiae s.l.	*An. funestus*
Cows	0	0.0 (0)	0.0 (0)
Goats	61	0.96 (385)	0.93 (44)
Cows and goats	10	0.96 (68)	1.00 (3)
No cows/goats	24	0.89 (75)	0.86 (28)
Total	95	0.95 (528)	0.91 (75)

Proportion of blood meals positive for human IgG.

Figure 5.1 Hourly Indoor and Outdoor biting catches for An. gambiae s.l at Mtondia



5.4 Discussion

Anopheles gambiae s.l. and *An. funestus* fed primarily on humans despite the presence of cows and goats at 11% and 75% of the houses, respectively. The high human blood index for indoor resting populations in unlike the situation in western Kenya (Beier *et al.,* 1988; Petrarca *et al.*, 1991; Petrarca & Beier, 1992) and in Mwea irrigation scheme (Ijumba *et al.*, 1990) where the availability of cows is a major determining factor for blood-feeding. These findings are unexpected. Of the 3 species in the *An. gambiae* complex in Kilifi *(An. gambiae, An. merits*, and *An. arabiensis), An. merus* and *An. arabiensis* are at least partially zoophilic and partially endophilic (Iyengar, 1962; White, 1974; Mosha & Petrarca, 1983; Mutero *et al..,* 1984). At this point, there is no evidence that any of the 3 species of the *An. gambiae* complex in Kilifi feed to a significant degree on hosts other than humans. Further efforts are necessary to identify bloodmeals for each of the 3 species in the *An. gam biae* complex and to examine bloodmeals from outdoor resting populations.

The high degree of human-feeding may be a primary factor contributing to the efficiency of *Plasmodium falciparum* transmission on the Kenyan coast. In the initial studies, it was found that a high incidence of severe malaria associated with extremely low vector densities and entomological inoculation rates <10 per year (Chapter

3). Transmission can be maintained year-round despite low vector densities because high rates of human feeding facilitate direct contact with gametocyte carriers and sporozoite-positive mosquitoes "waste" few infective bites on domestic animals.

Analysis of the biting rhythm behaviour revealed that *An. gambiae s.l.* started biting people both indoors and outdoors as early as 1800 hours and thereafter only about 30% of the entire human-biting population was active until 2300 hours, after which biting became intense up to dawn. *An. gambiae s.l.* was equally likely to bite people indoors or outdoors. The close similarity in the indoor and outdoor biting cycles of An. *gambiae s.l.* could indicate either a homogenous population indoors and outdoors, or that biting cycles depend mainly on intrinsic rhythm, rather than environmental conditions. However, the risk of contracting malaria infections is greater indoors than outdoors because most people are in bed at the peak biting time.

Man is the principal host for *An. gambiae s.l.* at Kilifi and consequently in the absence of cattle outdoors man-vector contact is greatly enhanced both indoors and outdoors. In Kilifi most people go indoors after 2100 hours and also during occasional social functions they stay outdoors for any considerable time at night. Under normal circumstances people sleep outdoors in Kilifi and since in the current study outdoor biting starts when most people are outdoors, exophagy appears to be an important problem in Kilifi.

The diel biting pattern observed for *An. gambiae s.l.* resemble those reported in western Kenya (Githeko, 1992). A 16% of the total biting indoors of *An. gambiae s.l.* occurred before people retire to, and after rising from bed, indicating that in areas where bednets may be used for control of biting mosquitoes, this population would still be exposed to the risk of contracting malaria.

In conclusion, evidence is presented that indoor-resting malaria vectors on the coast of Kenya feed predominantly on humans irrespective of the availability of cattle and other domestic animals. This situation is unusual given the complexity of the malaria vectorial system (Mosha & Petrarca, 1983). In Kilifi, one reason why the incidence of severe *P. falciparum* malaria is high under conditions of low vector densities (Chapter 3) is that high rate of human-feeding and biting at the time when people are asleep facilitate efficient malaria parasite transmission by vector populations.

CHAPTER 6

EVALUATION OF LIGHT TRAPS FOR SAMPLING ANOPHELINE MOSQUITOES

6.1 Introduction

A number of sampling techniques have been used to measure human exposure to anopheline mosquitoes, including human-biting catches, pyrethrum spray collections and light traps. Several studies have found that light traps underestimate host-seeking anophelines (Service, 1976; Zaim *et al*, 1986; Hii *et al*, 1986), while others have used light traps with relative success (Odetoyinbo, 1969; Service, 1970; Chandler *et al*, 1975; Garrett-Jones & Magayuka, 1975; Joshi *et al,* 1975). Recently, Lines *et al* (1991) in Tanzania showed that CDC light traps used inside houses, in combination with bednets, yielded results comparable to standard human-biting collections and could be used to estimate anopheline abundance. Host-seeking females attracted to humans are diverted by the bednets and are readily caught by light traps. This method also offers logistical advantages ovgr human-biting collections.

To test this hypothesis, the sampling efficiency of light traps was evaluated and compared to standard human-biting catches. The goals were to evaluate mosquito detection sensitivity, numerical

trapping bias, and bias in the portions of the mosquito population that was sampled.

6.2 Materials and Methods

Children from the study area presenting with malaria infections at Kilifi District hospital were selected and entomological sampling was conducted in their respective houses. The study was conducted for 11 months from August 1991 to June 1992. In June and July 1991, preliminary mosquito sampling were conducted using light traps set inside houses without bednets to examine the significance of bednets in the proportion of blood-feeding stages of mosquitoes caught.

Mosquito collections were made in 302 houses by all-night biting and light trap catches. Light traps were used on the first two nights, followed by one all-night biting collection in each house. A CDC light trap (Model 512, John W. Hock Company, Gainesville, FL) with a lid was hung inside houses where children sleep, about 1.5 m from the floor and about 50 cm from the child's bednet. All the occupants of these bedrooms were given untreated mosquito nets which were provided specifically for this study.

