

**SEASONAL VARIATIONS IN INFECTIONS OF *ANOPHELES*  
*GAMBIAE* MOSQUITOES WITH *PLASMODIUM* OOCYSTS AND  
ITS EFFECTS ON HIGHLAND MALARIA IN KISII TOWN AND  
ITS SURROUNDINGS.**

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Applied Sciences of Kenyatta University.**

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**DECLARATION**

I hereby declare that this is my original work and has not been presented for degree or any other award in any other University.

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**DEDICATION**

I dedicate this thesis to my parents who gave me financial support, my wife Maureen, daughter Michelle and son Miguel for their understanding and encouragement during the study time.

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**ABBREVIATIONS AND ACRONYMS**

AIDS	Acquired Immune Deficiency Syndrome
ANOVA	Analysis of variance
HIV	Human Immune Deficiency Virus
ICIPE	International Center for Insect Physiology and Ecology
KARI	Kenya Agricultural Research Institute
MIR	Minimum infection rate
MoH	Ministry of Health
P.E	Pre-erythrocytic cycle
pH	Hydrogen ion concentration
SAS	Statistical Analysis System
WHO	World Health Organization
G6PD	Glucose-6-Phosphate dehydrogenase
ST	Subtertian Malaria
MT	Malignant tertian Malaria

**ABSTRACT**

Malaria is a serious threat to human life in sub-Saharan Africa. For a long time the disease occurred in areas with an altitude below 5000 feet, but with time it has encroached highland areas. The western Kenya highlands which were in the early 1900s considered as a safe haven from the surrounding malaria prone areas have also been invaded. In Kisii highlands with an altitude of about 6000 feet, the disease has been getting more prevalent with time, claiming many lives and causing the greatest morbidity as compared to other infectious diseases. Transmission of the disease depends on the presence of a female *Anopheles gambiae* mosquito which acts as the vector. The female *Anopheles gambiae* mosquito acts as the definitive host of *Plasmodium* protozoa, and allows sexual reproduction to take place in its gut. Infection rates may vary seasonally due to temperature changes, but this occurrence is not well studied in the Kenyan highlands. This study therefore aimed at investigating the seasonal variations in infection of *Anopheles gambiae* by *Plasmodium* oocysts and its implications on malaria prevalence in human beings. The study was conducted in three estates namely, Darajambili, Mwembe, and Maili Mbili which surround Kisii town in Nyanza province. Three villages in Keumbu location which is in the outskirts of Kisii town were also sampled to represent the rural population. Bi-weekly collection of mosquitoes during both wet and dry seasons was done for a period of 2 months in each season. Data were collected by demonstrating the presence or absence of *Plasmodium* oocysts in the mosquito gut. About 1625 mosquitoes were collected from a total of 288 houses. The collected mosquitoes were sorted to species level using physical appearance before dissection to demonstrate *Plasmodium* oocysts in the gut of the vector. All patients visiting Kisii and Keumbu hospitals respectively from the estates with malaria related symptoms were examined for *Plasmodium* in blood smear stained with Giemsa. Differences between infections in mosquitoes and mosquito densities in the two seasons were analyzed by t-test. The correlation between human parasitemia and mosquitoes infections were tested using Pearson Correlation coefficient. It was observed that the densities of mosquitoes increased with increase in rainfall, temperature, vegetation cover and presence of human hosts. It was also observed that malaria infections in humans are dependent on oocyst density in the invertebrate host ( $P < 0.05$ ). This information forms a basis of educating the community on potentiality of mosquitoes to transmit malaria parasites at different times of the year. It also forms a ground for developing a malaria control strategy.

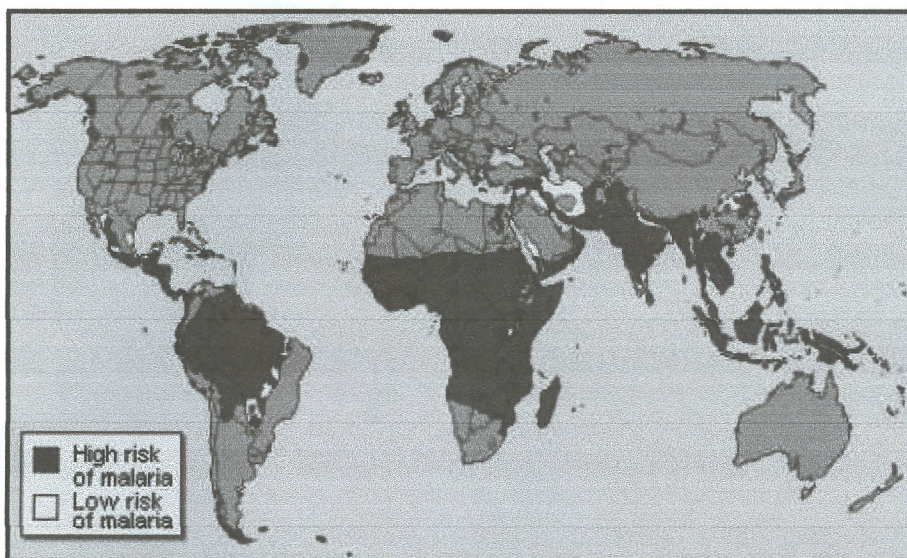
## CHAPTER ONE: INTRODUCTION

### 1.1 Background Information

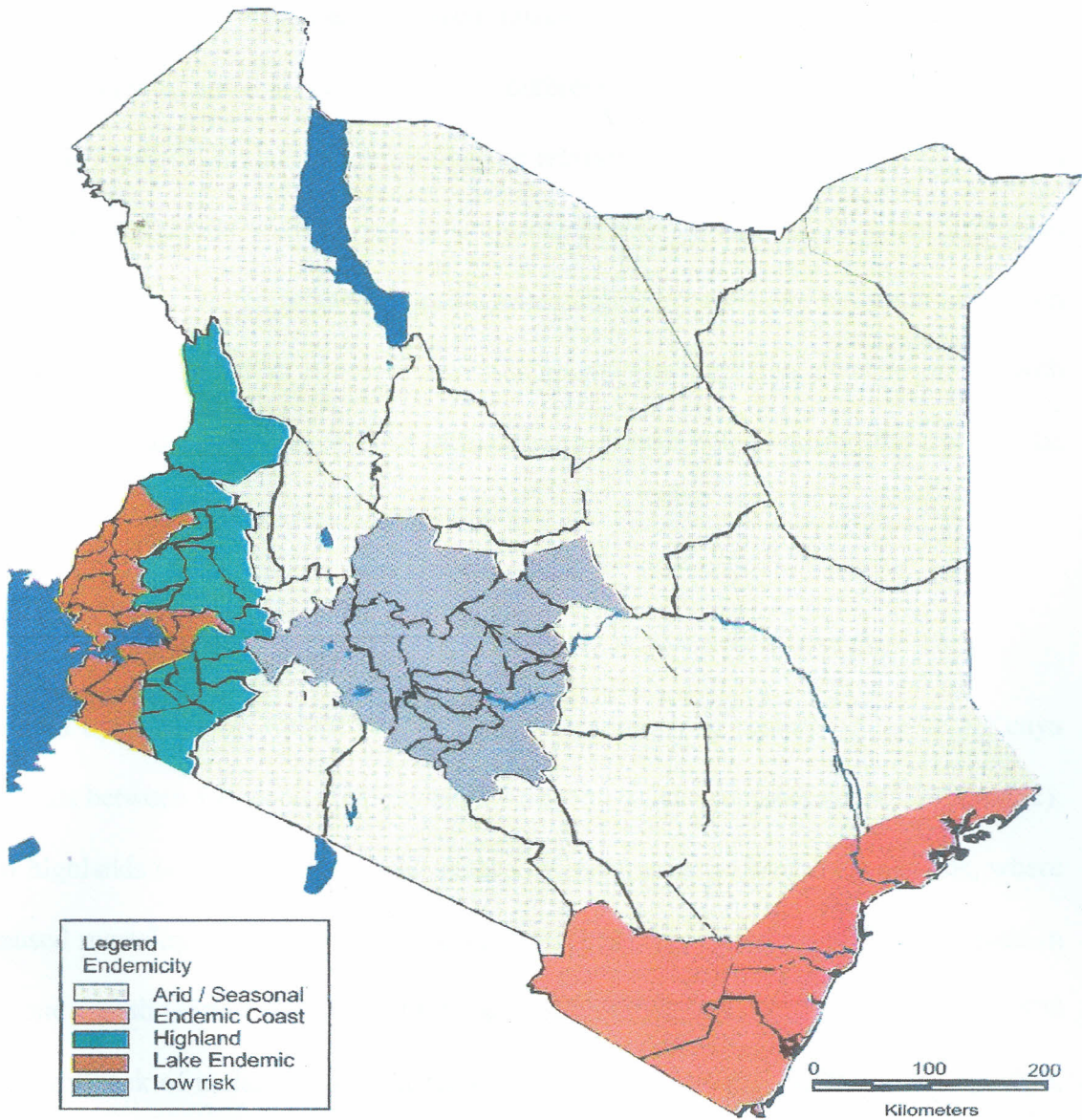
The highlands of Kenya were regarded by the colonial settlers as safe from the surrounding lowland malaria areas of Uganda and Kenya. Between 1980 and 1999, a series of malaria “epidemics” were reported in Kisii and other communities located on the highlands (Hay *et al.*, 2002a). At an altitude of about 6000 feet, Kisii had been considered too high for the survival of the mosquito that harbors the parasite (Fisher, 2008). After the World War II (1939-1941), malaria encroached into the highland communities as a result of wide scale population settlement that was linked to increased transportation which enabled the inhabitants to access areas that were endemic for malaria. The emergence of malaria in the highlands has also been related to the increased agricultural development in the region with the arrival of colonial settlers (Githeko, 2011). Climate changes provided transiently suitable conditions for development of mosquitoes in regions that were not infested before (Simon *et al.*, 2002).

Malaria is endemic in 106 countries all over the world (Figure 1.1) with about 3.3 billion of the world’s population at risk and an estimated 300-500 million people are affected/ infected with malaria annually resulting in about 655,000 deaths (WHO, 2011). Majority of these people are children under five years and pregnant mothers (WHO, 2011). In Kenya some 25 million people are at risk of malaria which causes more deaths than HIV and AIDS in the country as well as providing highest morbidity and mortality cases of all infectious diseases (GOK, 2001). Between May and July 1999, in Kisii District Hospital alone, 300 people died of malaria and about 18,000 cases were admitted.

The prevalence of the disease in the area is increasing with time and it is listed among the most vulnerable areas in the country (Fisher, 2008; Figure 1.2). Malaria in the western highlands is commonly spread by *Anopheles gambiae* which account for about 80% of the mosquito vector in the region (Fisher, 2008). This study investigated whether infection in the mosquito vector *Anopheles gambiae* by malaria parasites has effect on the number of infections in humans in Kisii district.



**Figure 1.1: Distribution of malaria in the world, 2010 ( Source: Leeds University, 2012)**



**Figure 1.2: Endemicity of malaria in Kenya (USAID-KENYA, 2012)**

## 1.2 Statement of the problem

In the past years, there was an alarming increase in malaria cases in the highlands which were considered a haven away from infectious lowland diseases (Hay *et al.*, 2002a). For long there has been research on *Anopheles gambiae* mosquito that transmits malaria in the highlands, but the infections of the *Anopheles gambiae* by *Plasmodium* oocysts has

not been documented particularly for Kisii District. Kisii, being one of the areas that are greatly affected by malaria, experiences the outbreak of malaria in specific seasons in the year. This research aimed at determining the relationship between seasonal variation in mosquitoes infections with oocysts and the occurrence of malaria parasites in human patients. The data will be important in designing targeted control and optimum utilization of resource strategy. The research also documents the infection of mosquitoes with *Plasmodium* oocysts and occurrence of malaria in human beings during the dry and the wet seasons for Kisii town and its surroundings.

