RELATIONSHIP BETWEEN BITING FREQUENCY AND SURFACE PROTEINS OF CIRCUM-SPOROZOITE AND MEROZOITE STAGES IN HIGHLANDS OF WESTERN KENYA

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DECLARATION AND RECOMMENDATION

DECLARATION

I declare that this research thesis is my original work and has not been submitted wholly or in part for any award in any other institution of learning.

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RECOMMENDATIONS

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DEDICATION

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ABSTRACT

Vector control in the highlands of western Kenya has resulted in a significant reduction of malaria vectors. This has made the current entomological parameters being used to measure transmission to become insensitive and challenging. Antibodies to the Circum-sporozoite protein (CSP) have been associated with transmission intensity and have corresponded well with EIR. The study objective was to determine the association between the level of circum-sporozoite protein and merozoite surface protein antibodies and the entomological inoculation rates in the Western Kenya Highlands. Indoor resting adult mosquitoes and blood samples were collected in Western Kenya highlands in four selected villages categorized into two valley systems. These were the U shaped (Iguhu and Emutete) and the V shaped valleys (Marani and Fort Ternan). This study was done for eight months, from September 2009 to April 2010.

Members of the *Anopheles gambiae* complex were identified by PCR. Blood samples were collected from children 6–15 years old and the densities categorized by site of home location and age of the children. Exposure to malaria was tested using Circum-sporozoite protein and Merozoite surface protein (MSP) immunchromatographic antibody test. Malaria infection was tested by microscopic examination of thick and thin smears. Sporozoite ELISA was conducted for detection of circum-sporozoite protein which was used to calculate the sporozoite rates and estimate the entomological inoculation rates. Overall indoor resting densities of *Anopheles gambiae* varied significantly between all the sites except between Emutete and Marani ($P<0.05$). *Anopheles gambiae s.s.* composed of 91.3% while *Anopheles arabiensis* composed 8.7%. Sporozoites were detected in only two of the Sites Iguhu and Emutete with an annual EIR of 3.7 and 2.05 respectively. Prevalence of CSP-MSP antibodies was 29.7%, 38.6%, 16.3 and 12.3% in Iguhu, Emutete, Marani and Fort Ternan Respectively. There was a significant correlation between indoor resting densities of *Anopheles gambiae* and the Circum-sporozoite and Merozoite surface protein antibodies prevalence in Iguhu and in Fort Ternan, ($r^2=0.567$) and ($r^2=0.570$), ($P<0.05$) respectively. The study indicates that the biting frequencies were very low with no sporozoites detected in the V shaped valleys. The presence of the Circum-sporozoite and merozoite surface protein indicated an ongoing transmission. This was detected two to three months earlier compared to the EIR. The changing vectorial system may favour *Anopheles arabiensis* which may pose challenges in vector surveillance due to its zoophilic and exophilic behaviour.

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LIST OF ABBREVIATIONS

CSP	Circumsporozoite
EIR	Entomological inoculation rate
ELISA	Enzyme linked immunosorbent assay
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IRS	Indoor residual spraying
ITN	Insecticide treated nets
LSA	Liver Stage Antigen
MSP-1	Merozoite Surface Protein 1
PCR	Polymerase Chain Reaction
PSC	Pyrethrum spray collection
rDNA	Recombinant Deoxy-ribo Nucleic Acid.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Malaria causes over 90% of the mortalities in Africa. Out of this, 85% of the deaths are of children under the age of five according to the WHO world malaria report, 2010. Climate change which has been shown to affect the rate of malaria transmission in the western Kenya highlands is expected to lead to an increase of latitudinal and altitudinal warming. An increase of environmental temperature to 18°C, which is the threshold for malaria transmission will increase the rate of parasite development in and also increase the mosquito development (Githeko, 2009). Lack of proper vector control measures have greatly resulted in an increase in the transmission of malaria as the measures that have been put in place do not target all the life stages of the mosquitoes. Findings by Fillinger *et al.* (2009) suggest that integrated vector management can be a highly effective tool for reducing malaria in the future in many parts of sub-Saharan Africa. Land use change has also contributed to an increase in malaria in the highlands (Githeko, 2007). As the population in the highlands increases, there is an increased demand for agricultural and settlement land which leads to swamp cultivation (Minakawa *et al.*, 2004) and deforestation in western Kenyan. This leads to an increase in indoor temperature, and consequently, enhances the net reproductive rate of *Anopheles gambiae* mosquitoes (Afrane *et al.*, 2006). A study by Zhong *et al.* (2008) suggest that drug resistance of malaria parasites in the highlands could be contributed by the mutations of the malaria parasites and their occurrence in high frequencies as found especially in the lowland regions. However, the role of drug resistance as a driving force for malaria outbreaks in the highlands has not been established which is compounded by a healthcare system that is overwhelmed by other diseases and lack of funding (Malakooti *et al.*, 1998).

The prevalence of asymptomatic *P. falciparum* infection in the western Kenya highlands is high and consistent with gradual acquisition of immunity. This shows with increasing age upon repeated infection (Baliraine *et al.*, 2009). Munyekenye *et al.* (2005) carried out a cross-sectional study in a highland site where malaria epidemics had been reported in the past. The study showed that transmission is intense, particularly after the rainy season, but variable with regard to season and distance from major mosquito proliferating habitats. Spatial analysis of asexual

parasite densities indicated clustering in relation to distance from major larval breeding habitats and in relation to age.

Fillinger *et al.* (2009) found out that the risk of acquiring new malaria infections in children was substantially and independently reduced by insecticide treated nets use and microbial larvicide application. A study by Guyatt *et al.* (2002) suggested that indoor residual spraying may be more effective and cheaper than insecticide treated nets in communities subjected to low, seasonal risks. This is because bed nets confer high protection against parasite infection in such areas where baseline transmission is low, however, the absolute reductions in parasiteamia due to wide-scale net use is relatively small (Noor *et al.*, 2008). A combination of both insecticide treated nets and indoor residual spraying contributes most to reducing malaria morbidity (Nyarango *et al.*, 2006).

A low level of transmission of malaria in the highlands suggests that it may be disrupted by vector control methods such as residual spraying that reduce vector abundance after a control has been implemented (Ndenga *et al.*, 2006). Entomological inoculation rates, parasite prevalence and anti-malaria antibodies prevalence can be used to measure malaria transmission intensity (Stewart *et al.*, 2009). As entomologic parameters are often much more difficult to accurately measure than human infection, the parasite prevalence, or fraction of hosts with detectable blood stage infections, is often used as a measure of the intensity of malaria transmission (O'Meara *et al.*, 2008). Therefore it was the objective of this study to compare the effectiveness of the use of the CSP and MSP proteins in the detection of early transmission of malaria in relation to the use of Entomological parameters in similar environmental settings of the highlands of Western Kenya.

1.2 Statement of the problem

Malaria transmission intensity can be measured from entomological inoculation rates, malaria antibodies and also parasite prevalence, though the collection of mosquitoes in regions where vector control measures have been put up is relatively complex. This makes the use of entomological inoculation rates only to estimate the levels of transmission to very challenging. The use of Entomological inoculation rate is also one of the oldest methods of measuring malaria transmission and it is quite challenging. This study tried to find an association between the prevalence of circum-sporozoite protein and merozoite surface protein antibodies and

entomological inoculation rates to try see if CSP-MSP antibodies could be used in a rapid diagnostic kit to detect early malaria transmission.

1.3 Objectives

1.3.1 General objective

To determine the association between the level of circum-sporozoite protein and merozoite surface protein antibodies and the entomological inoculation rates in the Western Kenya Highlands.

