

# Infections of *Anopheles Gambiae* Mosquitoes with *Plasmodium* Oocysts and its Effects on Highland Malaria in Kisii Town and its Surroundings

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**Abstract** Malaria is a serious threat to human life in sub-Saharan Africa, claiming many lives and causing the greatest morbidity as compared to other infectious diseases. 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, Daraja mbili, 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 of infection in mosquitoes and mosquito densities in the two seasons were analyzed and correlation between human parasitemia and mosquitoes infections were tested. 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 oocysts 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.

**Keywords:** malaria, *Plasmodium*, oocysts

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## 1. Introduction

Malaria is endemic in 106 countries all over the world 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 [21]. Majority of these people are children under five years and pregnant mothers [21]. 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 [7]. Between 1980 and 1999, a series of malaria "epidemics" were reported in Kisii and other communities located on the highlands. 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 [3].

The highlands of Kenya were regarded by the colonial settlers as safe from the surrounding lowland malaria areas of Uganda and Kenya. At an altitude of about 6000 feet, Kisii had been considered too high for the survival of the mosquito that harbors the parasite [3]. 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 [5]. Climate changes provided transiently

suitable conditions for development of mosquitoes in regions that were not infested before [19].

Malaria in the western highlands of Kenya is commonly spread by female *Anopheles gambiae* which account for about 80% of the mosquito vector in the region [3]. The malaria epidemics occurs at given seasons of the year, but there has been no documentation on the infections of *Anopheles gambiae* mosquitoes with *Plasmodium* oocysts in this seasons. This research therefore 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.

## 2. Materials and Methods

### 2.1. 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. 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 . Daily mean temperature range from 10°C to 30°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*. *Anopheles gambiae* forms about 80 percent of mosquito density and is the main malaria vector in the highlands.

### 2.2. Sample Size Determination

Sample size was determined by use of the formula;

$$n = \frac{Z^2 pq}{d^2}$$

Proposed by Fisher [4]

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 mosquitoess sampled were determined as follows;

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

For the two seasons, the number of mosquitoes required was 288 x 5 which equals to 1440, but the study targeted 1625 female anopheles mosquitoes.

### 2.3. 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.

#### 2.3.1. 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. 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, and subsequently sorted according to sex. The female *Anopheles gambiae* were dissected, while the other species not required in the study, were discarded.

#### 2.3.2. 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.

### 2.4. Examination of Mosquitoes for *Plasmodium* Oocysts

The oocysts 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 Merochrome 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.

## 2.5. 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.

## 2.6. 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.

## 3. Results

### 3.1. Mosquitoes Collected in the Town Estates and its Outskirts for the Two Seasons

A total of 1625 female Anopheline mosquito 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, while 456 were collected from the estates surrounding the town. 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. 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 ( $P < 0.05$ ).

### 3.2. 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%).

### 3.3. 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. 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.

Twenty six (11%) out of the 236 *An. Gambiae* dissected from estates within the town were found to have *Plasmodium* oocysts. One was collected 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. Two of the mosquitoes with *Plasmodium* oocysts were collected in the month of March, 2010, one in the month of August, 2010.

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 ( $P < 0.05$ ).

### 3.4. 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. 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. 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.