### **1.3 Justification for the study**

Research by Hay *et al.* (2002b) on clinical epidemiology of malaria in western Kenya highlands between 1980 and 2000 revealed worrying trends of malaria cases (Figure 1.2). Kisii highlands is one of the worst hit places by malaria in the western highlands, where it caused mortality to 300 people and caused morbidity to 18,000 in a single hospital in only one month (Fisher, 2008). Multiple agencies aimed at raising \$500 million in 1998 for the rollback of malaria across Africa by 2010 had raised only \$200 million by 2002. Two years later, the rate of malaria infection had not reduced but had instead increased by 12% (Kamau, 2011). This calls for cheap and proper strategic approaches to malaria control in this area. Though minimized contact between mosquitoes and human beings has been suggested as the major means of control of the disease, the number of infected mosquitoes and the number of oocysts carried by the mosquitoes in different seasons of the year are important in pointing out the trends of the disease in human beings during the seasons hence would enable early preparation to combat the disease (Hay *et al.*, 2002b).

This study brings out the relationship between infection in mosquitoes by *Plasmodium* oocysts and the occurrence of malaria in human beings.

#### 1.4 Research Questions

- a) What proportions of *Anopheles gambiae* mosquito are infected with *Plasmodium* oocysts during the wet and dry seasons of the year in Kisii town and its surroundings?
- b) What is the occurrence of malaria in patients attending Kisii and Keumbu hospitals during the wet and dry seasons of the year?
- c) How do the infections in *Anopheles gambiae* mosquito correlate with Malaria cases in humans during the wet and dry seasons.

#### 1.5 Research hypothesis

**H<sub>0</sub>:** There is no relationship between infections in *Anopheles gambiae* with *Plasmodium* oocysts and the occurrence of highland malaria in humans in Kisii town and its outskirts.

#### 1.6 Objectives

##### 1.6.1 General Objective

- a) To determine the relationship between *Plasmodium* oocysts infections in *Anopheles gambiae* mosquito and its effects on highland malaria in Kisii town and its outskirts.

## 1.6.2 Specific Objectives

- a) To determine infections of *Anopheles gambiae* mosquitoes with *Plasmodium* oocysts during the dry and wet seasons of the year in Kisii town and its outskirts.
- b) To determine malaria cases in patients attending Kisii Level 5 hospital and Keumbu sub-district hospital during the dry and wet seasons of the year.
- c) To determine the relationship between infections in mosquitoes and occurrence levels of malaria in patients attending Kisii Level 5 and Keumbu sub-district hospitals.

## 1.7 Significance of the study

According to Simon *et al.* (2002), transmission of *Plasmodium falciparum* in high altitude communities is limited by low ambient temperatures. Changes in climate may therefore provide transiently suitable conditions for transmission of the disease in populations that may have acquired little functional immunity, or the ability to harbor parasites in the body without causing a clinical condition. Determining the relationship between the numbers of people infected and the number of infected mosquitoes during different seasons of the year can be of great importance in determining the future occurrences of the disease. The data generated from this study forms a basis of educating the community on the potentiality of mosquitoes in transmitting the malaria parasites during a given season, and advising the Ministry of Health to develop an appropriate disease targeted control and optimal resource utilization strategy.

## CHAPTER TWO: LITERATURE REVIEW

### 2.1 General Introduction

On world-wide basis, malaria is one of the most common human infectious diseases. It is the most prevalent vector-borne disease in the world, threatening some 3.3 billion people in more than 106 countries—40 percent of the world's population. Malaria is estimated to cause up to 500 million clinical cases and 655,000 million deaths each year (WHO, 2011). The vast majority of deaths occur among young children; other high-risk groups include pregnant women and non-immune travelers, refugees, displaced persons, and laborers entering endemic areas. Cerebral malaria produces seizures and unresponsive coma (Warrell *et al.*, 1990). These complications are most frequent among African children (with a peak incidence at 3–4 years of age), but they also occur in Southeast Asian children and among non-immune adults. Although cerebral malaria has a substantial mortality, an uncertain fraction of those who survive (approximately 10 percent) have persistent neurologic deficits at the time of hospital discharge (Carne *et al.*, 1993). Thus, the long-term effects of cerebral malaria on central nervous system development and function (including the ability to learn) are important unresolved questions with major implications for global health and development. Severe malarial anemia produces hemoglobin levels of less than 5 grams per 100 milliliters of blood — less than one-third the normal hemoglobin (Warrell *et al.*, 1990). Although severe malarial anemia is widely prevalent until age 3, its peak incidence occurs around 6–7 months of age (Miller *et al.*, 1994). Kidney failure is a common complication of malaria infection, and a major cause of death among non-immune population (Warrell *et al.*, 1990).

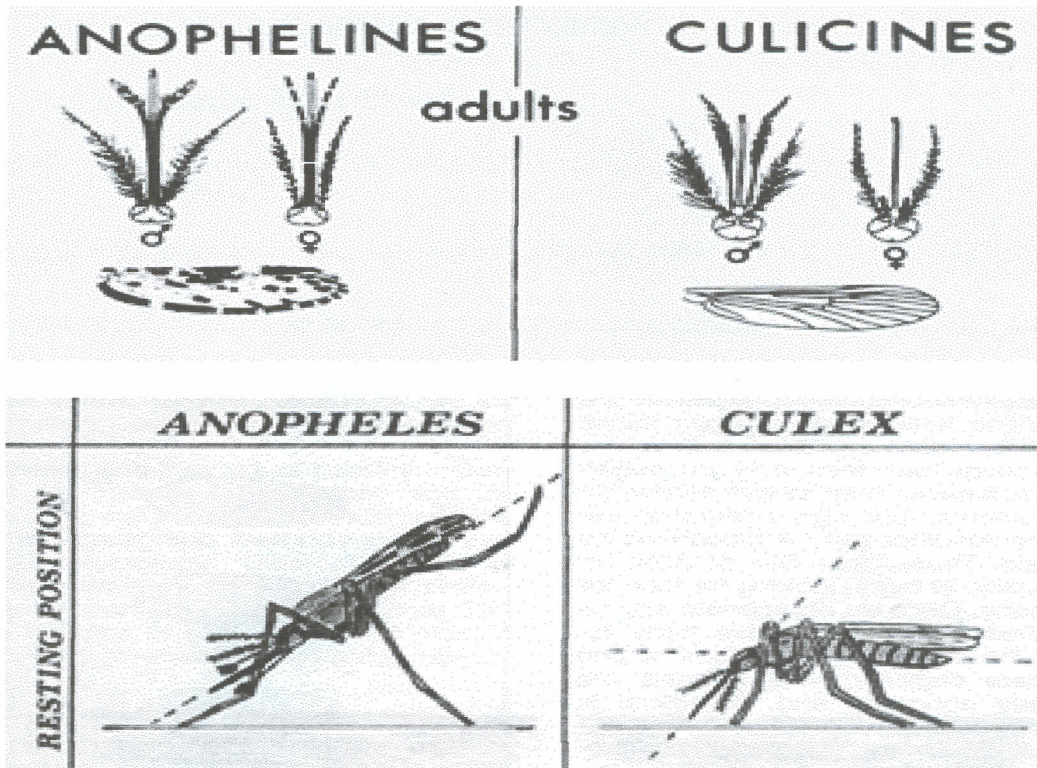
## 2.2 The Malaria Parasite

The malaria parasite belong to the Phylum Apicomplexa, order Haemospororida and family Plasmodiidae (Krief *et al.*, 2010). This family includes genera such as *Plasmodium*, *Haemoproteus* and *Leucocytozoon*, which are the malaria and malaria-like organisms. When in host cells, *Plasmodium* and *haemoproteus* which is a wild animal parasite produce a pigment called hemozoin from host hemoglobin, distinguishing them from the closely related *Leucocytozoon*. The parasite differs from closely related coccidia in that it lacks conoids and syzygy. In *Plasmodium*, the microgametocytes and macrogametocytes develop independently unlike the other two genera (Krief *et al.*, 2010). There are four species of the malaria parasite that parasitize human beings; *Plasmodium falciparum*, *Plasmodium ovale*, *Plasmodium malariae* and *Plasmodium vivax* (Waters *et al.*, 1991). *Plasmodium vivax* causes benign tertian malaria, also known as vivax malaria or tertian ague. *Plasmodium falciparum* causes malaria known as malignant tertian, subtertian or estive atumnal, which is the most virulent of the *Plasmodim* species in human beings. *Plasmodium malariae* causes Quartan malaria with paroxysms every 72 hours. *Plasmodium ovale* causes ovale or mild tertian malaria, which is the rarest of all the four parasites to human beings (Larry and Gerald, 2006). The early trophozoites of *Plasmodium vivax* are about 1/3 of the diameter of the red blood cells and appear as a chromatin dot which is heavy and with a prominent vacuole (Figure 2.1). Those of *Plasmodium ovale* and *Plasmodium malariae* are similar morphologically in the blood stage (Figure 2.2 and 2.3). Trophozoites of *Plasmodium falciparum*, are about 1/5 of the red blood cells, with small chromatin dot frequently occurring in twos (Figure 2.4).

Microgametocytes and macrogametocytes in *Plasmodium falciparum* are crescent shaped while for the other three, they are oval in shape (Larry and Gerald, 2006).

### **2.3 The mosquito vector and transmission of malaria**

The vector of human malaria belongs to the genus *Anopheles*. There are about 422 known species of *Anopheles* with only 70 being malaria vectors (Lane and Crosskey, 1993). Taxonomy of the genus *Anopheles* is complicated by the existence of several species of complexes (White, 2007). In Africa, members of the *Anopheles gambiae* and *Anopheles funestus* complexes are important vectors (Beaty and Marquaardt, 1996). Mosquitoes undergo complete metamorphosis, with egg, larva, pupa and adult stages. The adult of *Anopheles gambiae* is medium sized with irregular speckled legs, pale spot in 3<sup>rd</sup> dark area of wing vein 1 and lower branch of vein 5. The palps are smooth with three pale rings, thorax is grey, brown or black with creamy yellow scales along the centre of back (Smith and Judith, 1988). The wings have variable pale areas with pale patches on the Costa and two pale interruptions at the base of Costa. The legs are irregularly speckled with a narrow pale ring at tip of tibiae and segment 1-4 of the tarsi are pale. The abdomen is pale brown and hairy often with scales on the back of segment 7 or 8 (Smith and Judith, 1988).



**Figure 2.1 a and b: Morphology of Anopheles and Culex mosquitoes ( Impact malaria, 2013)**

Both the male and the female feed on plant juices, but only the female mosquito bites to get blood meal from animal host to allow oogenesis to be completed (Bruce and Edman, 2004). Of the 422 species, some prefer animal blood while others prefer human blood. *Anopheles gambiae* is host specific, and it bites only human beings to get blood for oogenesis, though they can bite other animals for survival without allowing for oogenesis (Wolfgang *et al.*, 2002). The preferred breeding and resting places are also very important as determinants of malaria transmission. The main mode of transmission is during blood meal, although accidental transmission can occur by blood transfusion and sharing of needles by IV drug users between infected and non-infected individuals. In

addition, infections of newborn from their infected mothers may occur through inter-placental exchange (Bruce-Ehwatt, 1980).

*Anopheles gambiae* prefer to breed in stagnant water around human habitations (Larry and Gerald, 2006). Use of bed nets and protective clothing therefore reduce transmission of malaria parasites by *Anopheles gambiae*. According to Lymo (1993), mosquitoes start entering human habitats at dusk as from 1800 hours. Activities are low until 2200 hours after which they increase gradually and hit a peak between midnight and 0400 hours. The mosquito will rest in the house, shortly after the blood meal to allow digestion to take place, before looking for ovipositing sites. A mosquito that has ingested blood appears red and is said to be fed. As the blood gets digested, the eggs develop. A mosquito with fully developed eggs is said to be gravid (Cruz, 1987). The choice of ovipositing sites is influenced by predators, vegetation, chemicals, bacteria, size and shape of the habitat. For example, presence of some chemicals, predators and bacteria, discourage oviposition, because they destroy the eggs and larva of the mosquitoes (Blaustein and Kotler, 1993).