1.3.2 Specific objectives

1. To determine the mosquito biting frequencies in the study sites
2. To determine the prevalence of circum-sporozoite protein and merozoite surface protein antibodies in study sites.
3. To determine the relationship between entomological inoculation rates and prevalence of circum-sporozoite protein and merozoite surface protein antibodies

1.4 Hypotheses

Null Hypotheses:

i. There is no significant difference in the biting frequencies between the study sites.
ii. There is no significant difference in the circum-sporozoite protein and merozoite surface protein antibodies prevalence between the study sites.
iii. There is no significant relationship between entomological inoculation rate and the prevalence of circum-sporozoite protein and merozoite surface protein antibodies.

1.5 Justification

This study was important in understanding the spatial patterns of vector distribution. A new tool of measuring malaria transmission was used during the study which will be useful in future early detection of malaria transmissions before the entomological parameters can detect any transmission which will be useful in early treatment of malaria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 A history of malaria epidemic in the highlands of Western Kenya

Malaria did not exist in the western Kenya highlands until the second decade of the 20th century (Shanks *et al.,* 2005). Completion of a railway from the Kenyan coast across the highlands and down to Lake Victoria in 1901 increased land transport and facilitated the gradual spread of infective mosquitoes into the highlands from the low-lying hyper-endemic-disease areas. The development of tea estates and agriculture in the highlands, with the concomitant clearing of the forests, provided suitable mosquito breeding grounds. Finally, infected labourers who moved in to the Kenya highlands from other regions completed the conditions necessary for malaria transmission (Malakooti *et al.,* 1998). In the late 1980s and early 1990s, a series of malaria epidemics were reported in the Western highlands of Kenya and other communities at high altitude in the sub-region (Hay *et al.*, 2002a). Ever since the Western Kenya highlands have experienced frequent malaria outbreaks in the last two decades as ambient temperature is relatively low (Afrane *et al.*, 2006). A study by Baliraine *et al*. (2009), found out that the prevalence of asymptomatic *P. falciparum* infection in a highland area of Western Kenya was high.

2.2 The role of climate change and variability in malaria transmission

Climate can influence all three components of the life cycle of the malaria parasite. It is thus a key determinant in the geographic distribution and the seasonality of malaria (Zhou *et al.*, 2004a). The transmission of malaria mainly depends on mosquito distribution, which in turn depends on habitat availability, suitability and productivity. Rainfall increases mosquito habitats while temperature increases the rate of both the mosquito and parasite development which results in an increase in malaria transmission (Githeko, 2007). This makes malaria one of the most climate sensitive vector borne diseases (Githeko *et al.,* 2000). It is in areas where transmission of malaria is low or absent that climate change is expected to have its greatest effect as in these areas immunity is low in all age groups and the protective genetic polymorphisms are rare because of lack of selective pressure to maintain them in the population. Climate change includes the change in the mean meteorological parameters and a change in the magnitude of departure from the mean climate variability. A change in mean temperature is a slow and long term process

while the deviation from the mean can be large, abrupt, short lived but frequent. This makes the climate variability to be episodic. Though the effects of gradual warming may be slow enough to allow the human populations to develop immunity to malaria, climate variability such as El Niño can result in severe malaria epidemics which can result in high morbidity and mortality (Githeko, 2007 and Balls *et al.*, 2004).

2.3 Land use change and malaria transmission

Need for more land for settlement and agriculture has led to activities such as deforestation in Western Kenya which in turn leads to an increase in indoor temperature. A slight increase of indoor temperatures enhances the net reproductive rate of *An. gambiae* mosquitoes (Afrane *et al.*, 2006). The highest ground surface temperature increases are in the areas where extensive land cover changes have occurred, such as the clearing of forests, increased forest fire activity, and conversion of prairie grassland to agricultural land (Bounoua *et al.*, 2002 and Munga *et al.*, 2009). Open-space irrigated vegetable fields in cities can provide suitable breeding sites for *An. gambiae*. This is reflected in higher numbers of adult *An. gambiae* (Afrane *et al.*, 2004a). Other land use activities such as abandoned fishponds are conducive to the presence of malaria vectors the *Anopheles* mosquitoes (Howard *et al.*, 2008). The larval habitats of *An. gambiae* in Western Kenya highland are characterized by warmer daytime temperatures of water, which were significantly affected by the habitat size and plant size. This indicates that the practice of swamp cultivation, in populated areas of the highlands, increases availability and enhances habitat conditions for *Anopheles* mosquitoes (Minakawa *et al.*, 2004).

2.4 Vectors dynamic and distribution in the highlands

Malaria vector abundance is an important determinant of the force of malaria transmission hence any factor that increases or decreases the vector densities have an impact on the prevalence of malaria (Ndenga *et al.*, 2006). Clustering of mosquitoes occur in houses below 400 m from a valley bottom as demonstrated by studies by Zhou *et al.* (2004b) and the temporal change in mosquito abundance is mainly caused by rainfall changes. *An. gambiae* adults are more abundant during the rainy season than during the dry season. The topography of the highlands comprises hills, valleys and plateaus. Rivers and streams run along the valley bottoms in the valley ecosystem and swamps are a common feature. Unlike in lowland plains where drainage is poor and mosquito breeding habitats have an extensive distribution, the majority of breeding habitats in the hilly highlands are confined to the valley bottoms because the hillside gradients

provide efficient drainage (Minakawa *et al.*, 2004). The topographic features of the highlands restrict the spatial distribution of vector breeding habitats. Larval breeding habitats are confined to the valley bottom hence a low intensity of exposure to malaria in hilltop residents resulting in a non-homogeneous parasite burden and probably the incidence of morbidity (Githeko *et al.*, 2006). The productivity of the mosquitoes has also been shown to be significantly higher in aquatic habitats located in farmlands compared with those in swamps and forests (Munga *et al.*, 2006).

2.5 Parasite prevalence in the highlands

In a study conducted at the Western Kenya highlands, the parasite prevalence and densities in the population decreased with age and distance from valley bottoms due to the availability of larval breeding sites (Munyekenye *et al.*, 2005, Githeko *et al.,* 2006 and Munga *et al.,* 2009). Baliraine *et al*. (2009) reported that prevalence of asymptomatic *P. falciparum* infections in the western Kenya highland is high, with PCR detecting a significantly higher number of infections than microscopy. The results were consistent with gradual acquisition of immunity with age upon repeated infection, and also showed that malaria transmission risk is highly heterogeneous in the highland. Zhou *et al*. 2004(c) found that in some highlands of East Africa, climate variability could explain a significant proportion of malaria cases. An increased amount of rainfall in the South western Uganda highlands during the 1997-1998 El Nino was positively correlated with malaria vector abundance and malaria incidence (Lindblade *et al*., 1999). In Ethiopia, malaria epidemics in the highlands were positively associated with elevated minimum temperature (Abeku *et al*., 2003).

2.6 Malaria control in the highlands

The re-emergence of epidemic malaria in the western Kenya highlands has been attributed to factors such as hydrology, climate variability, land-use or land-cover change, and drug resistance (Afrane *et al.,* 2006). Hence effective disease control calls for an understanding of the interaction between these epidemiologic factors. Malaria is looked at from two angles, the control of the parasite and control of the vector. Vector control for the prevention of malaria includes; insecticide-treated bed nets, indoor residual spraying and larval control (Zhou *et al.,* 2010). Insecticide-treated bed nets are a form of personal protection that has repeatedly been shown to reduce severe disease and mortality due to malaria in endemic regions. In community-wide trials

in several African settings, insecticide-treated bed nets have been shown to reduce all-cause mortality by about 20%. Insecticide-treated nets are a powerful malaria control tool and in 2006, only 3% of African children were sleeping under an insecticide-treated bed net, and only about 20% were sleeping under any kind of net (Hill *et al.*, 2006). In 2010, there was a larger coverage with 95% households owning at least one net. Though only 59% of household residents slept under a net while 77% of those who slept under a net used an insecticide-treated net (ITN) or long-lasting insecticide-treated nets (Githinji *et al.*, 2010).