Light traps were set at 1830 h and collected the following morning at 0700 h. Enquiries were made as to whether the trap fan and light

had both worked all night and catches from faulty traps were discounted. Human-biting catches were conducted on the third night. Mosquitoes coming to bite indoors between 1830 h and 0600 h were caught at half-hour intervals by two people seated with legs and feet bared to the knee (WHO, 1975).

All anopheline mosquitoes were identified, counted, and classified according to blood-feeding stages (WHO, 1975). Frequencies of the various blood-feeding stages from light trap catches were compared with preliminary results from light traps set in houses without bednets by X^2 analysis. Female anopheline mosquitoes were tested by enzyme-linked immunosorbent assay (ELISA) using 2A10 monoclonal antibodies to detect circumsporozoite (CS) protein of P. *falciparum* (Wirtz *et al.*, 1989).

6.2.1 Statistical Analysis

To test the efficiency of light traps in estimating mosquito abundance, we utilized graphical and parametric methods of Altman & Bland (1983)" to examine bias and error in methods. Light trap (LT) and human-biting (HBI) catches were log transformed as $\log_{10}(LT+1)$ and $\log_{10}(HBI+1)$. The difference in abundance between $\log_{10}(HBI+1)$ and $\log_{10}(LT+1)$ was graphed relative to the average of the log of abundance for the two methods. The presence of a

significant slope was used as evidence of bias in the collection technique (Altman & Bland, 1983). To examine the sensitivity of the two methods for detecting mosquito presence, mosquitoes captured by light traps and human-biting collections were summed for each house and the abundance divided into quartiles. The percentage of houses that had at least one mosquito collected by each method was calculated for each quartile as a measure of detection threshold for each method as the mosquito population size changed.

6.3 Results

A total of 343 *An. gambiae s.l.* and 19 *An. funestus* were captured in 164 houses without bednets while 736 *An. gambiae s.l.* and 6 *An. funestus* were captured in 262 houses with bednets (Table 6.1). The comparison of blood-feeding stages of *An. gambiae s.l.* and *An. funestus* caught by light traps with or without bednets indicates that bednets significantly alter the feeding pattern of mosquitoes. The proportion of blood-fed *An. gambiae s.l.* caught by light traps was lower in houses with bednets (8.7%) than in houses without bednets (16.3%) (X^2= 13:78, df=l, P=0.0002). More empty *An. gambiae s.l.* were caught by light traps set in houses with bednets (84.1%) than in those without bednets (73.8%) (X^2=16.14, df=l, P< 0.0001).

Light trap failures or refusals for human-biting collections occurred

Table 6.1 Bloodfeeding status of *Anopheles gambiae s.l* and *Anopheles funestus* caught b untreated bednets to those used without bednets

	% of mosquitoes caught (n) by ELISA		
	An. gambiae s.l		
Bloodfeeding status	Bednets	No bednets	Bednets
Unfed	84.1 (619)	73.8 (253)	50.0 (3)
Bloodfed	8.7 (64)	16.3 (56)	33.3 (2)
Haif-gravid	0.3 (2)	2.3 (8)	0.0 (0)
Gravid	6.9 (15)	7.6 (26)	16.7 (1)
Total	(736)	(343)	(6)

in 40 of the original 302 houses. Of the remaining 262 houses, only 104 (39.7%) yielded at least one mosquito by either light traps or biting catches (Table 6.2). Light traps yielded 736 *An. gambiae s.l.* and 6 *An. funestus* in 28.2% of the houses, while 985 *An. gambiae s.l.* and 40 *An. funestus* were captured by human-biting collections in 33.1% of the houses. Light traps underestimated presence of *An. funestus* at low vector densities (X^2=3.89, df=l, P=0.049). There was a significant correlation in the number of *An. gambiae s.l.,* captured by the two sampling methods (r=0.64, df= 260, P< 0.0001). The potential bias in estimating anopheline mosquito abundance by light traps was examined graphically. There was a significant tendency for the ratio of HBI/LT to increase (Figure 6.1) with increasing mosquito densities (r=0.29, df=260, P< 0.0001). *An. funestus* was not included in the analysis because too few were caught.

Figure 6.2 shows that the proportion of houses that had mosquitoes collected by either method increased with the number of mosquitoes captured in the houses with at least one mosquito. Overall, light traps detected mosquitoes in 67.3% of the houses compared to 71.2% by human-bitirtg collections (X~=0.36, df=l, p=0.55). At the lowest quartile (1 mosquito), light traps detected mosquitoes in 45.7% of the houses, while human-biting catches detected mosquitoes in 54.3% of the houses (X^2=0.31, df=l, P=0.58). At the upper quartile of mosquito abundance (11 +), light traps detected mosquitoes 88% of the time compared with 96% by human-biting collections.