#### **2.4 Life-cycle of the malaria vector**

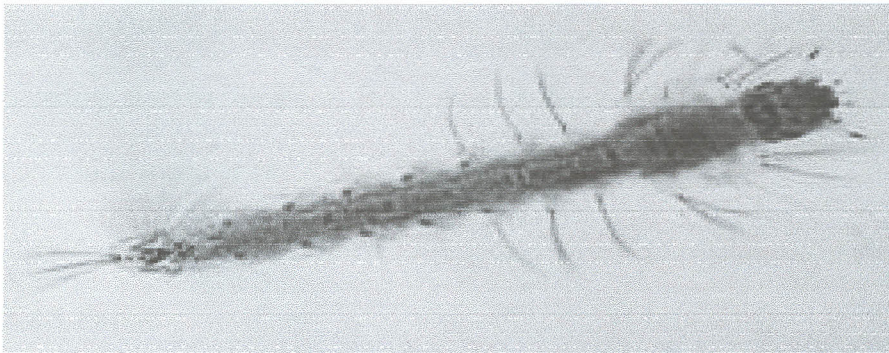
Like all mosquitoes, anophelines go through four stages in their life cycle: egg, larva, pupa, and imago. The first three stages are aquatic and last 5–14 days, depending on the species and the ambient temperature. The adult stage is where the female *Anopheles* mosquito acts as malaria vector.

### 2.4.1 Eggs

Adult females lay 50–200 eggs per oviposition. The eggs are quite small ( $\sim 0.5 \times 0.2$  mm). Eggs are laid singly and directly on water. They are unique in that they have floats on either side. Eggs are not resistant to drying and hatch within 2–3 days, although hatching may take up to 2–3 weeks in colder climates (Cross, 2004).

### 2.4.2 Larvae

*Anopheles* larva is about 8 mm long. Mosquito larvae have a well-developed head with mouth brushes used for feeding, a large thorax and a nine segmented abdomen. They have no legs. In contrast to other mosquitoes, *Anopheles* larvae lack a respiratory siphon and for this reason they position themselves so that their body is parallel to the surface of the water (Figure 2.6).



**Figure 2.2: Photograph of an *Anopheles gambiae* mosquito larva (source: McCafferty, 2010).**

Larvae breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently. The larvae spend most of their time feeding on algae, bacteria, and other microorganisms in the surface microlayer. They dive below the

surface only when disturbed. Larvae swim either by jerky movements of the entire body or through propulsion with the mouth brushes. Larvae develop through 4 stages, or instars, after which they metamorphose into pupae. At the end of each instar, the larvae molt, shedding their exoskeleton, or skin, to allow for further growth. First stage larvae are ~1 mm in length; 4th stage larvae are normally 5–8 mm in length. The process from egg laying to emergence of the adult is temperature dependent, with a minimum time of 7 days (McCafferty, 2010).

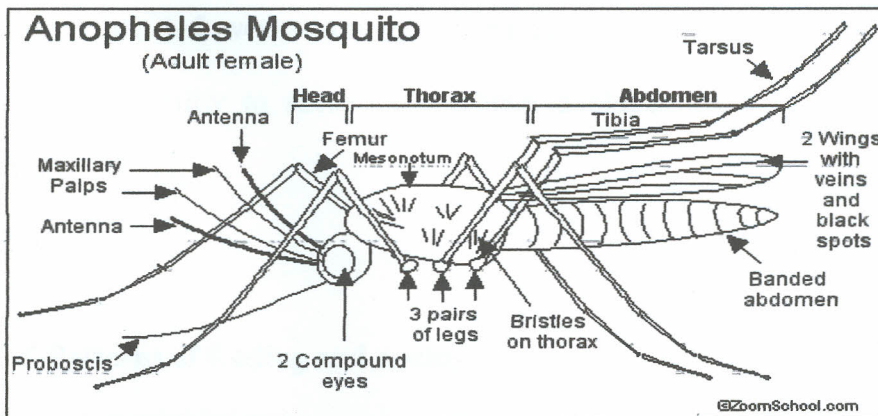
The larvae occur in a wide range of habitats but most species prefer clean, unpolluted water. Larvae of *Anopheles* mosquitoes have been found in fresh- or salt-water marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers, and small, temporary rain pools. Many species prefer habitats with vegetation. Others prefer habitats that have none. Some breed in open, sun-lit pools while others are found only in shaded breeding sites in forests. A few species breed in tree holes or the leaf axils of some plants (Shushinzimana *et al.*, 2006).

### **2.4.3 Pupae**

The pupa is comma-shaped when viewed from the side. The head and thorax are merged into a cephalothorax with the abdomen curving around underneath. As with the larvae, pupae must come to the surface frequently to breathe, which they do through a pair of respiratory trumpets on the cephalothorax. After a few days as a pupa, the dorsal surface of the cephalothorax splits and the adult mosquito emerges (Cross, 2004).

### 2.4.4 Adults

The duration from egg to adult varies considerably among species and is strongly influenced by ambient temperature. Mosquitoes can develop from egg to adult in as little as 5 days but usually take 10–14 days in tropical conditions. Like all mosquitoes, adult *Anopheles* have slender bodies with 3 sections: head, thorax and abdomen (Figure 2.7). The head is specialized for acquiring sensory information and for feeding. The head contains the eyes and a pair of long, many-segmented antennae. The antennae are important for detecting host odors as well as odors of breeding sites where females lay eggs. The head also has an elongated, forward-projecting proboscis used for feeding, and two sensory palps. The thorax is specialized for locomotion. Three pairs of legs and a pair of wings are attached to the thorax. The abdomen is specialized for food digestion and egg development. This segmented body part expands considerably when a female takes a blood meal. The blood is digested over time serving as a source of protein for the production of eggs, which gradually fill the abdomen (Gillet and Smith, 1988).



**Figure 2.3: Structure of an adult *Anopheles gambiae* mosquito (source: Impact Malaria, 2013).**

*Anopheles* mosquitoes can be distinguished from other mosquitoes by the palps, which are as long as the proboscis, and by the presence of discrete blocks of black and white scales on the wings. Adult *Anopheles* can also be identified by their typical resting position: males and females rest with their abdomens sticking up in the air rather than parallel to the surface on which they are resting. Adult mosquitoes usually mate within a few days after emerging from the pupal stage. In most species, the males form large swarms, usually around dusk, and the females fly into the swarms to mate (Cross, 2004).

Males live for about a week, feeding on nectar and other sources of sugar. Females will also feed on sugar sources for energy but usually require a blood meal for the development of eggs. After obtaining a full blood meal, the female will rest for a few days while the blood is digested and eggs are developed. This process depends on the temperature but usually takes 2–3 days in tropical conditions. Once the eggs are fully developed, the female lays them and resumes host seeking. The cycle repeats itself until the female dies. While females can live longer than a month in captivity, most do not live longer than 1–2 weeks in nature. Their lifespan depends on temperature, humidity, and also their ability to successfully obtain a blood meal while avoiding host defenses (Lyimo, 1993).

## **2.5 Patterns of feeding and resting**

Most *Anopheles* mosquitoes are crepuscular (active at dusk or dawn) or nocturnal (active at night). Some *Anopheles* mosquitoes feed indoors (endophagic) while others feed outdoors (exophagic). After feeding, some blood mosquitoes prefer to rest indoors

(endophilic) while others prefer to rest outdoors (exophilic), though this can differ regionally based on local vector ecotype, and vector chromosomal makeup, as well as housing type and local microclimatic conditions (Lyimo, 1993). Biting by nocturnal, endophagic *Anopheles* mosquitoes can be markedly reduced through the use of insecticide-treated bed nets (ITNs) or through improved housing construction to prevent mosquito entry (e.g. window screens). Endophilic mosquitoes are readily controlled by indoor spraying of residual insecticides. In contrast, exophagic/exophilic vectors are best controlled through source reduction (destruction of the breeding sites) (Burkot, 1998).

## 2.6 Vector distribution

Geo-distribution of the *Anopheles* mosquito vector of malaria is affected by complex interaction of biogeography, including biotic (e.g. competition and dispersal) and abiotic factors (e.g. climate and topography) that vary in both time and space. Africa has over 140 recorded *Anopheles* species, of which at least eight are considered to be effective vectors of malaria (Gillies *et al.*, 1987). Two of the most efficient vectors of human malaria, *Anopheles gambiae sensu stricto* (hereafter *An. gambiae*) and *Anopheles arabiensis* are members of the *An. gambiae* complex (White, 1994). Members of the *Anopheles gambiae* have been reported from most countries of Western, Central, Eastern and Southern Africa and other adjacent islands including Madagascar as well as South Arabia and Yemen (Minakawa *et al.*, 2005) Figure 2.4 shows the distribution of *Anopheles gambiae* in Kenya.

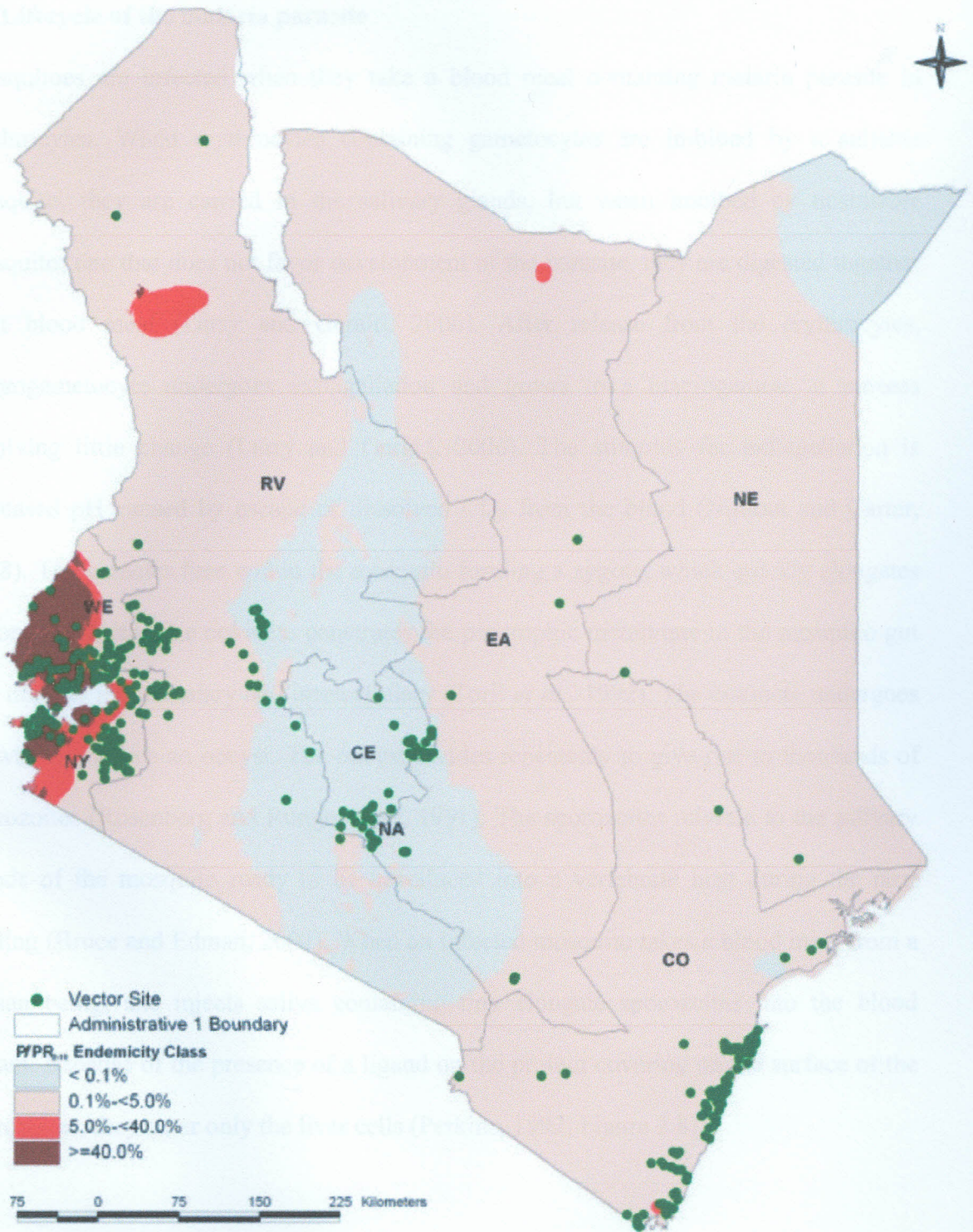


Figure 2.4: Map showing vector distribution in Kenya (Minakawa *et al*, 2005)