The application of a residual insecticide greatly enhances the protective efficacy of bed nets. The introduction of house-spraying with residual contact insecticides has changed the whole outlook of mosquito control though incorrect use can lead to development of insecticide resistance. Chemoprophylaxis can be used to control malaria in travellers who come from non-endemic region, pregnant women and children. Some of the drugs used include Atovaquone plus Proguanil, Chloroquine, Proguanil, Doxycycline and Mefloquine (Brown, 1993). Control of malaria is also done by treating the infected human population with chemotherapeutic drugs. Some of the drugs that are in use include chloroquine , quinine sulfate, hydroxychloroquine, combination of sulfadoxine and pyrimethamine, mefloquine, combination of atovaquone and proguanil, Doxycycline, Artemisinin derivatives such as Artesunate and amodiaquine, artemether–lumefantrine , artesunate plus sulfadoxine/pyrimethamine, halofantrine and Primaquine. The World Health Organisation recommended that artemisinin combination therapies (ACT) be first-line therapy for *P. falciparum* malaria worldwide (WHO, 2006). The history of antimalarial medicine has been marked by a constant struggle between evolving drug-resistant parasites and the search for new drug formulations. Artemisinin combination therapy has been widely adopted as first-line treatment for uncomplicated falciparum malaria (Nosten and White, 2007). A study by Bhattarai *et al*. (2007*)* where they used a combination of artmesinin based combination therapy and insecticide treated mosquito nets showed a highly significant reduction of 52% in crude under-five mortality. Artesunate and amodiaquine and a fixed combination of artemether–lumefantrine were used for the first line and second line of treatment. A significant reduction was found with regard to hospitalization of malaria patients and incidence of blood transfusions, which may be considered proxy indicators of severe malaria. Combinations are effective because the artemisinin component kills the majority of

parasites at the start of the treatment while the more slowly eliminated partner drug clears the remaining parasites (White, 2004).

2.7 Protecting vulnerable groups, children and pregnant mothers from malaria infections

Pregnant women, nursing mothers, infants and small children are considered as a vulnerable group to malaria infections especially in highly endemic areas. The pathophysiology of malaria in pregnancy is greatly due to the altered immunity and availability of a new organ, the placenta. A breakdown of acquired immunity occurs in pregnancy, especially in primigravidae. While fully effective antimalaria immunity is transferred to the child (Nosten *et al.,* 2004). Loss of antimalarial immunity is consistent with the general immunosupression of pregnancy; reduced lymphoproliferative response, sustained by elevated levels of serum cortisol. This is designed to prevent the fetal rejection but renders the pregnant woman susceptible to infection (Bellamy, 2004). In multigravid women, what is lost is cell mediated immunity, but what is transferred is the passive antibody mediated immunity and therefore the pregnant mother suffers. Placenta is a new organ in the primigravidae and allows the parasites to by-pass the existing host immunity or allows placenta specific phenotypes of *P. falciparum* to multiply (Shulman and Dolman, 2003). Recently, it has been discovered that multigravid women can form strain-independent antibodies against CSA-specific parasites, and they demonstrate greatly diminished parasite load. The unique susceptibility of primigravids to placental infection can be explained by their immune inexperience with the parasite subpopulation (Luxemburger *et al.,* 2001). Pregnant women display a bias towards type- 2 cytokines and are therefore susceptible to diseases requiring type 1 responses for protection like. However, in infected pregnant women, a change of balance of the local placental environment from TH2 to TH1 has been observed, consistent with large number of monocytes in infected placenta. IL-10 levels are decreased, while IFN-g, IL-2, and TNF-α level of a type-1 cytokine response-are elevated. These pro-inflammatory cytokines account for the pathology of maternal malaria: Elevated levels of TNF- α are associated with severe maternal anaemia; symptoms of malaria and localized cytokine elevation contributes to adverse pregnancy outcomes (Anya, 2004).

Severe malaria associated disease is more common in areas of low to moderate transmission such as the highlands of East Africa and other areas of seasonal transmission (Githeko, 2007). Regular drug administration should be a priority for these groups as a form of chemoprophylaxis (Geerligs *et al.,* 2003). Intermittent preventive treatment is a very promising approach to the

management of clinical malaria burden in infants and children. However, concerns as to whether immunity is being allowed to build up during treatment, or if disease risk is actually just being postponed to later ages, persist. Intermittent preventive treatment in children has a significant potential to reduce malaria transmission, specifically in areas where it is already low to moderate (Aguas *et al.*, 2009). Pregnancy associated malaria is a major public health problem and pregnant women are at a risk of developing placental malaria. Placental malaria is associated with increased risk low birth weight as well as reduced levels of maternal hemoglobin during pregnancy. Increased public education, the use of insecticide-treated bed nets and intermittent preventive treatment as well as adequate treatment of malaria with effective anti-malarial drugs during pregnancy can help to control pregnancy associated malaria (Ofori *et al.*, 2009). This consists of administration of curative dose of an efficacious anti-malarial drug. The drug is administered under supervision of a doctor during antenatal care visits. Sulfadoxine-pyrimethamine is required at least twice during the second and third trimesters of pregnancy during routinely scheduled antenatal clinic visits regardless of whether the woman is infected or not (Akinleye *et al.*, 2009).

2.8 Monitoring malaria morbidity and mortality

Although malaria incidence has been increasing in the East African highlands (Lindsay and Martens, 1998), the extent and distribution of malaria infections in the asymptomatic human population is highly unknown. Monitoring malaria morbidity and mortality is mainly done using hospital data. This can be seen in a study that was done by Malakooti *et al*. (1998) in which they used data from hospitals for both inpatient and outpatient illnesses. To determine the local risk for malaria infection and number of people admitted during that month, daily logbooks on inpatient wards were used. These records however did not provide a population based, epidemiologic profile or information on the unbiased prevalence of malaria in local highland populations. The bias in hospital data arises due to poverty, self medication and lack of access to hospitals. Detailed parasitological data are also not normally available from clinical records. Because data on malaria prevalence in local residents is unavailable, it's not known whether whole populations are susceptible to malaria or if the infections are skewed towards children as seen in malaria holoendemic regions (Hay *et al*., 2002b).

2.8.1 Malaria transmission by entomological inoculation rate (EIR)

The EIR is the number of infectious bites per person per unit time, usually measured or expressed per year. It is the product of the human biting rate and the sporozoite rate.