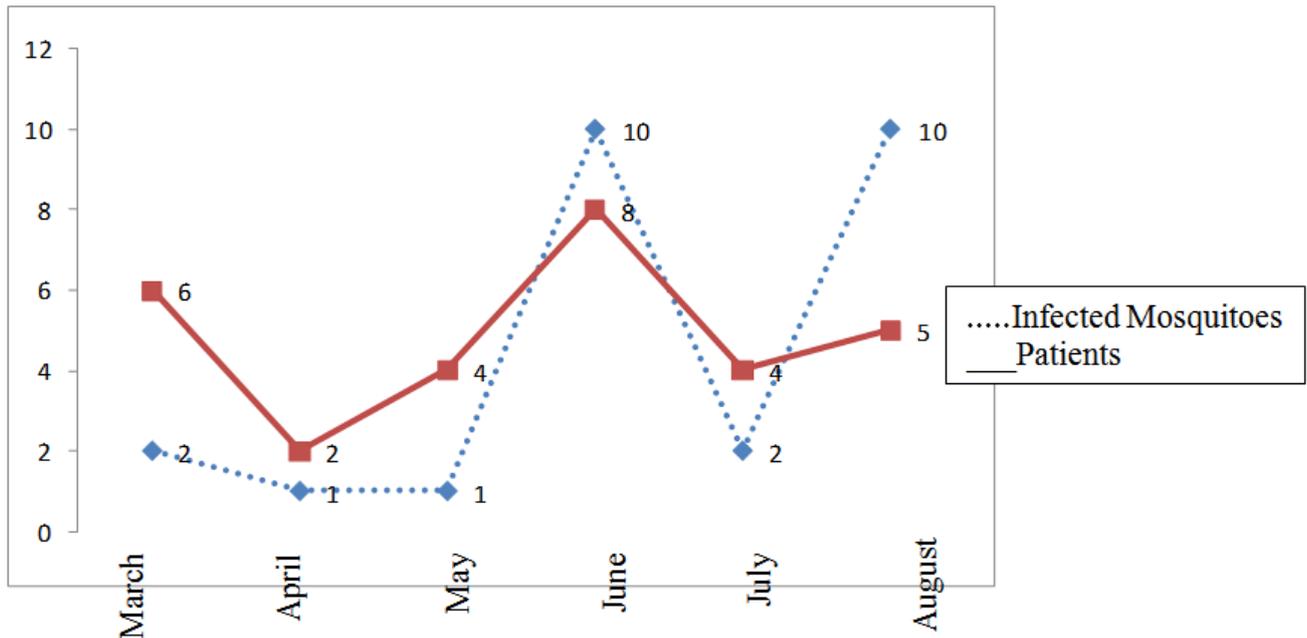
The mean for patients during the wet season was 13, compared to the dry season when it was 19. A student t-test showed a significant difference between the two samples ( $P < 0.05$ ).