## 2.7 Lifecycle of the malaria parasite

Mosquitoes are infected when they take a blood meal containing malaria parasite in erythrocytes. When erythrocytes containing gametocytes are imbibed by a suitable mosquito, they are carried in the salivary glands, but when imbibed by unsuitable mosquito; one that does not favor development of the parasite, they are digested together with blood meal (Larry and Gerald, 2006). After release from the erythrocytes, macrogametocyte undergoes exflagellation and grows to a macrogamete, a process involving little change (Larry and Gerald, 2006). The stimulus for exflagellation is increased pH caused by escape of dissolved CO<sub>2</sub> from the blood (Nijhout and Carter, 1988). The gametes fuse within the mosquito forming a zygote, which quickly elongates to form ookinete. The ookinete penetrates the peritrophic membrane in the mosquito gut and migrate intracellularly and intercellularly (Torii *et al.*, 1992). The ookinete undergoes growth to produce an oocyst. The oocyst divides repeatedly to give rise to thousands of sporozoites (Rosenberg and Rungsowgse, 1991). The sporozoites migrate to the salivary glands of the mosquito ready to be introduced into a vertebrate host during the next feeding (Bruce and Edman, 2004). When an infected mosquito takes a blood meal from a human being, she injects saliva containing tiny elongate sporozoites into the blood stream. Because of the presence of a ligand on the protein covering on the surface of the sporozoites, they enter only the liver cells (Perkins, 1992; Figure 2.8)

## 2.8 Pathology in falciparum malaria

Malaria caused by *Plasmodium falciparum* is referred to as falciparum malaria, formerly known as subtertian (ST) or malignant tertian (MT) malaria. It is the most widely spread

accounting for up to 80% of malaria cases in the world. This species is the most pathogenic of the malaria species with untreated infections causing severe disease and death, particularly in young children and pregnant women (Larry and Gerald, 2006). Penetration into the cells of the host is facilitated by extrusion of protein from the rhoptries (Perkins, 1992).

According to WHO (2000), the pathogenicity of this infection is mainly due to: the cytoadherence of *falciparum* parasitized red cells, causing the cells to adhere to one another and to the walls of the capillaries in the brain, heart, spleen, intestines, lungs and placenta; sequestration of parasitized cells in the microcirculation causes congestion, hypoxia, blockage and rupturing of small blood vessels and high levels of parasitaemia result in the activation of cytokines and destruction of many red blood cells. *Falciparum* malaria parasitaemia can exceed 250,000 parasites per microlitre of blood, while up to 30-40% of the red cells may become parasitized. Severe *falciparum* malaria is associated with cerebral malaria, black water fever, severe anaemia, hypoglycaemia and complications in pregnancy (WHO, 2000).

Cerebral malaria is the commonest cause of coma and death in *falciparum* malaria, especially in children and non-immune adults. In the case of cerebral malaria, many parasitized cells can be found in the capillaries of the brain and in the late stages, hemorrhage from small vessels occur (Erick, 1999). Black water fever is a rare but acute condition where there is rapid and massive intravascular haemolysis of both parasitized and non-parasitized red blood cells resulting in haemoglobinaemia, haemoglobinuria and

a fall in haemoglobin (Larry and Gerald, 2006). It is accompanied by high fever, vomiting and jaundice, and is often fatal due to renal failure. Haemoglobinuria can also occur when persons with glucose-6-phosphate dehydrogenase (G6PD) deficiency are treated with primaquine or other oxidant drugs (Larry and Gerald, 2006). Anaemia in falciparum malaria is caused by destruction of parasitized red cells.

Parasitized cells lose their deformability and are rapidly phagocytosed and destroyed in the spleen. In addition, production of red cells in the bone marrow is reduced and there is slow reticulocyte response (Erick, 1999). Falciparum malaria is reported to occur commonly during pregnancy when the immunity is reduced, while in areas of stable malaria transmission, a pregnant woman will have acquired partial immunity to malaria. The immunity normally protects against serious clinical falciparum malaria, but does not prevent heavy parasitic infections of the placenta. Anaemia normally results in a low birth weight babies who in turn have low survival rates (Ma *et al.*, 2009). The first pregnancy is normally at the greatest risk. In areas of unstable malaria transmission, pregnant women lack protective immunity, and are at serious risk of developing severe life-threatening falciparum malaria, particularly in the last months of pregnancy. Such infections if untreated result in abortion, still birth, pulmonary edema and hypoglycemia (Ma *et al.*, 2009).

## **2.9 Clinical manifestations and diagnosis of malaria**

The major clinical signs and symptoms of malaria include fever, headache, shivering, vomiting and diarrhea (Davey, 2010). Fever is due to inflammation caused by toxins

released when erythrocytic schizonts rupture, stimulating the secretion of cytokines from leukocytes and other cells. In the early stages of infection, the fever is irregular (Erick, 1999). Splenomegally occurs in all forms of malaria with repeated attack causing enlargement of the spleen. Anemia and jaundice are also features of *falciparum* malaria (Davey, 2010). In severe malaria (caused by *Plasmodium falciparum*), clinical findings (confusion, coma, neurologic focal signs, severe anemia, respiratory difficulties) are more striking and may increase the index of suspicion for malaria (WHO, 2000).

Diagnosis of malaria is normally based on clinical symptoms, Though the most commonly used diagnostic test for malaria is microscopy to detect parasites in stained blood films. Thick blood films are used in routine diagnosis and as few as one parasite per 200  $\mu$ l blood can be detected. The method can be used to differentiate between different parasite species and stages of the life cycle. In cases of low parasitemia visualizing the parasites after staining with a fluorescent dye can be done. Antigen Detection is another method of diagnosis. Various test kits are available to detect antigens derived from malaria parasites. Such immunologic ("immunochromatographic") tests most often use a dipstick or cassette format, and provide results in 2-15 minutes. These "Rapid Diagnostic Tests" (RDTs) offer a useful alternative to microscopy in situations where reliable microscopic diagnosis is not available. (Larry and Gerald, 2006). The third method used in diagnosis is Molecular Diagnosis where Parasite nucleic acids are detected using polymerase chain reaction (PCR). Although this technique may be slightly more sensitive than smear microscopy, it is of limited utility for the diagnosis of acutely ill patients in the standard healthcare setting. PCR results are often not available quickly

enough to be of value in establishing the diagnosis of malaria infection. PCR is most useful for confirming the species of malarial parasite after the diagnosis has been established by either smear microscopy or RDT.

## 2.10 Chemotherapy and Immunity in Malaria

Chemotherapy refers to use of drugs in treatment of malaria. Most malaria control strategies today depend on safe and effective drugs, as they have done for decades (Irwin, 2010). There are only a limited number of drugs which can be used to treat or prevent malaria (Table 2.1). The most widely used are quinine and its derivatives and antifolate combination drug (BLoland *et al.*, 1999).

**Table 2.1: summary of clinically used anti-malarial drugs (G.O.K, 2001)**

Infection	Drugs used in treatment.	Drugs in chemoprophylaxis
All plasmodial infection	Chloroquine	Chloroquine or proguanil
Infection with Chloroquine-resistant <i>P.falciparum</i> .	(i)Mefloquine, (ii)Quinine + pyrimethamine-sulphadoxine(Fansidar) or Doxocycline or berberine (iii)Halofantrine	(i) Mefloquine (i) Chloroquine + proguanil or pyrimethamine-dapsone(maloprim)
Chronic infection	Primaquine	Primaquine

Protective immunity to malaria is primarily a premunition; resistance to super infection while the host immune response controls the number of parasites persisting in the body. Premunition is only efficient as long as not all the parasites are cleared from the body. If a person is completely cured, susceptibility to re-infection returns (Owuso-Agyei *et al.*, 2002). Thus, in endemic areas, infants are protected by maternal blood and children are at risk after weaning. Immunity of children who survive after first attack is stimulated by the bites of infected mosquitoes as long as they live in malaria areas. It is probable that an important mechanism for *Plasmodium* to evade the host defense system involves epitopes (Day and Kimarsh, 1991). In this case, the parasite synthesizes a protein called *P. falciparum* erythrocyte membrane protein/PfEMP1 that appears on the surface of red blood cells. These proteins anchor the red blood cells to the walls of the vessels so that they are not swept and destroyed in the host's spleen. The protein elicits immune responses, and to evade this, the parasite periodically alters the composition of the protein to avoid being recognized by the antibodies (Day and Kimarsh, 1991).

## 2.11 Drug Resistance

Antimalarial drug resistance has been defined as the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the Subject. Resistance of *Plasmodium falciparum* to chloroquine, the cheapest and the most used drug is spreading in almost all the endemic countries. Resistance to the combination of sulfadoxine-pyrimethamine which was already present in South America and in South-East Asia is now emerging in East Africa (WHO, 2000). The malaria parasite is capable

of becoming resistant to the action of anti-malaria drugs. This is due to small changes in the parasite DNA (point mutations). Over-prescription of anti-malarial (confusion with other febrile diseases) and the uncontrolled selling of poor quality drugs contribute to the increase in drug resistant parasites (BLoland *et al.*, 1999). Many antimalarial drugs in current usage are closely related chemically and development of resistance to one can facilitate development of resistance to others. Chloroquine and amodiaquine are both 4-aminoquinolines and cross-resistance between these two drugs is well known. Development of resistance to mefloquine may also lead to resistance to halofantrine and quinine. Antifolate combination drugs have similar action and widespread use of sulfadoxine/ pyrimethamine for the treatment of malaria may lead to increased parasitological resistance to other antifolate combination drug (BLoland *et al.*, 1999).

Since the discovery of the first effective anti-malarial drug in the 16<sup>th</sup> century, people treated for malaria develop relapses, weeks, months or years after an apparent cure. In species that undergo relapse, there are two populations of erythrocytic forms, where one develops rapidly into schizonts but the other remains dormant as hypozoites, which are capable of initiating schizogony later (Alfred, 2005). The World Health Organization has classified resistance into RI, RII, and RIII. In RI, treatment with chloroquine clears parasitaemia but a recrudescence occurs 1-3 weeks later. For RII, following treatment, there is no reduction in parasitaemia. In the absence of effective treatment, the parasitic density continues to rise. In RIII, there is reduction but not a clearance of parasitaemia after treatment with chloroquine. When treatment ceases, the patient condition worsens (Chesbrough, 2002).

## CHAPTER THREE: MATERIALS AND METHODS

### 3.1 Study area

The study was conducted in three estates within Kisii town; Mwembe, Maili mbili, and Daraja mbili, and three villages in Keumbu Location in the outskirts of Kisii town (Appendix i). This study was carried out between the months March and May 2010, during the rain season and June and August 2010, during the dry season. Kisii is one of the seven districts of Nyanza Province, located in the southwest of Kenya. The district lies on a highland equatorial climate which receives rain almost throughout the year with two major rainy seasons; March to May which is the long rainy season and October to November which is the short rainy season. The average rainfall is over 1500 mm (BLoland *et al.*, 1999). Daily mean temperature range from 10<sup>0</sup>C to 30<sup>0</sup>C. The district is highly dissected by rivers flowing west into Lake Victoria which serve as the breeding ground for the vectors. The main malaria vectors in the area are *Anopheles gambiae* and *Anopheles funestus* (Belar *et al.*, 1990; Phillips- Howard *et al.*, 2003). *Anopheles gambiae* forms about 80 percent of mosquito density and is the main malaria vector in the highlands.

### 3.2 Sample size determination

Sample size was determined by use of the formula;

$$n = \frac{Z^2 pq}{d^2} \quad (\text{Fisher, 2008})$$

Where,  $n$  is the desired sample size,  $p$  is the mosquito prevalence in the study area, which was taken to be 0.25% hence;  $p=0.25$  and  $q=1-p$ , hence 0.75,  $d$  is the level of statistical significance corresponding to 95% confidence.