Table 6.2 *Anopheles gambiae s.l* and *An. funestus* collected by CDC light traps and human-biting catches, August 1991- June 1992

	Light trap catches		Biting catches	
Species	No. collected	% Positive houses	No. collected	% positive houses
An. gambiae s.l	736	26.7	985	28.2
An. funestus	6	1.5	40	4.9
Total	742	28.2	1025	33.1

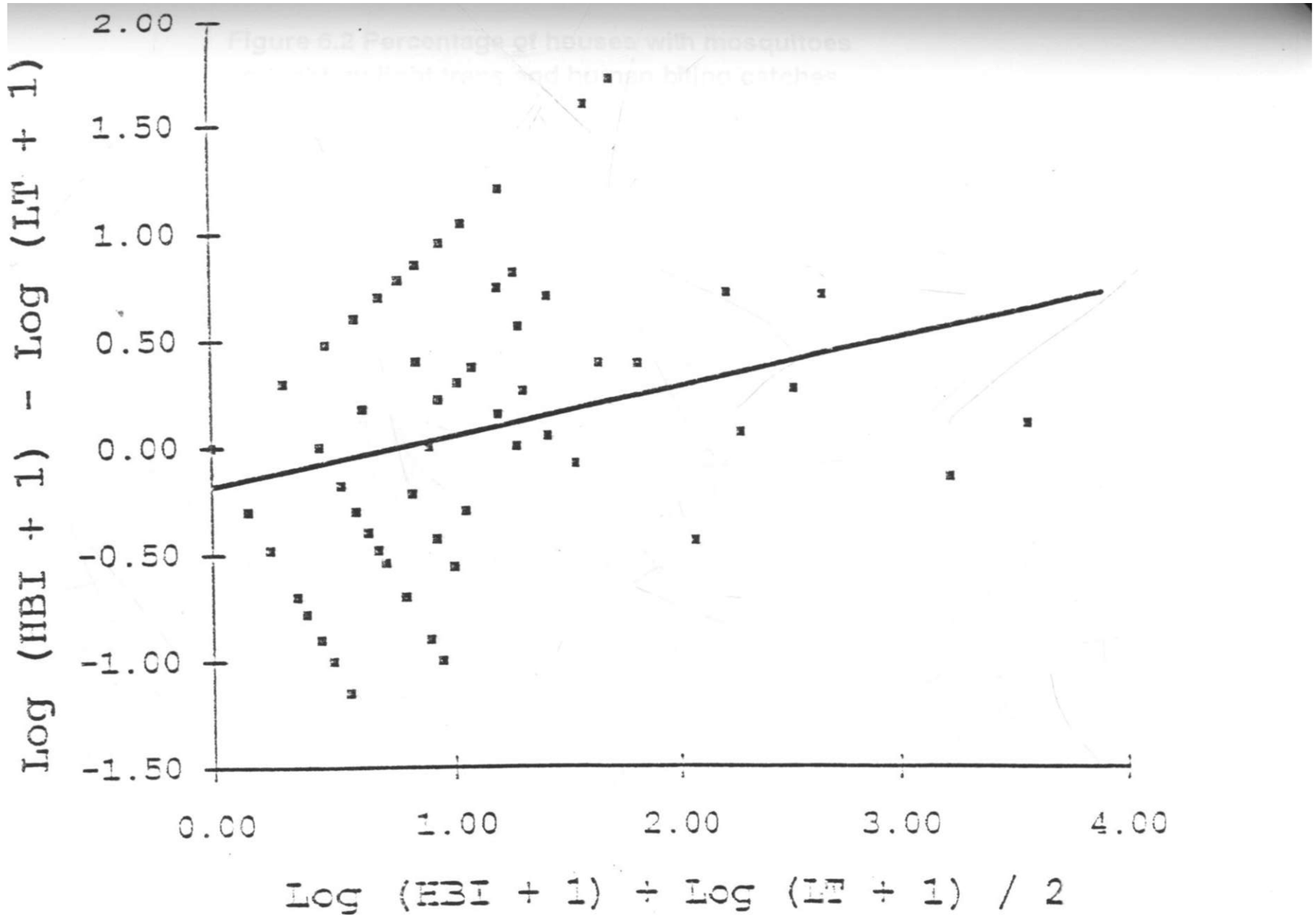
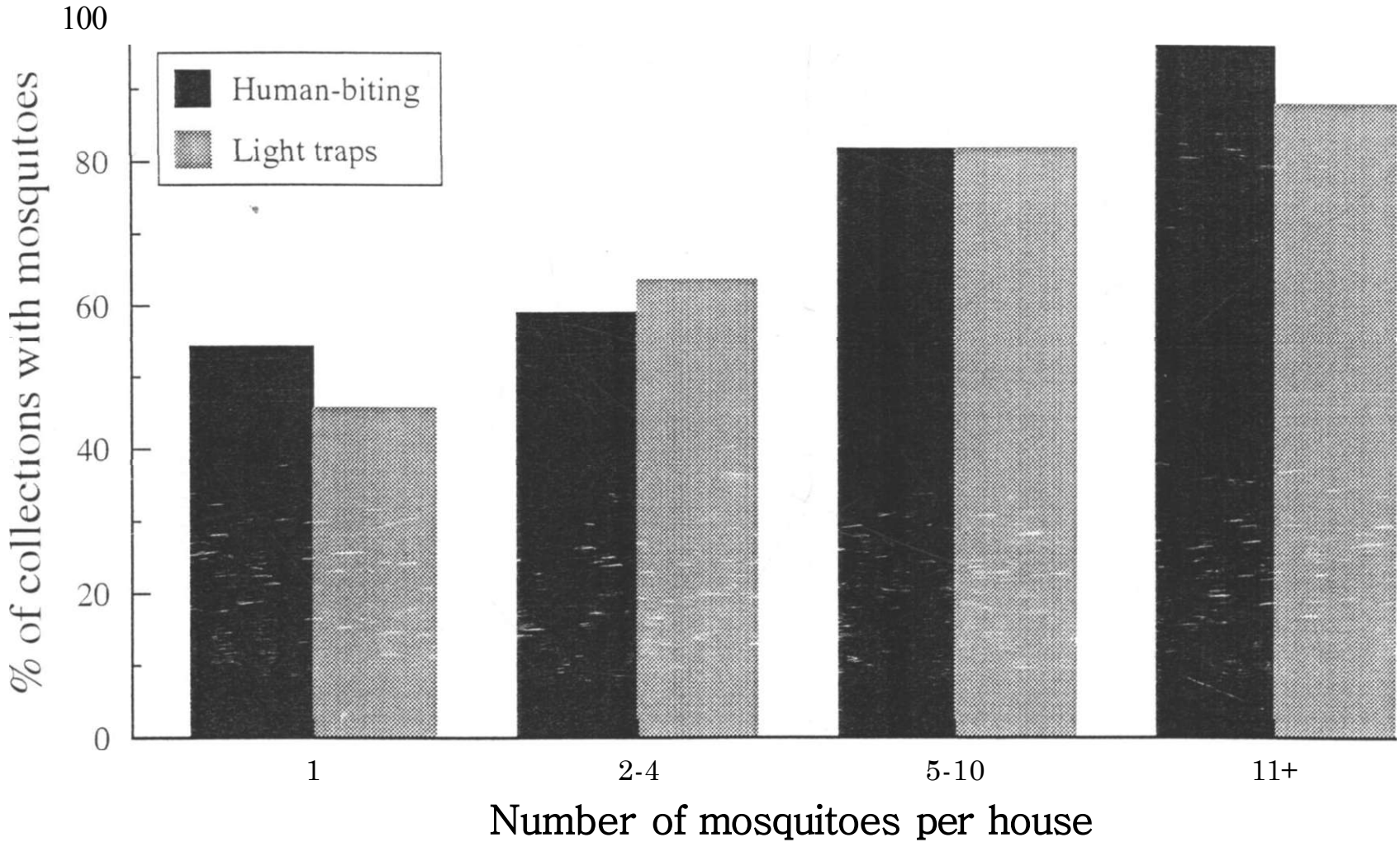