EIR = *MaS* (Shaukat *et al.,* 2010)

The human biting rate (*Ma)* is the number of vectors biting an individual over a fixed period of time. *M* equals the number of *Anopheles* per person and *a* equals the average number of persons bitten by one *Anopheles* in one day. The sporozoite rate (*S*) is the fraction of vector mosquitoes present and biting that are considered infectious, i.e. *Anopheles* with sporozoites in their salivary glands (Warrell and Gilles, 2002, Snow and Marsh, 2002). Reducing any of these values would decrease the EIR. Several methods measure the human biting rate, including using capturers also known as human landing catches, pyrethrum spray catches, exit trap collections, and CDC light traps (WHO, 1975). Many errors can emerge in estimating both the human biting rate and sporozoite rate. These result from variation in method used, attraction of mosquitoes to the capturer, and diligence of the technical teams (Fontenille *et al.*, 2001). Numerous factors influence the EIR, including temperature, altitude, rainfall, and urbanization (Warrell and Gilles, 2002). In general, the EIR is directly proportional to temperature because heat accelerates the sporogonic cycle, the time necessary for ingested gametocytes to develop into infectious sporozoites. The optimal temperature for malaria transmission is 25-27°C and an average monthly relative humidity above 60% (Pampana, 1969). For the same reason, the EIR is inversely proportional to altitude because temperature decreases as altitude increases. The EIR is directly proportional to rainfall because female *Anopheles* mosquitoes lay their eggs in water. Generally, the EIR is inversely proportional to urbanization. This is because urbanization leads to construction on land that could have stagnant water and greater pollution of water sources (Robert *et al.,* 2003). Therefore, tropical areas with warm temperature, heavy rainfall, high humidity, and efficient *Anopheles* vectors are ideal for malaria transmission (Breman, 2001). These factors explain a large part of the variability in the EIRs across Africa. An adult mosquito's lifespan is particularly important to transmit malaria. The mosquito must survive long enough for the parasite to complete sporogonic development from the point where gametocytes are ingested with the blood meal to the time when infectious sporozoites appear in the salivary glands. This process typically takes 10 days for *P. falciparum* (Killeen *et al.,* 2002). Therefore, decreasing the lifespan of mosquitoes substantially decreases the EIR.

2.8.2 Malaria exposure by CSP and MSP surface protein antibodies

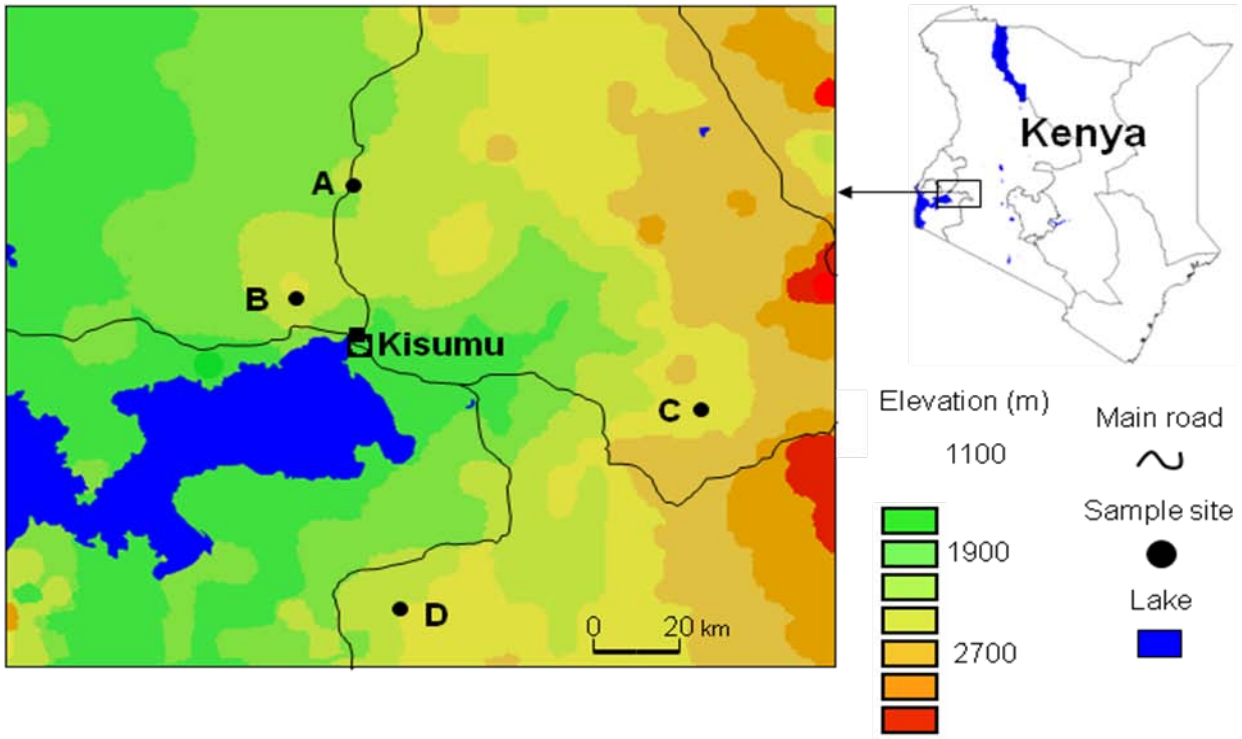
A study by Baliraine *et al*. (2009), found out that the prevalence of asymptomatic *P. falciparum* infection in a highland area of Western Kenya was high. These occurrences in the population play a significant role in acting as a reservoir for the transmission of malaria infections and the results of these occurrences were consistent with the acquisition of immunity with increasing age upon repeated infection. Transmission intensity influences the course of development of both clinical and parasitic immunity. Where malaria transmission is intense, young children bear the brunt of the disease. As they grow older, they build up an acquired immunity and are relatively protected against disease and blood stage parasites (Okiro *et al.*, 2009). Noland *et al*. (2008), documented that only 3.3% of those individuals living in an area of unstable malaria transmission such as the western Kenya highlands had a high-level IgG antibodies to both circum sporozoite and liver stage antigen 1, compared to 44.3% of those from an area with stable malaria transmission. These findings suggested that the persistent risk of clinical malaria in older children and adults in areas of unstable transmission may relate in part to the absence of high-level IgG antibodies to circum sporozoite and liver stage antigen 1. A study by Elgadir *et al*. (2007) showed that the prevalence of MSP antibodies was associated with the clinical outcome of malaria infection, and the antibody prevalence was age-dependent. More importantly, the prevalence of MSP antibodies against the test antigens was lower in severe malaria compared to uncomplicated malaria, suggesting a protective role for these antibodies against severe malaria. Furthermore, the antibody responses between individual complications of severe malaria were significantly different.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study area was located in selected villages in two valley systems, U-shaped and V-shaped valleys in the Highlands of Western Kenya. In the U-shaped valley, there were two sites, Iguhu in Kakamega district and Emutete in Emuhaya district. In the V-shaped valley, there were two sites, Fort Ternan in Nyando district and Marani in Kisii district. The study sites are shown in the figure below.



A. Iguhu
B. Emutete
C. Fort Ternan
D. Marani

Figure 1: The study area

Site 1: Iguhu, Kakamega

Iguhu village (N 0°10'.8" and E 34°45'.3" and elevation 1,430–1,580 m above sea level) (Munyekenye *et al.,* 2005) is located in Kakamega, Western Kenya. The topography of the region is U-shaped valleys along river Yala. The bottom of the valley gives poor drainage hence retains water for long making breeding grounds for mosquitoes. More breeding sites are created by the cultivation of swamps along several streams and the river Yala banks. The hillside is mostly dotted with maize plantations, with beans and potatoes being planted on the cultivated swamps. Most of the forests in the region have been cleared to give way for settlements though a natural forest, constituting about 15% of the total area, covers the east side of the study area (Afrane *et al.*, 2004a). Iguhu has two rainy seasons, the long rainy season usually occurs between mid-March and May, with an average monthly rainfall 150–260 mm, and the short rainy season which usually occurs between September and October, with an average monthly rainfall of 165 mm. Malaria prevalence peaks usually lag 1–2 months after the rain. The mean annual daily temperature is 20.8°C (Munyekenye *et al.*, 2005). The malaria situation in this site provides an ideal setting for investigating the relationship between anthropogenic landscape changes and malaria transmission (Munga *et al.*, 2009). Iguhu is an epidemic prone area for malaria. This site has been experiencing malaria outbreaks and is representative of highland areas in Kenya. Moreover, these areas have been experiencing drastic deforestation and landscape changes due to dramatic increases in the populations of humans, and thus are ideal for studying the impact of land use changes on malaria transmission. (Afrane *et al.,* 2008, Munga *et al.,* 2009)

Site 2: Emutete, Kakamega

Emutete (34° 64'E 00° 22'N and elevation 1,463-1,604m above sea level) is located in Emuhaya district and it is at the valley bottom of the U-shaped valley. It has a mean temperature of 29°C. This area experiences 2 rainy seasons and averages 2,500 mm rainfall per year. Malaria prevalence peaks usually lag 1–2 months after the rain. The mean annual daily temperature is 20.5°C.