The number of houses sampled were determined as follows;

$$n = \frac{(1.96)^2 \cdot 0.25 \cdot 0.75}{(0.05)^2} = 288$$

For the two seasons, the number of houses required was  $288 \times 2$  which equals to 576, but the study targeted 600 houses.

### 3.3 Study design

The study took a randomized block design. Three estates in Kisii town and three villages in the outskirts of Kisii town were selected purposively based on differences in infrastructure, agricultural practices, and activities of the inhabitants, presence of water bodies and previous malaria outbreaks. Within each village 48 homes out of the about 100 homes were sampled at random depending on proximity and willingness of the owners. The density of *A. gambiae* in Kisii is estimated at 0.25 per house (Lindblade *et al.*, 2000).

#### 3.3.1 Mosquito collections

Mosquitoes were collected using flashlight and aspirator technique. The mosquitoes were collected during the period March to May 2010, representing the long rainy season and June and August 2010, representing the dry season. A total of 600 homes within the estates and their outskirts were visited to collect mosquitoes once every two weeks

between 1800 and 2000 hours. This is the time when mosquitoes start entering human habitats (Lyimo, 1993). Three collectors moved randomly in the homes within the estates, depending on proximity and willingness of the owners, and collected both blood engorged and unfed adult mosquitoes. The collected mosquitoes were allowed to rest within cages in the KARI-Kisii laboratory, for 24 to 48 hours to ensure complete blood digest. The mosquitoes were then transported to ICIPE-Mbita where they were identified to species level using the taxonomic key prepared by Sorawat (2009), and subsequently sorted according to sex. The female *Anopheles gambiae* were dissected, while the other species not required in the study, were discarded.

### **3.3.2 Patient recruitment**

Patients from the estate/villages of the study attending Kisii Level 5 Hospital and Keumbu Sub-District Hospital with malaria related complications were recruited in the study. 72 people were recruited for sampling in Kisii Level 5 while 298 were recruited and sampled at Keumbu Sub-District Hospital. The patients recruited were determined based on the estimated number of malaria cases expected for each hospital from the hospital records. The two Hospitals are the only government hospitals in the area, and they are preferred by the local community because of their cheaper medical costs.

### **3.4 Examination of mosquitoes for *Plasmodium* oocysts.**

The oocyst of malaria protozoa were detected by dissection of the vector gut. A drop of 1X PBS was placed on a glass slide mounted under the light microscope, a mosquito to be dissected was placed on the prepared slide by stabbing the mosquito thorax with a

needle-tip probe, while holding down the mosquito with the probe, forceps were used to grasp the second to the last abdominal segment. The segment was pulled gently off the mosquito abdomen in a single motion, leaving the midgut attached to the immobilized thorax. The abdomen was discarded, using the forceps the midgut was detached from the thorax. Malphigian tube and other accessory tissue and debris were removed leaving the midgut alone. A drop of Merckochrome was placed on a slide, and then the midgut was placed on the stain and covered with a clean cover slip. The slide was observed under high power dissecting microscope and oocysts identified as brightly stained purple structures.

### **3.5 Data on human malaria cases**

Data on occurrence of malaria cases for the previous year in human patients was obtained from Kisii Level 5 Hospital and Keumbu Sub-District Hospital daily current records, where most of the patients from the estates and villages attend. The data showed the proportion of malaria cases. Only malaria data of patients from target estates was consolidated. Data on the number of patients diagnosed and treated for malaria during the study period from the two Hospitals was also recorded. This was done by examining Giemsa stained blood smears from the patients and detecting parasitemia.

### **3.6 Data analysis**

Student t-test was used to test for any significant difference in the density of mosquitoes during the rainy season compared to the dry season. The number of mosquitoes infected with plasmodium oocysts during the rainy and dry seasons were compared using

Graphpad Prism Version 4.00 for windows (Graphpad Software, San Diego California USA). T-test was also used to test for difference in mosquito densities in the rural and urban settings. Pearson Correlation Coefficient was performed to determine the relationship between number of malaria cases in humans and the number of mosquitoes infected with *Plasmodium* oocysts.

## CHAPTER FOUR: RESULTS

### 4.1 Mosquitoes collected in the town estates and its outskirts for the two seasons

A total of 1625 female Anopheline mosquitos species were captured both indoors and outdoors in Keumbu and Kisii for the two seasons. The population had more *Anopheline* mosquitoes than the other species. One thousand two hundred and sixty eight (78%) were *Anopheles gambiae*, three hundred and thirty three (20.5% ) were *Anopheles funestus*, twenty four (1.5%) were other species of mosquitoes. Out of the 1268 *Anopheles gambiae* collected, 812 (64%) were from the villages in the outskirts of Kisii town (Figure 4.1), while 456 were collected from the estates surrounding the town (Figure 4.2). This showed that in the town estates and its outskirts, *A. gambiae* were the majority.

During the study, the highest amount of rainfall was experienced during the month of April while the lowest was in the month of July (Figure 4.3). The mean mosquitoes collected during the dry season were more (240) compared to the wet season (183). A test done to test the two samples showed a significant difference ( $P < 0.05$ ).

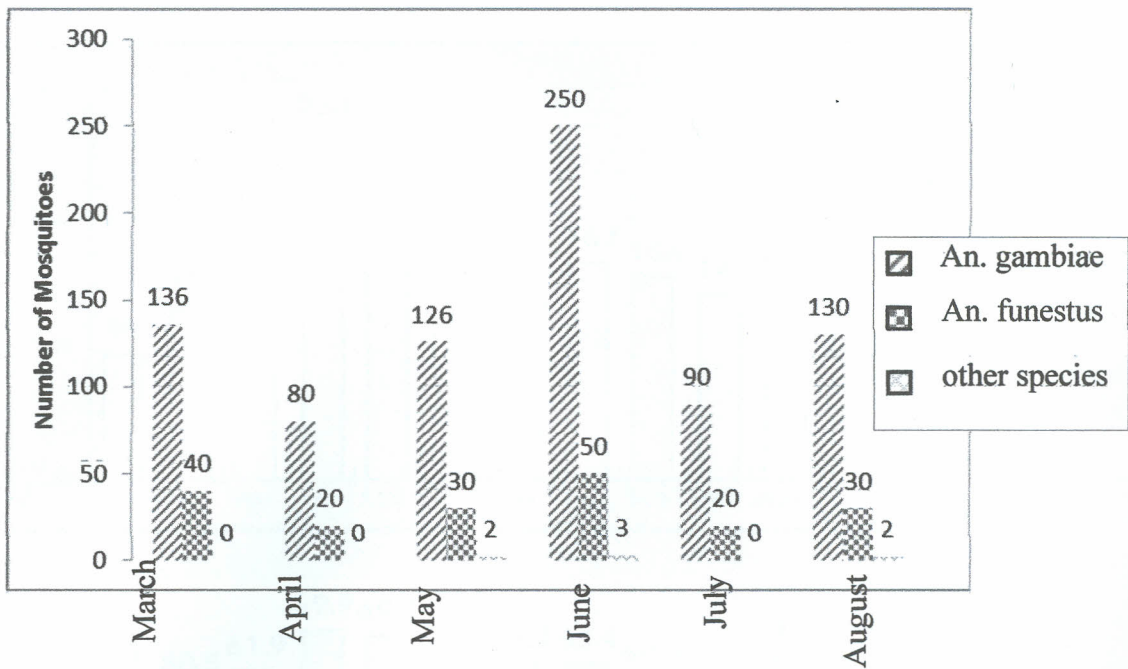


Figure 4.1 Mosquito collections from villages in Keumbu for each month in 2010.

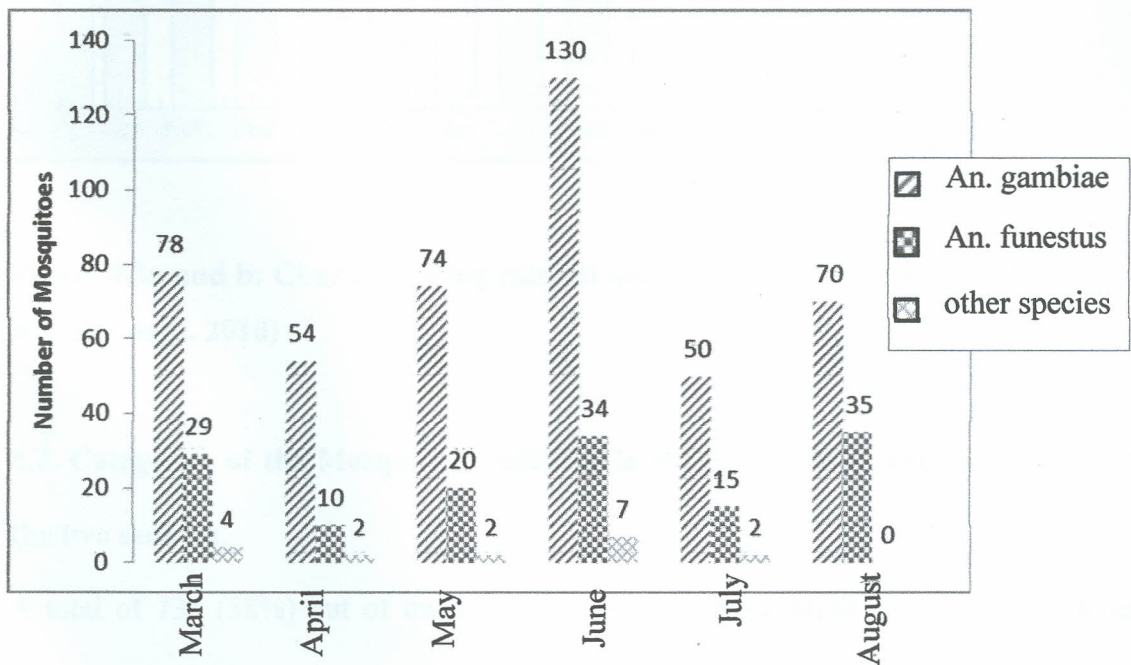
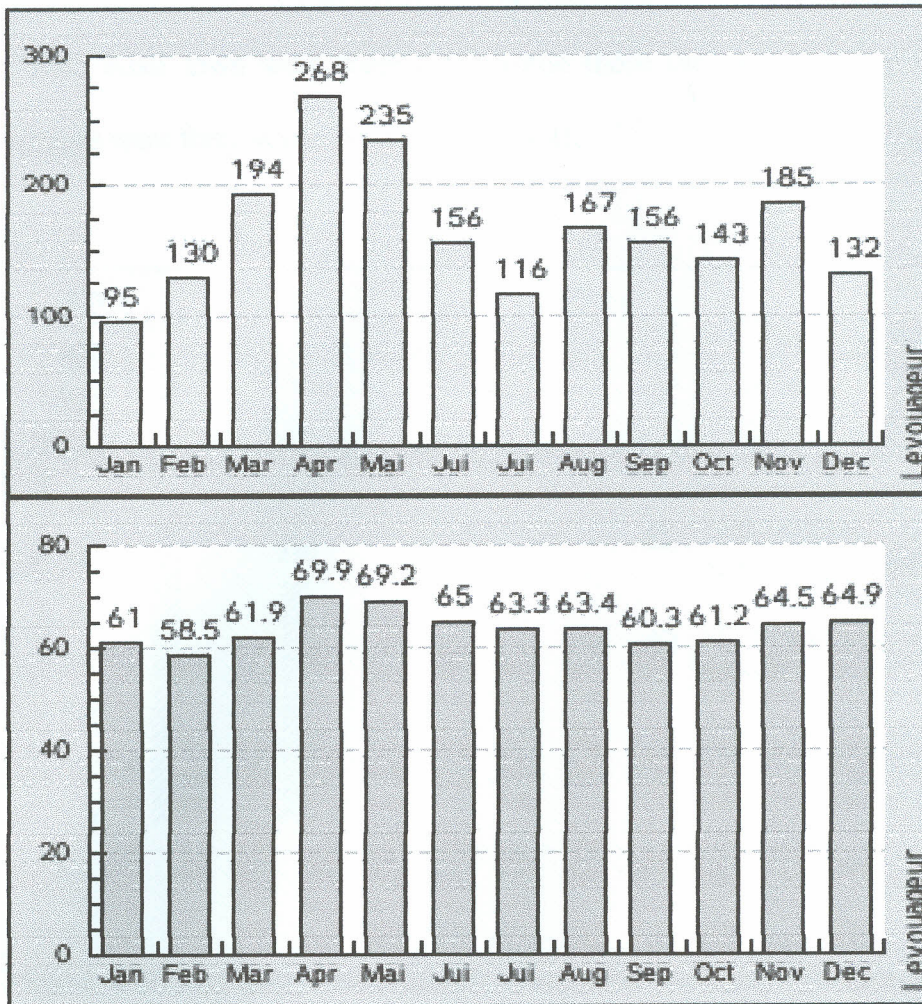