2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
Log (HBI + 1) - Log (LT + 1)
0.00
1.00
2.00
3.00
4.00
Log (HBI + 1) + Log (LT + 1) / 2

Figure 6.2 Percentage of houses with mosquitoes caught by light traps and human biting catches relative to mosquito abundance



P. falciparum infection rates for ***An. gambiae s.l.*** and ***An. funestus*** caught by light traps and human-biting collections are shown in Table 6.3. The proportion of infected ***An. gambiae s.l.*** was 2.3-fold higher for mosquitoes from light traps (5.7%) compared with those caught by human-biting collections (2.5%) (X^2=11.5, df=l, P=0.0007).

6.4 Discussion

This study evaluated light traps as a sampling method for estimating human exposure to host-seeking anopheline mosquitoes in Kilifi District, an area with extremely low vector densities along the coast of Kenya. The intention was to determine whether light traps could be used to estimate human-biting rates in individual houses during epidemiologic studies of malaria parasite transmission. Even though Lines ***et al.*** (1991) in Tanzania demonstrated clearly the potential value of light traps used in conjunction with bednets, it was recognized that there may be special considerations in Kilifi where the same vectors occur but at much lower densities.

There were several sources of bias associated with light trap sampling. Although there was a significant correlation in densities of ***An. gambiae s.l.*** between light trap and biting collections, light traps underestimated densities and this bias increased with increasing densities. Light traps were also less efficient than biting-

Table 6.3 ***Plasmodium falciparum*** infection rates for ***Anopheles gambiae s.l*** and ***An. funestus*** captured by light traps and human-biting collections.

	% positive (n) by ELISA		
Sampling technique	***An. gambiae s.l***	***An. funestus***	Total
Light traps	5.7 (722)	0.0 (6)	5.6 (728)
Human bait	2.5 (971)	5.3 (36)	2.6 (1007)

collections for detecting the presence of ***An. funestus***, a minor vector in Kilifi District. Importantly, light traps caught mosquitoes with higher P. ***falciparum*** infection rates than those from biting collections. Even though the use of light traps in conjunction with bednets decreased the number of blood-fed mosquitoes caught so that more than 80% of the mosquitoes were "empty" and presumably host-seeking, there is a good possibility that light traps attracted a substantial portion of the indoor-resting populations. In Kenya, such mosquitoes generally have higher parity rates and higher infection rates than host-seeking mosquitoes (Petrarca ***et al.*** 1991).

Understandably, the use of light traps under conditions of low vector densities were less efficient than biting catches. It is important to note that the sources of error in light trap sampling were observed in comparisons of 2 light trap collections relative to one man-biting catch in each house (i.e., one man-night). Clearly, estimations of anopheline densities by light traps are extremely sensitive to sampling effort (Altman & Bland 1983, Lines ***et al***, 1991). This highlights the probability that collections from a single night of trapping will underestimate densities even further.

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There are several important logistical considerations for the use of light traps. Compared with biting catches, light traps are more convenient and can be replicated many times if necessary. Even though care must be taken in the physical placement of light traps

within houses and the supervision of trap collections, it is possible to achieve a satisfactory degree of standardization with light traps. The design of this study involved short-term mosquito sampling in each house. In general, residents were more receptive to the use of light traps than all-night, visiting mosquito collectors.

As noted by Lines ***et al.*** (1991), it is important to determine relationships between light trap and biting collections for each geographic area. Apparently, light traps efficiency varies as a function of vector densities. Findings that light traps are less efficient than biting collections under conditions of low vector densities does not necessarily preclude the use of light traps for monitoring population densities in vector control or epidemiologic studies when the scale of interest is the village-level. Provided that a validation study is done, regression equations derived for each species can be used to estimate biting densities based on light trap collection data.

In Kilifi, the focus of the field studies was to evaluate malaria parasite transmission in individual houses. The extremely low vector densities in this area necessitate intensified mosquito sampling. The findings that light traps provided a biased collection of host-seeking mosquitoes both in terms of densities and sporozoite rates emphasize that light trap sampling is not suitable for epidemiologic studies when vector-related information is required at the household level.