The main economic activity in the region is subsistence farming, where there is a cultivation of reclaimed land from swamps and irrigation of farms with streams passing in between the farms that border swamps which creates breeding sites for the malaria vectors, the mosquitoes.

Site 3: Fort Ternan, Nyando

Fort Ternan (35° 21' E, 00° 12' and elevation 1,559m above sea level) is located in the Nyando district. This study site is in a narrow valley with good drainage systems with farming cash crops such as maize and sugar cane as their main agricultural activity. Malaria prevalence in this area is unstable varying between 10% and 60%, due to this instability, epidemics are common with a high morbidity and mortality rates

Site 4: Marani, Kisii

Marani (34°48' E, 00°35' S and elevation 1,520–1,700m above sea level) is located on the highland plateau adjacent to the Lake Victoria Basin and 17 km north of Kisii. Malaria epidemics have occurred frequently in this area for the last 15 years (Minakawa *et al.*, 2006). The area has Agriculture as the main economic activity with cash crop farming such as maize plantations, banana trees and other subsistence farming. It has narrow valleys hence the drainage of rain water is very fast at the valley bottoms.

3.2 Study population

The children included in this study were both males and females between the ages of six to fifteen years who were enrolled in class one to class six in selected primary schools in these regions. Studies have documented that cellular and humoral protection are present in older children who are over five years and adults in malaria endemic areas. However, the frequency of these immune responses in children aged below one year and four years is not well described (Luty *et al.*, 1999; Chelimo *et al.*, 2003). Authority of the guardian or parent was sought and they signed the informed consent forms for the child to be included in the study. Lack of consent from parent or guardian and any child who did not want to be in the study regardless of the qualifications was excluded from the study.

3.3 Sampling

Ndenga *et al.* (2006) showed that the prevalence of malaria in children of between ages 3-12 years in the western Kenya highlands to be about 50% in regions with U-shaped valleys while that in V-shaped valleys is about 10%. The sample size was calculated using the formula given below (Munyekenye *et al.*, 2005).

$$n = \frac{Z^2 \times (p) \times (1-p)}{C^2}$$

n= sample size

Z= Z value (1.96 for 95% confidence level)

p = Expected prevalence (10-50 %)

c = precision value (10%)

The sample size needed in the U shaped valley regions of Iguhu and Emutete was 88 children while those in the V shaped valleys of Marani and Fort Ternan was 190 children.

Cohorts of 96 children from Iguhu and Emutete were recruited and 210 children from Marani and Fort Ternan. The expected attrition rate was 10% and thus the sample size was adjusted to accommodate the expected deficiency. The cohorts were followed monthly for 8 months.

A sample population was picked of children under the age of 15 but not below the age of six years for the monthly *P. falciparum* surveys for the highlands in the four sites where the historical malaria prevalence data exists. This was done in Waluka Primary School in Emutete and Iguhu primary school where a cohort of 96 children was sampled. A cohort of 210 children was recruited in Kiareni Primary School and Chilchilla Primary School in Fort Ternan. The parents, guardians and teachers were given an overview of the study objectives, methodology and the risks and benefits of the study. This was in their native languages which are Luhya, Kisii and Kalenjin as some of the parents and guardians do not understand English and Swahili very well.

3.4 Ethical Clearance

The informed consent forms were given to parents of the students who were willing to participate in the study. Scientific and ethical clearance was given by the KEMRI Ethical Review Board.

3.5 Mosquito collection and Identification

Mapping of the location of the houses around the study region was done by the global positioning system (GPS) and the coordinates were recorded using differential GPS. The

altitudes were divided into different strata. According to their distribution, location and altitude there was stratified random selection of 10 houses per site.

After recording the number of sleepers in the houses during the previous night, adult mosquito sampling was conducted using the indoor pyrethrum spray collection method (WHO, 1975). White sheets were spread all over the house for the easy collection of the mosquitoes before any spraying was done. The pyrethrum was mixed with paraffin and by use of a hand spray, the sample houses were sprayed for collection of mosquitoes resting in the house during the day. This is the indoor resting densities. *Anopheline* mosquito abundance was measured as the number of female mosquitoes per house; only female mosquito abundance was analyzed because they are responsible for malaria transmission and have a strong endophilic resting behaviour. Female anopheline mosquitoes collected were classified according to their gonotrophic stages as unfed, bloodfed, halfgravid and gravid. These mosquitoes were transported in cool boxes back to the Climate and Human Health laboratories in KEMRI Kisumu where they were identified morphologically.

3.6 Estimation of Entomological Inoculation Rates (EIR)

The head and thorax were dissected from the abdomen of the female mosquitoes and individually placed in a 1.5ml Eppendorf microcentrifuge tubes. These were crushed using blocking buffer adding NP40. Sporozoite ELISA was conducted for detection of circum-sporozoite protein as described by Wirtz *et al.* (1987). Sporozoite rates were then calculated.

The entomological inoculation rate (mas) is the product of the man biting rate (ma) multiplied by the proportion of the sporozoite infected female vectors. The fraction of biting females was obtained from the bloodfed fraction of the PSC collections (Githeko *et al.,* 1994). The man biting rate was obtained by dividing the blood fed fraction of females per house by the number of sleepers. The half gravid fraction was not included in the calculation as they had not fed on the house occupants in the night before the vector collection. Blood digestion takes longer in the cool highlands (Githeko, 2007).

3.6.1 Species Identification

Legs and wings of female *Anopheles gambiae* were frozen at -20°C in labelled vials before molecular identification by PCR into *Anopheles gambiae* and *Anopheles arabiensis* according to Scott *et al.* (1993). Studies in high-elevation areas in western Kenya with an elevation above

1,500m above sea level have shown that the abundance of *An. arabiensis* is either very low or absent (Afrane *et al.*, 2007).

3.7 Parasite prevalence

After swabbing the finger with an alcohol pad, blood from a finger prick was collected in microvet tubes and two blood drops were also taken on a glass slide for the thick and thin smears to check the levels of parasitemia.

Blood smears which were stained using 4% Giemsa solution for thirty minutes and examined for *Plasmodium* species by microscopists under X100 oil immersion. Parasite density/µl of blood was calculated by counting the number of parasites per 200 white blood cells and multiplying by 40, assuming an average white blood cell count of 8000/µl (Slutsker *et al.*, 1994).