Figure 4.2: Mosquito collections from estates surrounding Kisii town for each month in 2010

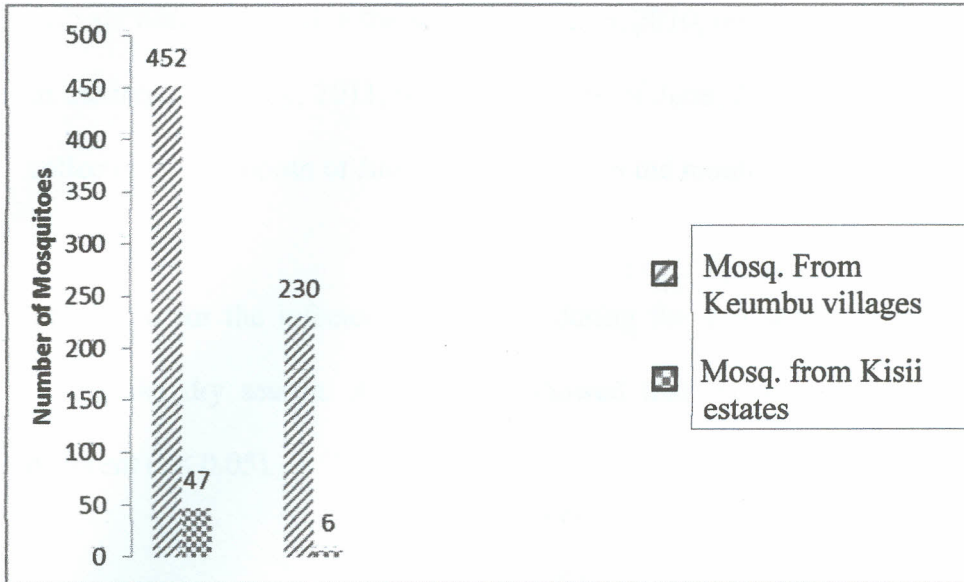


**Figure 4.3a and b: Charts showing rainfall and humidity patterns for 2010 (Source: weather dept, 2010)**

#### **4.2. Categories of the Mosquitoes collected in the town estates and its outskirts for the two seasons.**

A total of 735 (58%) out of the 1268 *An. gambiae* mosquitoes collected were female mosquitoes while 533 (42%) were male. Four hundred and ninety nine (68%) of female anopheline mosquitoes were from the rural area of Keumbu, while the remaining 236 (32%) were collected from the estates surrounding Kisii town.

Fifty three (14%) of the female *An. gambiae* mosquitoes collected from the estates around Kisii town were freshly fed while those that were freshly fed from Keumbu villages were forty seven (89%) (Figure 4.4).



**Figure 4.4: Fed and unfed female *Anopheles gambiae* mosquitoes collected from Kisii and Keumbu in 2010**

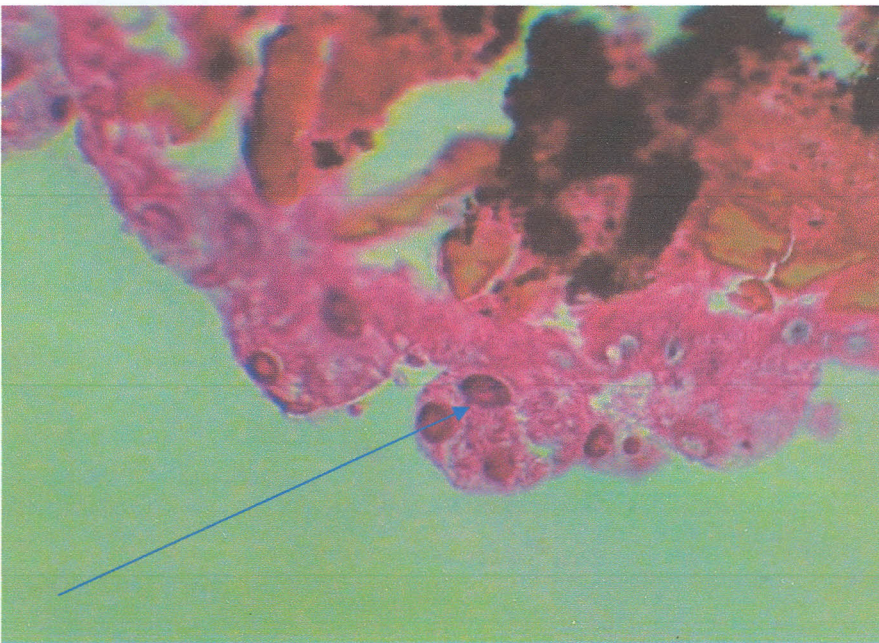
#### **4.3 Mosquitoes infected with *Plasmodium* oocysts from the town estates and its outskirts in the two seasons.**

A total of 499 mosquitoes from the villages in Keumbu were dissected to check for oval oocysts which stained brightly purple under a microscope (Figure 4.5). Forty mosquitoes had *Plasmodium* oocysts which represented 8% of the dissected mosquitoes. Seven of the mosquitoes with oocysts were captured in the month of March, 2010, three in the month

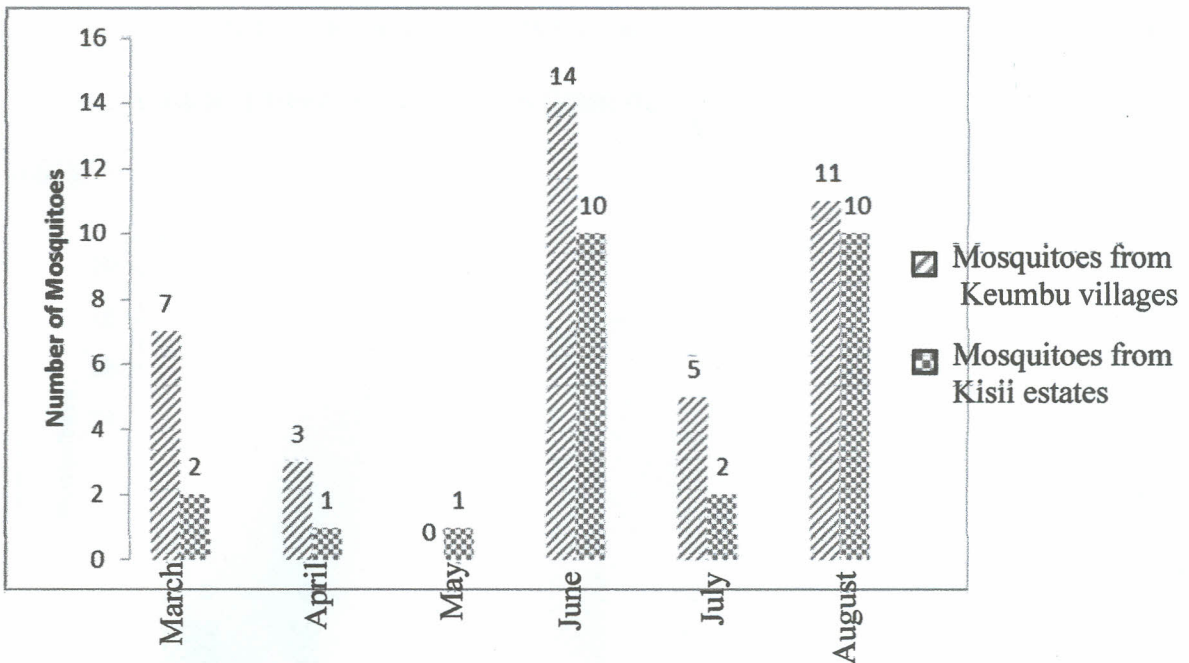
of April, 2010, fourteen in the month of June, 2010, five in the month of July, 2010, and eleven in the month of August, 2010 (Figure 4.5).

Twenty six (11%) out of the 236 *An. Gambiae* dissected from estates within the town were found to have *Plasmodium* oocysts. Two of the mosquitoes with *Plasmodium* oocysts were collected in the month of March, 2010, one in the month of April, 2010, one in the month of May, 2011, ten in the month of June, 2010. Two of the mosquitoes were collected in the month of July, 2010 and ten in the month of August, 2010 (Figure 4.6).

The mean for the infected mosquitoes during the wet season were 4, compared to 14 during the dry season. A test done showed that the two samples were significantly different ( $P < 0.05$ ).



**Figure 4.5: Photograph showing oocysts on a mosquito gut.**

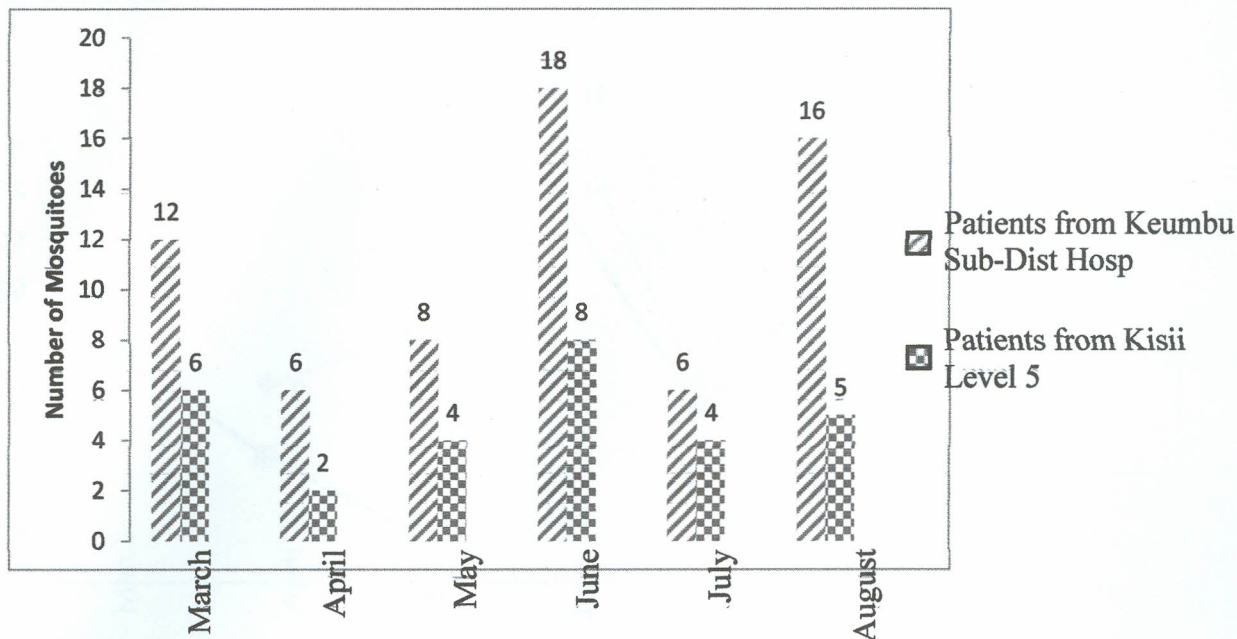


**Figure 4.6: Number of Anopheline mosquitoes found with oocysts for each month in 2010**

#### 4.4 Human malaria cases from Kisii estates and its outskirts in the two seasons

In Keumbu Sub-District Hospital, between March and August, 66 patients were diagnosed and treated for malaria. Of the 66, 12 were treated in the month of March, 6 in the month of April, 8 in May, 18 in June, 6 in July and 16 in the month of August, (Figure 4.6). In Kisii Level 5, within the same duration, 29 patients were diagnosed and treated for malaria. 6 of the patients were treated in the month of March, 2 in April, 4 in May, 8 in June, 4 in July, and 5 in the month of August, (Figure 4.7). The rainy season in Kisii occurs in months of April-May and Dry season June-August, meaning that cases of Malaria will be higher at the end and beginning of wet season.