CHAPTER 7

GENERAL SUMMARY AND DISCUSSION

The purpose of the present study was to quantify malaria parasite transmission in two areas of Kilifi, and to explore relationships between transmission challenge and disease patterns. Malaria parasite transmission by vectors; ***An. gambiae s.s., An. arabiensis, An. merits,*** and ***An. funestus,*** on the Kenyan coast is evidenced by low infection rates and transmission intensity, high human feeding and high incidence of severe malaria. ***An. gambiae s.l.*** appears to predominate and exhibits two seasonal peaks which coincides with peaks of severe malaria, although vector activity occurred throughout the year. ***An. funestus*** played a minor role in transmission. In the study area there were very low indoor resting densities of ***An. gambiae s.l.*** probably due to lack of suitable breeding sites, but a significant role in malaria transmission is assumed during the wet season. Transmission shows spatial and temporal trends with peaks between June and September, and December to January. This coincides with the peaks of the incidence of severe disease.

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The feeding habits of An. ***gambiae s.l.*** and ***An. funestus*** as shown by ELISA method indicated that these vectors are predominantly anthropophilic even in the presence of other hosts. The high proportion of human bloodmeals (95.2%) demonstrates the restricted host range of the ***An. gam biae*** complex. This increases their chances

to transmit malaria. In general ***An. gambiae s.l*** and ***An. funestus*** shows a high degree of endophily and anthropophagy.

Vector over-dispersion was evident in Sokoke. Small-area variations in vector abundance have been documented elsewhere (Burkot ***et al.,*** 1989; Lindsay ***et al.,*** 1989; Smith ***et al.,*** 1993) and the implications of such heterogeneity for models of malaria transmission have been described by Dye and Hasibeder, (1986). However, accounting for the effects of marked heterogeneity in vector abundance during sampling procedures for randomised control trials of vector control measures has not been adequately addressed. The intensity of sampling was greater than that used by other investigators in areas with higher vector abundance (Sexton ***et al.,*** 1990; Magesa ***et al.,*** 1991; Githeko ***et al.,*** 1993; Hill ***et al,*** 1993; Smith ***et al,*** 1993; Trape ***et al,*** 1993). Despite this intensive sampling the numbers of ***An. gambiae s.l*** caught were so low that estimates of annual EIRs have to been taken into caution. Surely, transmission was occurring year-round, sometimes at rates below our threshold for detection. Given the degree of over-dispersion of vectors within the study area such sampling could" easily misrepresent the true transmission profiles within a small geographical scale.

As reported here transmission intensity as reflected by the EIRs are

relatively low compared with other parts of Africa (Lindsay ***et al.,*** 1989; 1993; Beier ***et al.,*** 1990; Githeko ***et al.,*** 1993; Smith ***et al.,*** 1993). Most individuals can expect to receive less than 10 infective bites per year. Despite the low intensity of ***P. falciparum*** transmission malaria still causes 1 in 15 children to develop severe, life threatening malaria infections before they reach 5 years of age (Snow ***et al..,*** 1993). Under such conditions, a proportion of infants will pass their first birthday without ever being challenged with malaria. Inspite of the low-level transmission intensity, malaria in Kilifi can be categorised as stable, i.e. malaria that continues to be endemic even at low vector densities. Furthermore, the differences in transmission intensity is further evidenced by age patterns of parasitaemia and splenomegaly (C.G. Nevill, personal comm.).

Several issues warrant further discussion for future entomological study designs. Clearly, it is obvious that the reliability of the measure of sporozoite rates depends on sample size and hence the estimates of EIRs in areas of extremely low vector abundance have wide confidence limits. The large variations between households in the EIR could influence clinical presentation, or the risk of transmission from the human host to the vector. However, there is little evidence to suggest vector exposure as a risk to clinical presentation of malaria. Despite this, minimum hospital-based rates of severe malaria in this area were still high with a 1.8% minimum

risk of developing severe malaria infections (Snow et al.,1993; 1994a). Spatial and temporal patterns of severe disease varied independently of vector abundance. An increase in vector abundance, does not correspondingly increase the incidence of severe malaria. Findings that most of the severe malaria cases occurred in houses where no or few vectors were captured highlights the possible role of human host- and parasite-related determinants of severe disease (Greenwood ***et al,*** 1991; Marsh, 1992).

The number of blood-seeking ***An. funestus*** and ***An. gambiae s.l.*** Collected indoors in CDC light-traps were highly correlated with human-biting rates. CDC light-traps collected about two times more female vectors in houses where bednets were used than in houses where they were not, confirming that where possible people should be protected by nets to increase the efficiency of light traps in houses.

This study demonstrates associations between low-levels of ***P. falciparum*** transmission and relatively high incidence rates of severe malaria on the Kenyan coast. These findings are atypical of other areas of stable endemicity in Kenya (Beier ***et al.,*** 1990; Githeko ***et al.,*** 1993). The only comparable data are those from The Gambia and Congo where transmission is also low but markedly seasonal, with

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site-specific EIRs ranging from 4 to 24 infective bites in The Gambia (Snow ***et al,*** 1988a; 1988b; Lindsay ***et al,*** 1989; 1990; Alonso ***et al,*** 1991), and 1 to <100 in Congo (Trape ***et al,*** 1987). The considerable

variation from 100 infective bites in the semi-rural, peripheral areas of Brazzaville and the central area, where people could expect to receive one infective bite every three years, was not associated with any differences in hospital admissions for "pernicious" malaria or hospital-based mortality from malaria.

Given the high burden of malaria disease on the Kenyan Coast, low transmission pressure, and specific behaviour of the local vectors (predominant indoor feeding on man) a bednet programme is a logical intervention to assess. Yet successes or failures may not be uniform throughout the selected study area given the marked heterogeneity in mosquito distributions.