3.8 Immuno-assay for antibody detection

Blood in the microvet tubes was transported to the Kenya Medical Research Institute laboratories in Kisumu and span for five minutes at 1800 rotations per minute for serum separation. The rapid malaria diagnostic kit from SCIMEDX (SCIMEDX Corporation, Deville, New Jersey, USA) contains two recombinant capture *Plasmodium falciparum and Plasmodium vivax* parasite antigens circum-sporozoite protein (CSP) and merozoite protein (MSP). The immunochromatographic rapid test qualitatively detects antibodies of all isotypes (IgG, IgM, and IgA) specific to *Plasmodium falciparum* and *Plasmodium vivax*. The recombinant malaria *Plasmodium falciparum* antigen (CSP and MSP) and *Plasmodium vivax* antigen (CSP and MSP) colloid gold conjugate and serum sample moves along the test membrane chromatographically to the test region and forms a visible line of the antigen-antibody gold particle complex. The test has a high sensitivity and specificity. Results were scored after 10 minutes as positive or negative. Reactions occurring after 10 minutes were rejected (Kakkilaya, 2009). The tests were only carried out for *Plasmodium falciparum and Plasmodium vivax* is not known to occur in this region

3.9 Rainfall Data

Monthly rainfall, maximum and minimum temperature data for the four study sites, for the period between January 2009-April 2010 was obtained from the Kenya Department of meteorology at the Kakamega, Kericho and Kisii stations.

3.10 Data Analysis

The data was analyzed using the statistical package SPSS version 12. The average abundance of *Anopheline* mosquitoes in a house was computed for eight months for each site. Among site variation in vector abundance was analyzed using the analysis of variance (ANOVA) and the annual entomological inoculation rates were calculated. The paired t-test was used to determine differences between the circum-sporozoite and merozoite surface proteins between sites. Parasite prevalence in each site was determined by expressing positive smears as a percentage of all examined smears. The positive slides were used to calculate the geometric mean parasite densities for each site and the paired t-test was used to determine differences between the sites. Correlation and regression was then used to determine the relationship between entomological inoculation rates and the prevalence of CSP and MSP antibodies. Comparisons were considered significantly different at p values <0.05.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Rainfall Data

An El Nino event had been predicted to occur in East Africa in 2009/10. The event is characterized by heavy rains in the months of November and December. Anomalous temperatures may prevail during this period. Such conditions lead to rapid vector breeding, high malaria transmission and epidemics in the western Kenya highlands.

Data collected from the Kenya Department of meteorology indicated heavy rains in all study sites in December 2009. The Kakamega station reported 178 mm, Kericho, 299mm and Kisii, 310 mm (Figure 2). This level of rainfall is expected to trigger an increase in vector densities. Kakamega reported high rainfall (322 mm) in September 2009 which 87 mm in November. Rainfall declined to less than 100 mm mean monthly rainfall was reported in all the stations in January 2010. Anomalous temperatures were not observed.

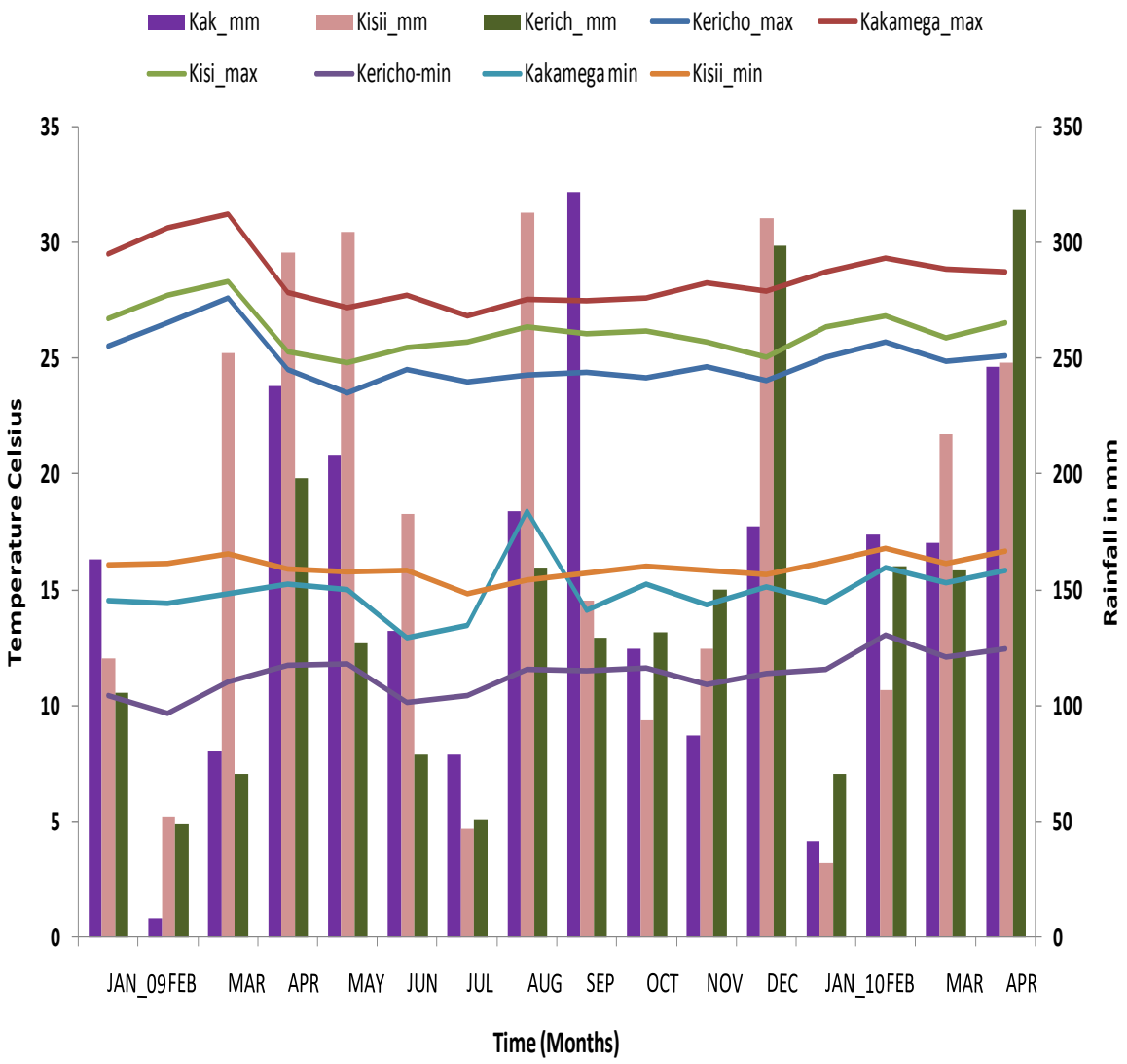


Figure 2: Rainfall and Temperature patterns

4.1.2 Vector species abundance

Anopheles gambiae complex and *Anopheles funestus* were the only vectors collected with the former comprising 98.7% of the population. *Anopheles funestus* was not collected in the V-shaped ecosystems of Fort Ternan and Marani in Kisii district.

During the study period between September 2009 and April 2010, a total of 226 Anopheles mosquitoes were caught. *Anopheles gambiae s.l.* composed of 97.1 % in Emmutete, 33.3% in Fort Ternan, 93.3% in Iguhu and 91.3% in Marani. *Anopheles arabiensis* was the predominant species in Fort Ternan with 66.7%, in Emmutete it was 2.9%, in Iguhu it was 6.7% while in Marani it was 3.6%.