The mean for patients during the wet season was 13, compared to the dry season when it was 19. A student t-test showed a significant difference between the two samples ( $P < 0.05$ ).

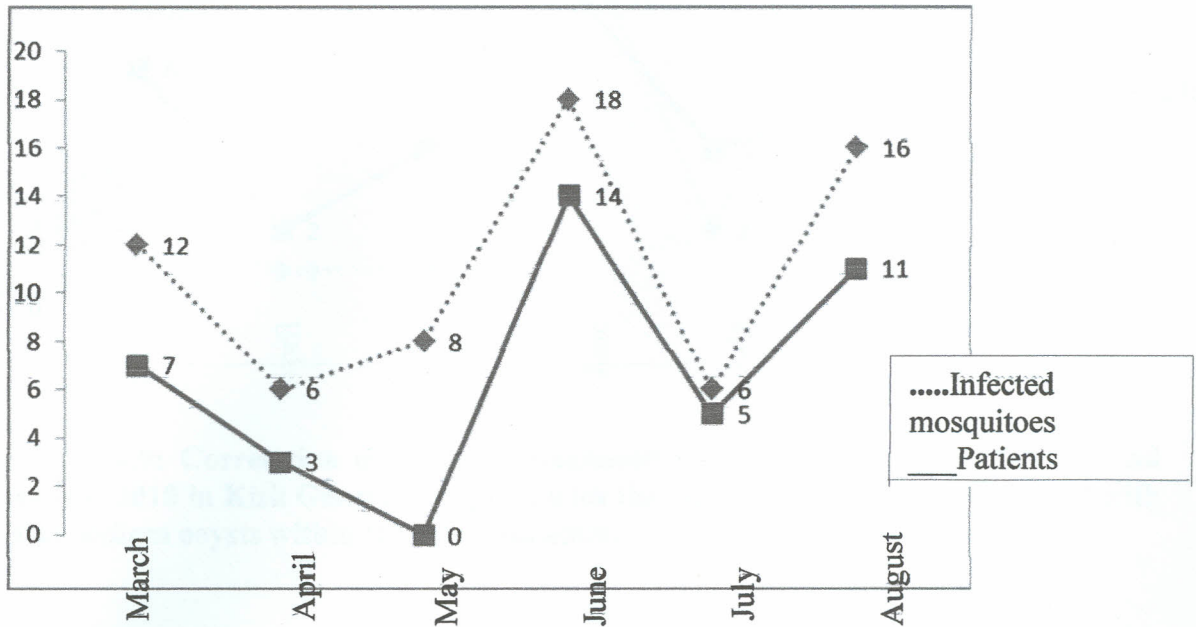


**Figure 4.7: Malaria patients for each month in 2010**

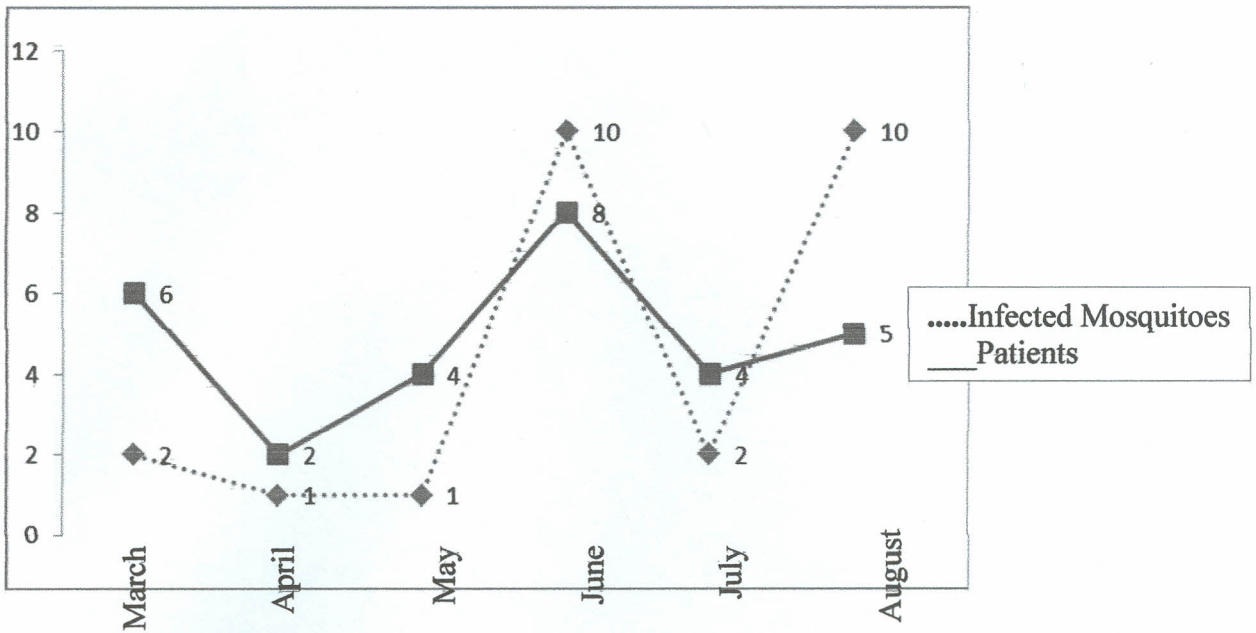
#### 4.5 Effect of Mosquito infection with Plasmodium oocysts on human malaria cases.

The number of mosquitoes from Keumbu villages found to be infected with Plasmodium oocysts were 40 out of the 499 (8%) collected. This was a higher number than 26 out of 236 (11%) collected from the estates in town. Within the same duration, 66 people tested positive for malaria in Keumbu Sub-District Hospital while 29 people tested positive for malaria in Kisii Level 5 (Figures 4.8 and 4.9). A Pearson's correlation test done to determine the relationship between presence of oocyst in mosquitoes and human infections with malaria at 95% and 99% confidence limits confirmed that increase in number of oocysts infected mosquitoes leads to increased human infections with Malaria.

( $P < 0.05$ ; Appendix ix). A similar correlation was observed between number of Malaria cases recorded in Kisii General Hospital and number of mosquitoes with oocysts dissected from Kisii estates.



**Figure 4.8: Correlation of Patients diagnosed with malaria between March and August 2010 in Keumbu Sub-district hospital, with number of infected mosquitoes with *plasmodium* oocysts within the same duration.**



**Figure 4.9: Correlation of Patients diagnosed with malaria between March and August 2010 in Kisii General Hospital, with the number of mosquitoes infected with *plasmodium* oocytes within the same duration.**

#### 4.6 Farming activities in the study area.

The research area had most of the areas covered with vegetation. The rural villages of Keumbu were however more densely covered than the town estates. The main crops grown were maize and bananas (Figure 4.10 and 4.11)



**Figure 4.10:** As Photograph showing banana plantations so close to house.



**Figure 4.11:** Photograph showing hollow parts of the banana plant where mosquitoes breed.

## CHAPTER FIVE: DISCUSSIONS

### 5.1 Proportions of mosquitoes infected during the dry and wet seasons

From the results of the study, the highest proportion of *An. Gambiae* were collected in the month of June. The mean mosquitoes collected during the dry season were 240 compared to 183 collected during the wet season. Lower vector productivity by the mosquitoes were observed during the mid-rain season (April), when temperatures were at mean of 20 degrees Celsius (weather dept., 2010). The results indicate that environmental changes have an effect on the vector population which can either increase or decrease vector abundance. Previous studies have observed that wet weather enhances breeding habitats and also enhances adult stage longevity and fecundity. According to Minakawa *et al.* (2005), at  $< 18^{\circ}\text{C}$  less than 0.28% of mosquitoes survive the 56 days required for sporogony to occur, while at  $30^{\circ}\text{C}$  more than 50% of mosquitoes survive the 56 days hence completing sporogony. During the study period, Kisii highlands had a major rainy season from March to May and a relatively dry season from June to August. The lower environmental temperature during the rainy season reduces survival chances for the mosquitoes. The mosquitoes may increase in number at the beginning and also at the end of the rainy season when there is sufficient water for breeding, increased humidity and increased temperatures (Figure 4.3).

Minakawa *et al.*, (2005) suggested that land use contribute to malaria vector productivity in the highlands. According to Mouchet (1978) and Patz (2000), increased land cover increases vector productivity. In the study areas, the rural areas had an estimated 70% vegetation cover compared to the town estates which was estimated to have 16% vegetation cover. Owing to the fact that land is limited in Kisii, the inhabitants of rural

areas who practice and survive on agriculture have been forced to make use of every single inch of land. Therefore, it is common for the rural inhabitants to plant crops right to the door step (Figure 4.10). In Keumbu area, the major crop is the banana plant. The banana has very large leaves and hollow points on the plant that accumulate water which creates sites for mosquito breeding (Kwamboka, 2010). The large leaves provides shade protection to the mosquito larvae. Minakawa *et al.*, (2004) and Minakawa *et al.*, (2005) suggest that larvae of malaria vectors occur more frequently in temporary sun-lit pools in cultivated areas than in indigenous forests and natural wetlands characterized by tall aquatic plants such as papyrus.

It was noted that there were a lot of un-cleared bushes around homesteads in the Keumbu villages as compared to the estates in town. This explains why the vector productivity was higher in the Keumbu villages as compared to the eastates in town where there minimal or no agricultural activities. Mosquito density is also affected by the vector's ability to access human blood. *Anopheles gambiae* species has a clear-cut preference for human blood hence their survival is heavily dependent on their ability to access the human host. In the two study areas it was noted that, in Kisii town businesses closed as from 2000 hours, while in Keumbu, the hawkers were on the road long after 2200 hours selling their commodities to late night Kisii-Nairobi travelers, hence exposing themselves to mosquito bites in the night.

## 5.2 Infection of mosquitoes with oocysts

The results showed that more mosquitoes captured in the Keumbu villages had oocysts (40/499) in contrast to those captured in Kisii estates (26/236). According to Githeko *et al.*, (1994), mosquito activity increases after 2200 hours and intensifies till 0400 hours in the morning. A research carried out by Kwamboka (2010) covering Kisii town and its environs indicated that up to 95% of the rural inhabitants do not have mosquito nets. The few mosquito nets that are available are either too old or untreated. This is due to low economical, social status and poverty in the rural areas. This increases human-mosquito exposure which may lead to an increased productivity of the vector. It is also interesting to note that a good number of rural inhabitants value their vegetables than their lives. The farmers use the free mosquito nets provided to fence their small vegetable gardens to protect them from chicken.

According to Gonzalez *et al.*, (1997) and Robert and Boudin (2003), infection of mosquitoes with malaria parasites is dependent on multiple characteristics of the parasite, vertebrate host, and mosquito vector. Parasite development in the mosquito can be inhibited by factors inherent to each *Anopheles* species, such as physiologic or physical barriers in midgut and salivary gland cells and mosquito survival, distribution, and feeding behavior (Kiszewski, 2004). Additionally, host immune responses, including antibodies, cytokines, and complement, may significantly modify parasite transmission rates in the mosquito (Ranawaka *et al.*, 1988; Naotunne *et al.*, 1991; Healer *et al.*, 1997). According to Snow and Marsh (1995), if *Anopheles* can acquire an infection with *Plasmodium* then three factors determine the vectorial capacity of the vector namely (a)

the intrinsic preference for biting of human host i.e. the degree of anthropophily, (b) longevity of the vector (i.e. the probability of the mosquito surviving through developmental period of the malaria parasite), and (c) the size of the vector population.