Although studies in Africa have shown decreases in childhood mortality by the use of insecticide-impregnated bednets (Alonso ***et al.,*** 1991), levels of transmission that are acceptable from the public health perspective have not yet been established. More data is required on the relationship between disease and transmission at higher levels of transmission but the findings that high rates of severe disease "can occur even at very low-levels of transmission raises questions regarding long-term impact of vector control efforts in Africa and elsewhere (Trape ***et al.,*** 1993). It is clear that reduction of high transmission, even to levels where annual EIRs are <10, will not prevent unacceptably high levels of severe disease.

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Appendix 1

HOUSE-ENVIRONMENTAL VARIABLES

Site I I I I Case-control number I I I I I I

Name of head of household

PEOPLE IN HOUSE

1. How many people sleep in the house? . . . . . I__I

   How many children under 7 years? . . . . . I__I

   How many from 7 to 20 years? . . . . . I__I

   How many over 20 years? . . . . . 1__I

2. How many rooms are there in the house? . . . . . I__I

   How many are sleeping rooms? . . . . . I I

   How many beds are in the house? . . . . . I__I

BEHAVIOUR/ACTIVITY

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

4. Kitchen: where is the cooking normally done? (1) seperate room inside the house (2) separate room outside the house (3) outside in open space (4) inside living quarters . . . . . I__I

5. Toilet: what type does the family use? (1) W.C. (2) private pan (3) public latrine (4) private pit (5) none_____I__I

6. Is water often stored in open containers? (Y/N)_____I__I
If yes, how long is it stored (in days)?. . . . . I__I

7. Are mosquito coils used (1) regularly (2) every night during peak insect abundance (3) occasionally (4) never?. . . . I__I
Were they burnt last night? (Y/N). . . . . I_I

8. Are insect sprays in cans used (1) regularly (2) every night during peak insect abundance (3) occasionally (4) never? I__I
Was it used last night (Y/N). . . . . I_I

9. How many mosquito nets in the house?_____I__I
If one or more, how many individuals slept under the net last night?. . . . . I_I

10. What time do the family normally retire to bed?I__I__I__I_I

ANIMAL HUSBANDRY

11. How many domestic and cash animals are kept close to the house (i.e., tethered, penned or corralled), count and list

numbers:

Donkeys/horses_

cows

Dogs

Cats

Sheep

Goats. . . . . I__I__I

Chickens. . . . . I__I__I

Ducks. . . . . I__I__I

O t h e r

(describe)_

HOUSE CONSTRUCTION

12. What are the two main construction material for the outer wall? (1) Mud (2) makuti (3) concrete (4)stone (5) sisal bags

(a)_____(b)_____I__I__I

Are the outer walls painted? (Y/N). . . . . I__I

If yes, is it completely painted (Y/N). . . . . I__I

»

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

15. Does the index coldest sleeping room have a ceiling (Y/N)I__I

If yes, describe the ceiling material (1) wood (2) mat (3) makuti (4) soft board (5) mud . . . . . I__I

If yes, what is the percent of ceiling coverage, (1) less than 20% (2) 20-50 % (3) 50-90% (4) over 90% . . . . . I_I

16. Are the interior walls of different material (Y/N) . . . . I__I

If yes, describe interior wall material (CS) . . . . . I I

Are the interior walls painted (Y/N) . . . . . I__I

17. Number of windows in the house . . . . . I__I

How many are over 90% screened . . . . . I__I

How many have wooden shutters . . . . . I__I

18. Number of outer doorways . . . . . I__I

How many have doors . . . . . I__I

How many have screens/curtains . . . . . I__I

19. Does the house have eaves (Y/N) . . . . . I__I

Are the eaves (1) small (2) medium (3) large . . . . . I__I

Do the eaves go all around the house (Y/N) . . . . . I__I

20. Do the walls have any large holes or openings to the outside, except windows, eaves, or doorways (Y/N) . . . . . I__I

If yes, describe (1) many (2) a few (3) very few . . . . . I__I

21. Has the house ever been residual sprayed (Y/N) . . . . I__I

If yes, when (MMYY) . . . . . I__I__I__I__I

ENVIRONMENT

22. Does the house stand in a clearing (Y/N) . . . . . I__I

23. Are there large fields farmed within 50 meters of the house (Y/N) . . . . . I_I

If yes, are these crops grown?

coconuts (Y/N) . . . . . I_I

cashewnuts (Y/N) . . . . . I_I

cotton (Y/N) . . . . . I_I

cassava (Y/N) . . . . . I_I

castor oil (Y/N) . . . . . I_I

maize (Y/N) . . . . . I_I

mangoes (Y/N) . . . . . I__I

other (describe) . . . . . I__I

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

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

Broken pots (Y/N) . . . . . II #

coconut husks (Y/N) . . . . . I__I

tires (Y/N) . . . . . I I

stones (Y/N) . . . . . I_I

other (describe)

Is there standing water within 20 meters of the house (YJN)I

If yes, are they large ponds (Y/N) . . . . . I__I

pools (Y/N) . . . . . I_I

swamps (Y/N) . . . . . I__I

other (describe) . . . . . I I

How many borrow pits are within 20 meters of the houseP__I

Appendix 2

SPOROZOITE ELISA SOLUTIONS:

Reagents preparation:

1. Phosphate Buffered Saline (PBS-plain):

This reagent is used for the dilution of capture monoclonal antibody (MAb) before the plates are coated and when preparing the blocking buffer and PBS-Tween.

(i) Rinse the preparatory plastic bottle with distilled water.

(ii) Add 1 litre of distilled water into the bottle,

(iii) Pour the whole content of 1 Dulbecco's PBS bottle (9.7gm) into the bottle and place a magnetic stirrer. Leave for 10 minutes to dissolve.