Overall indoor resting densities of *An. gambiae* varied significantly between Emutete and Iguhu(t=2.84, d.f=7, P=0.025), Emutete and Fort Ternan (t=2.799, d.f=7, P=0.027), Iguhu and Fort Ternan (t=4.25, d.f=7, P=0.04), Iguhu and Marani (t=4.169, df=7, P=0.04)and Marani and Fort Ternan(t=2.839, df=7, P=0.025). However, there was no significant difference between Emutete and Marani (t=2.302, df=7, P=0.055). During the study period, *An. gambiae* was highest in Iguhu and lowest in Fort Ternan. The U shaped valley of Iguhu had a higher density than that of Emutete and these densities were higher than those in the V shaped valleys of Fort Ternan and Marani (Table 1).

Table 1: Mean densities of *An. gambiae* per survey in Western Kenya, September 2009-April 2010.

Sites	**Species**	
	Anopheles gambiae	***Anopheles funestus***
Emutete	0.7875	0.02
Iguhu	1.3375	0.01
Fort Ternan	0.1375	0
Marani	0.5625	0

4.1.3 Temporal and spatial dynamics of Indoor resting *Anopheles gambiae* Densities

There was a variation of the indoor resting densities of *An. gambiae* across the study sites from the month of September 2009 to April 2010. Densities of *An. gambiae* peaked in the Month of January which was generally one month after the wet season that was in December. At Iguhu, the vectors increased by approximately 16-fold from the end of the dry season in November 2009 to the peak of malaria transmission season season in January 2010 (Figure 3). In Emutete, the vectors increased by approximately 6-fold, while in Marani, they also increased by 6-fold. In Fort Ternan the vectors were very low through out the study period with *An. arabiensis* as the abundant vector species. The vector densities were highest in January and February a month after the rains that occurred in December.

The U-shaped ecosystems had 3-fold more vectors compared to the V-shaped ecosystem, this being consistent with the availability of breeding habitats. Heavy rainfall in Kakamega triggered a surge in the *An. gambiae* population from a mean monthly indoor resting density of zero in September 2009 to 2.5 females per house in January 2010 (Figure 3). A similar trend was observed in Emutete with the population peaking in February 2010. The *An. gambiae* population in the V-shaped Fort Ternan had very low response to rainfall with the highest indoor resting density (0.6 females/house) being observed in November 2009. In Marani a surge of 0.6 females per house was observed in November followed by a peak of 1.3 females/ house in February 2010 (Figure 3). Despite continued rainfall in February to April 2010, the vector populations continued to decline. Within the U-shaped ecosystem the indoor resting densities were not

significantly different (P= 0.073) however the indoor resting densities in V-shaped ecosystem differed significantly (P = 0.025).

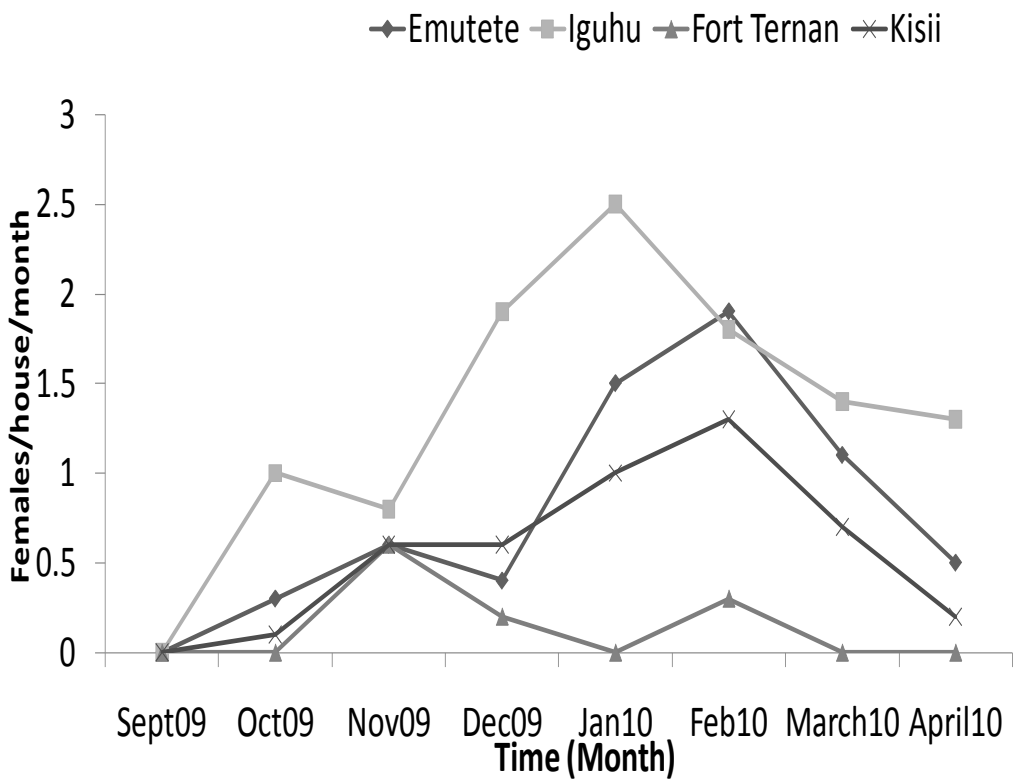


Figure 3: Indoor resting densities of *Anopheles gambiae*.

4.1.4 Distribution of Aopheles gambiae and Anopheles arabiensis

Results show that the *An. arabiensis* was the most abundant species in Fort Ternan. The highest proportion of *An. arabiensis* was observed in Fort Ternan (66.7%) followed by Emutete (6.7%), Marani (3.6%) and the lowest in Iguhu (2.9%). Species distribution was not related to the type of the ecosystem (Table 2).

Table 2: *Anopheles gambiae s.s.* and *Anopheles arabiensis* distribution in four sites in Western Kenya (PCR)

Valley	**Sites**	**Species**	
		***Anopheles gambiae*(%)**	***Anopheles funestus* (%)**
U-Shaped	**Emutete**	97.1	2.9
	Iguhu	93.3	6.7
V-Shaped	**Fort Ternan**	33.3	66.7
	Marani	66.7	3.6
	Mean Proportion	91.3	8.7

4.1.5 Sporozoite Rates and Entomological inoculation rates

Among the U-shaped ecosystems, the daily entomological inoculation rate at Emutete was 0.006 infected bites/person/night (ib/p) and in Iguhu 0.01 ib/p. In the V-shaped ecosystem, (Fort Ternan and Marani) no transmission was detected (Table 3) during the eight months period. At Iguhu, transmission was detected in December 2009 and January 2010 while at Emutete transmission was detected only in February and March 2010.

The estimated annual EIR at Iguhu was 3.68ib/p/yr and at Emutete 2.05 ib/p/yr. The mean sporozoite rate observed at Iguhu was 3.1 ib/p/yr and at Emutete 2.8 (Table3).

Table 3: Annual EIR of the study sites in Western Kenya Highlands

		U-shaped valley		V-shaped valley	
		Emutete	**Iguhu**	**Fort Ternan**	**Marani**
Year	**Mean sporozoite rate**	2.8	3.1	0	0
2009	September	0.00	0.00	0.00	0.00
	October	0.00	0.00	0.00	0.00
	November	0.00	0.00	0.00	0.00
	December	0.00	0.02	0.00	0.00
2010	January	0.00	0.06	0.00	0.00
	Feb	0.03	0.00	0.00	0.00
	March	0.01	0.00	0.00	0.00
	April	0.00	0.00	0.00	0.00
	Daily EIR	0.006	0.01	0.000	0.000
	Annual EIR	2.05	3.7	0.00	0.00

4.1.7 Prevalence of Malaria parasites in the study sites

The prevalence of parasites and the CSP and MSP antibodies peaked in January (26%, 20%, 9%, 6%) in Iguhu, Emutete, Marani and Fort Ternan respectively. The peak continued in February after the wet season in December 2009 (26%, 15%, 5%) in Iguhu, Emutete and Marani respectively.. In November 2009 and March 2010, there were no parasites seen in Marani in Kisii and the prevalence of parasites was generally low throughout the study period. In Fort ternan, there were no malaria parasites seen in the month of February and the study period was also consistent with very low parasite levels. In Iguhu and Emutete, parasite prevalence was high throughout the study period and only went down in December 2009 (Figure 4).