One must also consider various environmental factors, such as temperature and rainfall, which are known to modulate parasite development (Kleinschmidt *et al.*, 2000). The results showed that when the rains were low, it had a positive effect on presence and development of the malaria parasite. A t-test carried out to showed that more mosquitoes were infected with oocysts when the temperatures and rainfall were high.

### **5.3 Human malaria cases during the dry and wet season**

The positive malaria cases in Kisii estates and the Keumbu rural villages were 66 and 29 respectively. These cases were an underestimate because most of the inhabitants especially in the rural do not seek medical attention when sick, for example among the people questioned, it was noted that only two out of ten people sought medical assistance when sick. According to Kwamboka (2010), the disease is made worse because most people often take pain relievers or go to herbalists or even quarks who charge lower than the qualified medics, and once relieved off symptoms, they do not seek medical treatment, this may lead to resistance to various anti-malaria drugs especially Chloroquine, due to evolution of resistant strains of the parasite which effectively neutralize the drug via a mechanism that drains chloroquine away from the digestive vacuole.

During the wet season, 26 human cases were reported in Keumbu Sub-District Hospital compared to 12 cases that were reported in Kisii level 5. During the dry season, 40 human cases were reported in Keumbu Sub-District hospital compared to 17 human cases reported in Kisii level 5. The differences above could have been due to different activities especially economic activities the inhabitants are involved with. The town inhabitants are mostly informed people working while the rural comprises mainly of semi-illiterate people who labor in farms or engage in small businesses like hawking of farm produce. Due to their knowledge and economic ability, the town people are able to reduce infections by; use of bed-nets, use of insecticides, spraying their houses, use of mosquito repellants, and visiting the hospital in case of feeling unwell. According to Kamau, (2011) most of these people dispensed with the nets during the hot season which coincidentally is the active breeding season for the mosquitoes. The town dwellers, owing to the nature of the economic activities are mostly in their houses before the biting activities of the mosquitoes begin. A research carried out by Beler *et al.*, (1990), proposes that a mosquito bite whether infected or uninfected play a significant role in increasing the infectiousness of the human reservoir to mosquitoes vector.

#### **5.4 Relationship between proportion of mosquitoes infected and human malaria cases**

In this study human malaria cases are seen to be directly related to mosquito infection with *Plasmodium* oocysts. The numbers of mosquitoes infected with oocysts were seen to increase during the favorable conditions for mosquito survival, and decrease during the unfavorable conditions, this could be due to the fact that unfavorable climatic conditions

reduce the lifespan of the adult mosquitoes such that the probability of their surviving long enough to allow completion of sporogonic development is negligible (Emma *et al.*, 2009). According to Richard *et al.*, (2010), oocysts infected mosquitoes increase is attributed to increased mosquito bites. Snow and Marsh (1995) add that a subset of the human population becomes increasingly infectious to mosquitoes at the onset of mosquito activity and that increase in mosquito bites is an important factor in this. Thus increasing number of mosquito bites by the vector increases the infection of both the vector and human.

Increase in human infections was seen to be influenced by increase in oocyst infections in mosquitoes, for example there were higher malaria cases in the dry season coinciding with high number of mosquitoes infected with oocysts. From the correlation test it is likely that *Plasmodium* parasites affect both the mosquito vector and the human host in a way that might enhance transmission. Invasion of the mosquito's salivary glands by the sporozoites affect secretion of saliva which result in increased probing (Ribeiro *et al.*, 1998). The increased probing is likely to enhance host defensive responses and may therefore result in multiple feeding on the same host or on different hosts. Apart from the malaria vector being attracted to the human host by changes in body temperature and body odour, it has been suggested that they can also be attracted by presence of gametocytes in the blood of the human host (Burkot, 1998). If human attractiveness and susceptibility to *Anopheles* is affected by the presence of gametocytes, it then follows that asymptomatic carriers who do not seek treatment ensures that the disease is kept in the population continually. Such is the case in the villages in the outskirts of Kisii town

where the apparent number of Malaria cases were low compared to the large population of the mosquito vectors.

## CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

### 6.1 Conclusion

- i. Results obtained indicate that densities of *An. Gambiae* were higher during the end and beginning of rainy season. This because increased amount of rainfall increases breeding grounds. Apart from rainfall, there are other factors that increase survival chances of the mosquitoes and these factors include temperature, humidity, vegetation, and availability of human hosts.
- ii. Number of mosquitoes infected with oocysts changes with change in weather conditions; it was noted that favorable temperature for mosquito survival and availability of human host increased the proportion of mosquitoes infected with oocysts.
- iii. Number of malaria cases in humans increase with presence of rainfall and high temperature and humidity which allow mosquitoes to thrive hence allowing oogenesis to take place before the malaria parasite is introduced into the humans.
- iii. Malaria cases in humans are directly related to the number of mosquitoes infected with oocysts. This is partially attributed to the fact that very low temperatures reduce the lifespan of the adult mosquitoes such that the probability of their surviving long enough to allow completion of sporogonic development is negligible, hence malaria cases reduce during wet season when temperature is low. Another factor that could be leading to this phenomenon is the fact that oocysts increase in mosquitoes is attributed to increased mosquito bites. In non-immune people, this increased bites increase chances of infection.

## 6.2 Recommendations

i. High density of mosquitoes during the rainy seasons indicate that there is need to educate both the rural and the urban population on the importance of draining away stagnant waters, clearing bushes around the homes, proper use of mosquito nets and reduced human-mosquito contact. Since *Anopheles gambiae* mosquito is specifically dependent on human blood for completion of oogenesis, if a barrier is created between the two organisms, then developmental stages of *Plasmodium falciparum* will be interrupted, at the same time the mosquitoes will not be able to survive since they will lack the human blood required for oogenesis completion.

ii) The Ministry of Public Health and sanitation should consider the rearing and release to the wild sterile male *An. Gambiae* mosquitoes so as to stop fertilization of the female mosquitoes. This will reduce the host seeking behavior in mosquitoes hence stop oocyst formation in mosquitoes.

iii. Human population should be educated on the need to seek medical care in the case of sickness. From the findings, most of the rural population do not seek medication hence harbor the malaria parasites which are easily transmitted to other people in the event of a mosquito bite. It is therefore important that the people in malaria endemic areas be advised to take medical tests and treatment in order to reduce malaria parasites in the humans.

iv. The study shows that Malaria cases occur in Kisii despite being a highland previously expected to be free of Malaria. People from non-malaria areas traveling to malaria endemic areas should therefore be advised to use prophylaxis to prevent the spread of the disease to non-endemic areas.

### 6.3 Limitations of the Study

One limitation of this study is that most of the rural population studied did not seek medical assistance in case of illness, therefore the results obtained may not exactly bring out the gravity of the matter. Since mosquito bites were not observed, it was not possible to determine the number of bites received by the patients hence relate the parasitemia in human hosts with the number of the bites. The study captured only patients attending Kisii level 5 Hospital and Keumbu Sub-District Hospital and excluded those seeking medical attention in other Hospitals and those whose who opt for self-medication.

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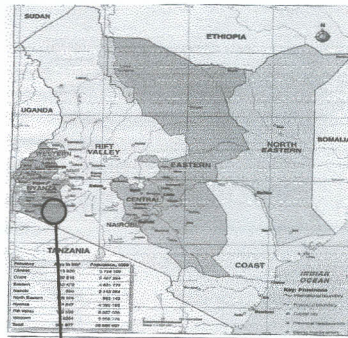
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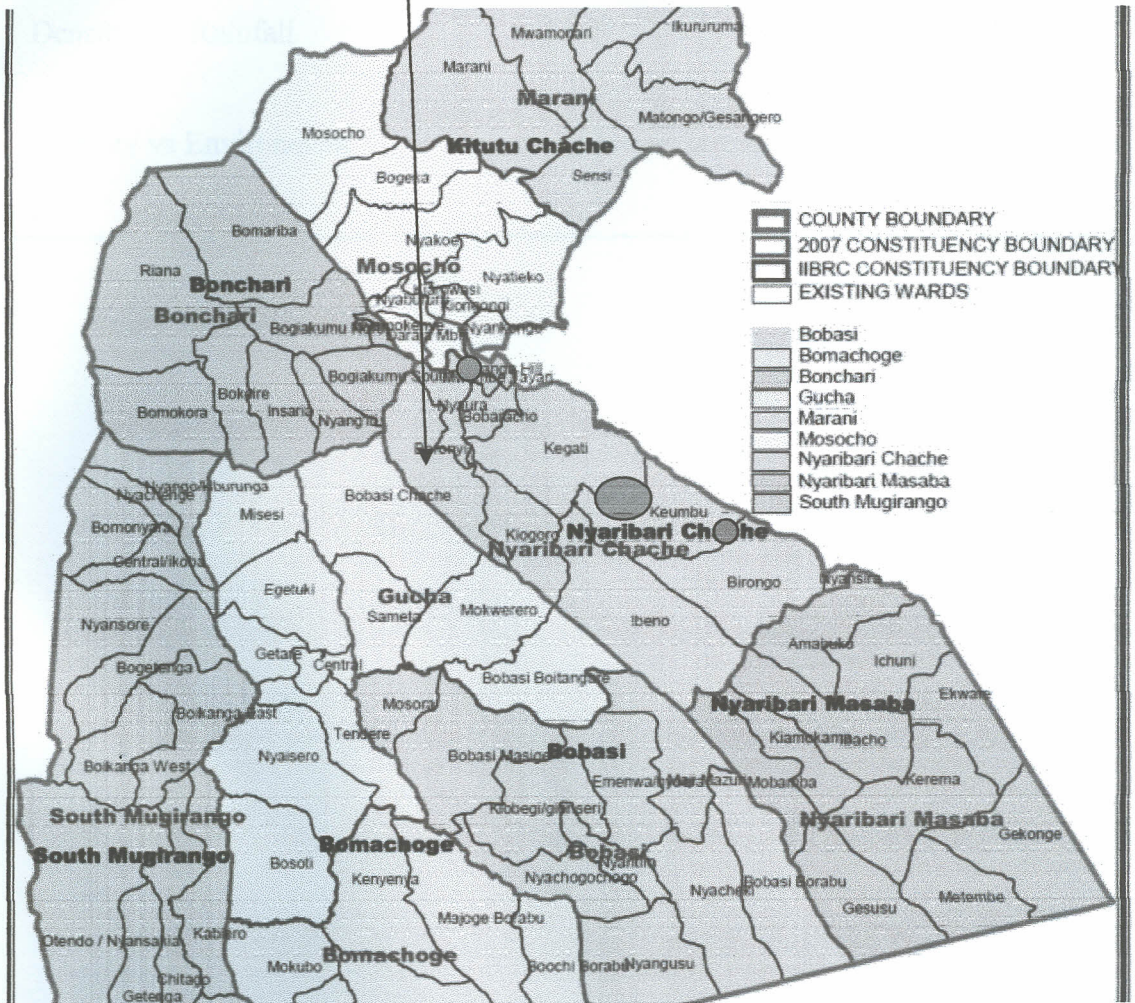
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Appendix i: Map of the study area



KEY:



KEY:



Area of study

(Source: Google maps (2013))

**Appendix ii: Statistical analysis for the dependence of mosquito density, rainfall and environment. ( $P < 0.05$ )**

Parameter	t cal	t tab	<i>P</i> value
Density vs Rainfall	1.909	2.228	$P < 0.05$
Density vs Environment	3.431	2.228	$P < 0.05$

**Appendix iii: Statistical analysis for the effect of rainfall and the environment on Oocyst density. ( $P < 0.05$ )**

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Parameter	t cal	t tab	<i>P</i> value
Density vs Rainfall	3.990	2.228	$P < 0.05$
Density vs Environment	5.300	2.228	$P < 0.05$

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**Appendix iv: Statistical analysis for the relationship between oocyst density and human malaria infections. ( $P < 0.05$ )**

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Parameter	r cal	r tab	<i>P</i> value
Density of oocysts vs human infections	0.9568	0.811	$P < 0.05$
Density of oocysts vs human infections	0.9568	0.917	$P < 0.01$

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