(iv) The solution is now ready for use. Store in fridge when not in use.

NOTE: Shelf life is 14 days.

2. **PBS-Tween 20:**

This is a wash Solution. It. is used to wash plates.

(i) Put 1 litre of PBS plain in the bottle,

(ii) Add 500 of Tween-20,

(iii) Mix well using magnetic stirrer.

(iv) Keep in the fridge when not in use.

NOTE: Make each day do not store.

3. **Blocking" Buffer or Boiled casein**

To 1 litre of PBS plain add,

(i) 10 g Bovine Serum Albumin (BSA)

(ii) 5 g casein

(iii) 0.1 g thimerosal

(iv) 0.02 g phenol red

OR

Suspend casein on 0.1 N Sodium hydroxide and bring to boil. After casein has dissolved, slowly add PBS, allow to cool and adjust the pH to 7.4 with Hydrochloric acid (HC1). Add thimerosal and phenol red. Thus;

Casein (0.5%)	2.5 g	5.0 g
0.1 N NaOH	50 ml	100 ml
PBS (pH 7.4)	450 ml	900 ml
Thimerosal	0.05 g	0.1 g
Phenol red	0.01 g	0.02 g

Mix well using a magnetic stirrer, leave it to mix for 2 hours or more. Keep in the fridge when not in use.

»

NOTE: This reagent is used to block the plates and to prepare the Nonidet P-40.

Shelf life is 1 week at 4"C.

4. Nonidet P-40 (NP-40):

To 40 ml of Blocking Buffer add 200 /d of NP-40, mix well and store in fridge when not in use.

5. **2A10 Monoclonal antibody (capture MAb):**

This is usually in **clear white bottles.**

(i) Put 5 ml of PBS plain into a tube (for only one plate).

(ii) Add 20 /JL\ of the capture MAb.

(iii) Mix well and dispense 50 /xl into each well of the PVC plate.

NOTE: The amount is adjusted according to the number of plates.

6. **Peroxidase Labelled Enzyme (2A10).**

This is usually in **dark brown bottles**

(i) Put 5 ml blocking buffer into a clear tube

(ii) Add 10 /xl of the 2A10 Peroxidase labelled enzyme (conjugate) into the tube and mix.

This is enough for only one plate.

7. **Peroxidase Substrate solution.**

Mix equal parts (1:1) of solution A and solution B; i.e. for 1 plate mix 5 ml of solution A and 5 ml solution B. It should be noted that this mixing is only required when using the 2 component substrate. In a one component substrate no mixing is required. Store in the fridge.

NOTE:

Solution A (ABTS) is 2.2' azino-di(3-ethyl-benzethiazoline sulphonate.

Solution B is Hydrogen peroxide.

8. Enzyme check:

Put 100 ij. 1 of substrate into a vial and add 3 of fresh prepared enzyme. Mix and observe colour change from colourless to blue.

9. **Positive control: R32tet$_{32}$ Reconstitution.**

Plasmodium falciparum

Xi) Dissolve lyophilized R32tet$_{S2}$ (10 /xg) with 1000 jul distilled water to yield 100ng/10jul. This is normally referred to as **Vial** I

(ii) Vial II stock solution.

(a) Put 1000 **/JL** Blocking Buffer into a tube

(b) Add 10 **/JL** (lOOng) of the R32tet$_{32}$ from vial I, and mix well. This gives 1000 pg/10 /xl BB.

Store in the freezer. It is also used to prepare the working control vial III when loading the triturates.

(iii) Vial III working solution.

(a) Put 1000 **/JL** BB into a vial

(b) Add 20 /il (2000 pg) R32tet„ from vial II, and mix well. This yields 100 pg/50 /ul BB (control I).

OR

(a) Put 500 fi\ BB into a vial

(b) Add 10 fi\ (1000 pg) R32tet:tt from vial II, and mix well. This gives 100 pg/50 /JL\ of BB (control I).

Make 1:10 dilution to get 10 pg/50 /JL\ blocking buffer.

e.g. Put 450 /JL\ BB into a tube and transfer 50 FX 1 from vial III (100 pg/50 /JL\) and mix well. This is control II. Keep in fridge.

10. **Negative controls:**

The negative controls are prepared from the head/thorax of the wild males of the same species or from the laboratory reared females.

- (i) Put 50 /JL\ of NP-40 into a vial
- (ii) cut the head/thorax and put into the vial
- (iii) Leave for sometimes to soften
- (iv) Grind with a pestle and adjust the volume by adding 200 jx\ of blocking buffer.
- (v) Keep in the freezer when not in use.

11. **Capture MAb (lyophilized MAb) and Peroxidase conjugated MAb Reconstitution.**

Dissolve the lyophilized MAb (0.1 mg/vial) and the peroxidase conjugated MAb (0.1 mg) in 0.2 ml (200 /xl) of diluent (1:1 distilled water and glycerine). Store at 4"C or -20"C.

TECHNIQUE

I: Triturate preparation

(a) Label the vials with corresponding numbers as marked in the ELISA working sheet.

(b) Add 50 jitl of BB-Np-40 into each vial

(c) Using a sharp clean surgical blade cut the mosquito between the thorax and the abdomen.

(d) Pick the head thorax with forceps and transfer to the sporozoite marked vial and the abdomen to the corresponding vial marked blood meal if the mosquito is blood fed. If not blood fed or no blood meal analysis required, discard.

(e) Leave the head/thorax to soak in the NP-40 for 20 minutes.

(f) Use a non absorbent glass or plastic rod (pestle) to grind the mosquito in the vial.

(g) Adjust the volume by adding 200 (A of the blocking buffer.

(h) Clean the pestle and wipe it dry with gauze before grinding the next sample to avoid contamination. This is repeated until all samples are prepared. Keep in the freezer until use.