### 3.5. Effect of Mosquito Infection with Plasmodium Oocysts on Human Malaria Cases

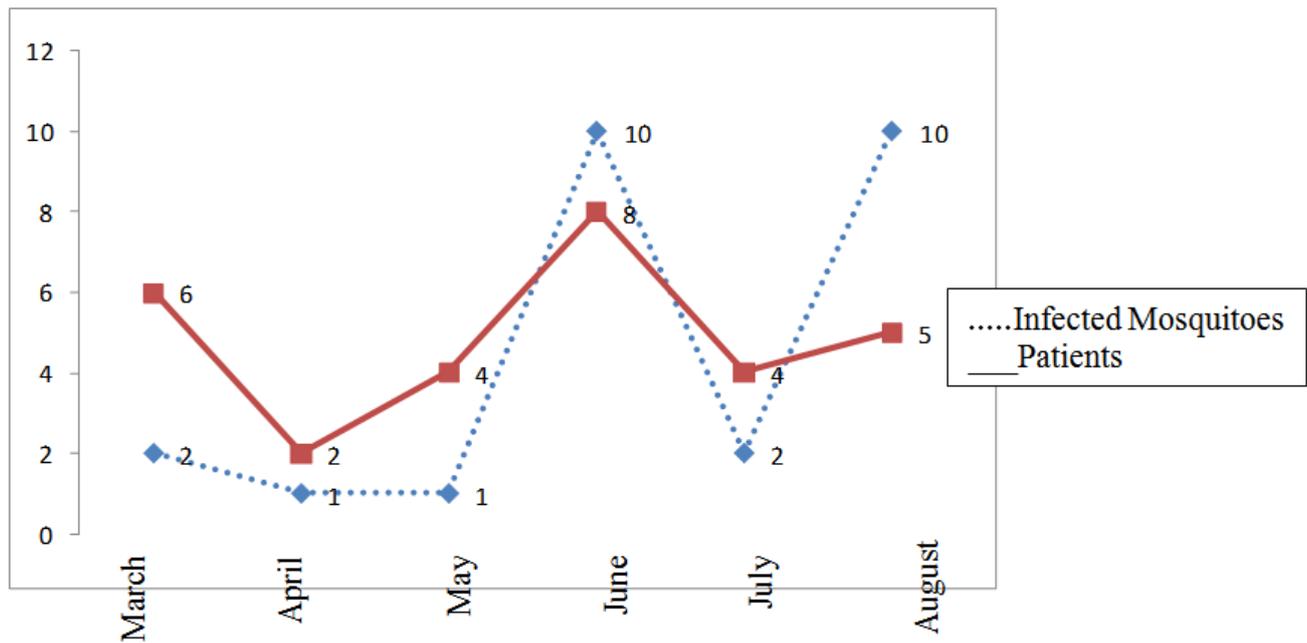
The number of mosquitoes from Keumbu villages found to be infected with *Plasmodium* oocysts were 40 out of the 499 (8%) collected. This was a higher number than 26 out of 236 (11%) collected from the estates in town. Within the same duration, 66 people tested positive for malaria in Keumbu Sub-District Hospital while 29 people tested positive for malaria in Kisii Level 5 (Figure 1 and Figure 2). A Pearson's correlation test done to determine the relationship between presence of oocysts 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. ( $P < 0.05$ ). 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 1.** 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 2.** Correlation of Patients diagnosed with malaria between March and August 2010 in Kisii General Hospital, with the number of mosquitoes infected with *plasmodium* oocysts within the same duration

### 3.6. 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.

## 4. Discussions

### 4.1. 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. 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. At < 18°C less than 0.28% of mosquitoes survive the 56 days required for sporogony to occur, while at 30°C more than 50% of mosquitoes survive the 56 days hence completing sporogony [12]. 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.

Land use contribute to malaria vector productivity in the highlands [12]. Increased land cover increases vector productivity [13]. 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. 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 [11]. The large leaves provides shade protection to the mosquito larvae. 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 [12,13].

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 estates 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.

#### 4.2. 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). Mosquito activity increases after 2200 hours and intensifies till 0400 hours in the morning [5]. A research carried out covering Kisii town and its environs indicated that up to 95% of the rural inhabitants do not have mosquito nets [11]. 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.

Infection of mosquitoes with malaria parasites is dependent on multiple characteristics of the parasite, vertebrate host, and mosquito vector [5,18]. 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 [10]. Additionally, host immune responses, including antibodies, cytokines, and complement, may significantly modify parasite transmission rates in the mosquito [8,14,15]. *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 [20].

One must also consider various environmental factors, such as temperature and rainfall, which are known to modulate parasite development [9]. 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 show that more mosquitoes were infected with oocysts when the temperatures and rainfall were high.

#### 4.3. 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. The disease is made worse because most people often take pain relievers or go to herbalists or even quacks 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 [11].

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. Most of the people dispensed with the nets during the hot season which coincidentally is the active breeding season for the mosquitoes [8]. The town dwellers, owing

to the nature of the economic activities are mostly in their houses before the biting activities of the mosquitoes begin.

#### 4.4. 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 [2]. Oocysts infected mosquitoes increase is attributed to increased mosquito bites [16]. 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 [20]. 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 oocysts 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 [17]. 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 [1]. If human attractiveness and susceptibility to *Anopheles* is affected by the presence of gametocytes, it then follows that asymptomatic carriers (healthy carrier of an infectious disease) 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.

## 5. Conclusions

Malaria cases in humans are directly related to the number of mosquitoes infected with oocysts within a given season. 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.

## Statement of Competing Interests

The authors have no competing interests.

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