II: Plate coating

(a) Label the PVC plates with appropriate number.

(b) Into each well of clean PVC plate add 50 fx\ of the diluted capture MAb.

(c) Cover the plates and incubate for 30 min at room temperature in subdued light.

III: Blocking the plates

(a) Using an eight-channelled manifold attached to a vacuum pump, aspirate the capture MAb from the microtitre plate.

(b) Bang the plate hard on an absorbent tissue paper or gauze to ensure complete dryness of the plate.

(c) Fill each well with blocking buffer (pH 7.4) using a manifold attached to a 60 /xl syringe. Incubate for 1 hour at room temperature in subdued light.

IV: Loading" the plates with triturates

(a) Aspirate the blocking buffer from the wells using the manifold attached to a vacuum pump and bang to complete dryness.

(b) Using labelled ELISA processing sheets, as a guide, put 50 /x 1 of 100, 50, 25, 12, 6, 3, 1.5, 0 pgs positive controls in the first column wells. Into the next column wells add 50 /JL\ of the negative controls.

(c) Load the first mosquito sample (50 triturate) into the third column well (A3) and continue in the horizontal order up to the last well in the plate.

(d) Cover thfe plate and incubate for 2 hours at room temperature in subdued light.

V: Addition of conjugate (Peroxidase enzyme)

(a) After 2 hrs aspirate the triturate from the wells

(b) Wash the plate 2 times with PBS-TWEEN 20.

(c) Add 50 of the peroxidase labelled enzyme and incubate for 1 h at room temperature.

VI: Addition of substrate

(a) Aspirate the enzyme from the wells and wash 3 times with PBS-Tween 20 and banging it to dryness.

(b) Using an octapete multichannel pipette add 100 /JL\ of the substrate mixture and incubate for 30 minutes. Results are read visually or at 414 nm using an ELISA plate reader.

Appendix 3

BLOODMEAL ELISA SOLUTIONS

1. Phosphate buffered saline (PBS), pH 7.4:

Use stock laboratory PBS or add 1 bottle of Dulbecco's BS to 1 litre of distilled water, mix and adjust pH if necessary. Store all of the following solutions at 4"C.

2. **Boiled Casein, 0.5% (BC):**	500 ml	1 litre
Casein	2.50 g	5.00 g
0.1 N NaOH	50.0 ml	100 ml
PBS, pH 7.4	450 ml	900 ml
Thimerosal	0.05 g	0.10 g
Phenol red	0.01 g	0.02 g

a) Suspend casein in 0.1 N NaOH and bring to boil.

b) After casein is dissolved, slowly add PBS, allow to cool and adjust the pH to 7.4 with HC1.

c) Add the thimerosal and phenol red.

d) Shelf lif£ - one week.

3. **Wash solution (PBS-Tw):**

PBS plus 0.05% Tween 20. Add 0.5 ml of Tween 20 to 1 L of PBS. MIX WELL. Do not store. Make each day.

4. **Enzyme Diluent (BC-Tw):**

100 ml + 25 /JL\ Tween 20. Do not store. Make each day.

HUMAN AND BOVINE HOST BLOODMEAL PROTOCOL FOR ELISA

A. Sample Preparation:

1. **Negative controls** - Grind male mosquitoes in 500 /JL\ of PBS per mosquito.
2. **Positive controls-** For each host serum: To 500 /JL 1 PBS add 5 i\ of host serum control
3. **Blood-meal Samples:-** Dilute each mosquito abdomen sample in 1000 n\ of PBS.

   -to ensure proper grinding, first put 100 of PBS and grind with a grinder, then add 900 ^ul PBS to raise to the required volume.

B. Technique:

1. To a PVC flex plate add:

   -column i add 50 ^tl/well of eight negative controls

   -column 2 add 50 ^I/well of eight positive controls (chicken human, pig, cat, horse, cow, goat & dog).

   -one well should be designated as a blank control and receive 50 æal of PBS alone.

   -The remaining wells should receive 50 ^il/well of mosquito

blood meal sample.

2. Incubate overnight at room temperature.

3. Wash plate with PBS-Tween 2 times.

Enzyme - Conjugate preparation:

To 5 ml of BC-Tw (Enzyme diluent) add:

HRP phosphate anti-human 2.5 /JL\ (1:2000 dilution)

Bovine phosphatase Conjugate . . . . . — 20.0 (1:250 dilution), and

Sera of each host except the one being tested for . . . . . 10.0 /JL\ (1:500 dilution).

NOTE: Add serum from all hosts except those for which enzyme-conjugate was added. All HRP conjugates should be diluted 1:2000. Cow which is a phosphatase conjugate should be diluted 1:250.

4. Add 50 /il/well of the prepared enzyme conjugate solution.

5. Incubate for 1 h at room temperature.

6. Wash plate with PBS-TW 3 times.

Peroxidase substrate preparation: Mix solutions A and B together, 1:1 i.e., 5 ml +5 ml per plate.

7. Add 100 /JL\ of substrate to each well

8. Incubate for 30 minutes

9. Read absorbance visually or at 414 nm with ELISA reader.

10. Wash plate with PBS-TW 3 times.

Phosphatase substrate preparation:

Add 2 tablets to 8 ml of distilled water and 2 ml of diethanolamine buffer.

11. Add 100 /JL\ of the phosphatase substrate to each well.
12. Incubate for 5 hrs and read absorbance at 414 nm (you can read from 2 hrs onwards).

Notes on bloodmeal ELISA

Preparations for PBS (7.4), BC 0.5% and PBS-Tween are as for sporozoite ELISA.

"For goat blood meal ELISA follow same directions except for use of conjugate use HRP goat dilution as in human ie 1:2000 (2.5 /JL\).

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