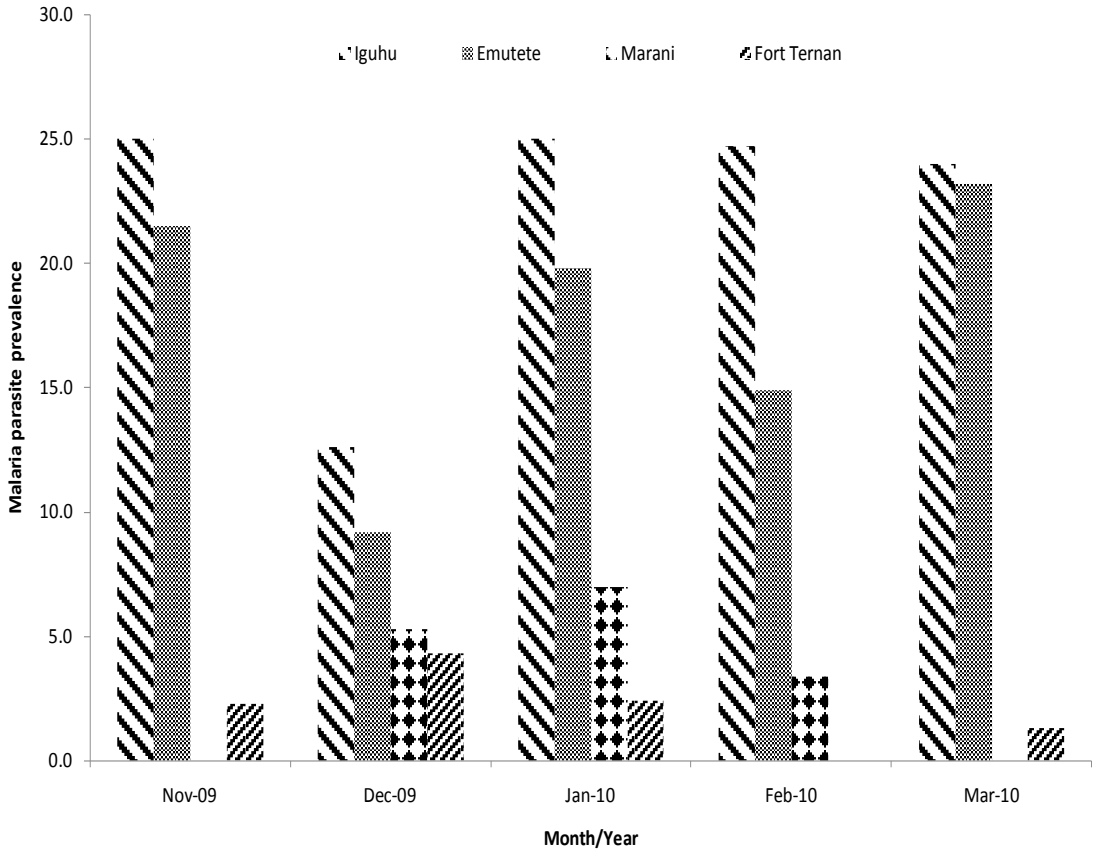


Figure 4: Prevalence of Malaria parasites across the study sites

4.1.8 Prevalence of CSP and MSP antibodies in the study sites

The prevalence of the antibodies was high in December 2009 (38%, 50%, 5%, 4%) in Iguhu, Emutete, Marani and Fort Ternan respectively and January 2010 (35%, 39%, 25%, 24%) in Iguhu, Emutete, Marani and Fort Ternan respectively. This shows that transmission increases during this months and it corresponds to the rainfall and the vector abundance during these months. Antibody levels were also generally higher in Iguhu and Emutete compared to Fort

Ternan and Kisii of the two different valley systems, the U and V shaped valleys respectively(Fig 5).

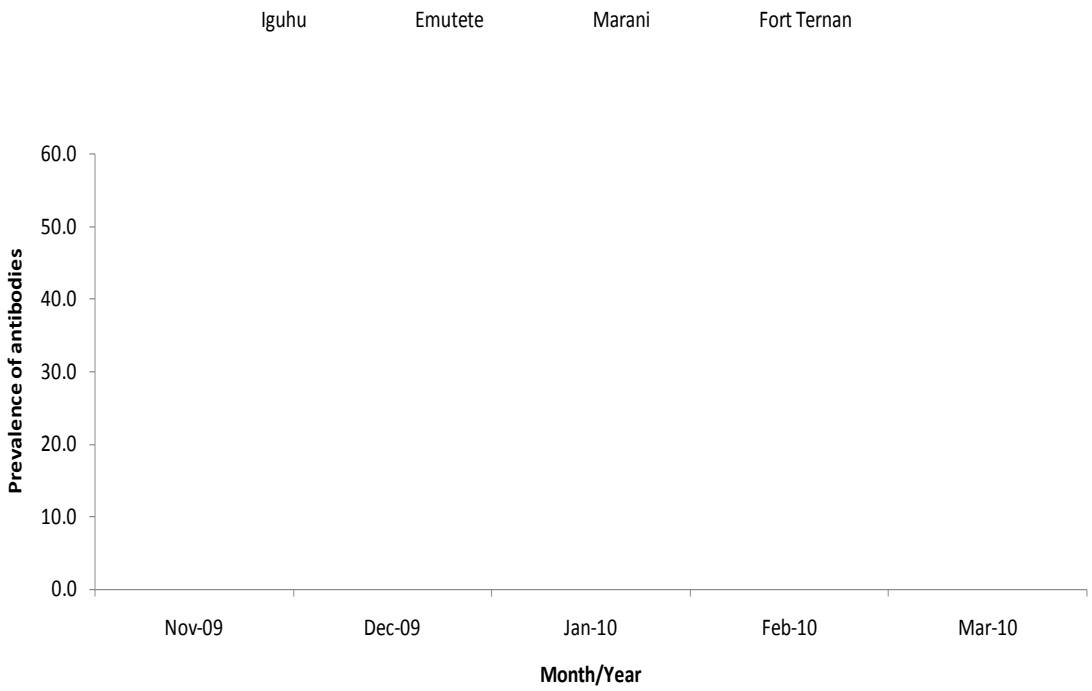


Figure 5: CSP and MSP antibody Prevalence across the study sites.

4.1.9 CSP_MSP antibodies and *P. falciparum* dynamics

An upward trend in the prevalence of CSP_MSP antibodies was observed in October 2009. Figure 3 shows the departure of the monthly prevalence from the long term mean calculate from 16 months of data. The increase in antibody prevalence was 12.6% in Iguhu, 7.5% in Emutete, 13.4% in Marani and 10.3% in Fort Ternan. At Emutete the peak prevalence (24%) was observed in December 2009, at Iguhu (15.3%) in January 2010. After a fairly linear and continuous increase in antibody prevalence a down ward trend in prevalence was observed until March 2010. In the V-shaped eco-systems of Fort Ternan and Marani the change in prevalence was characterized by a peak in December 2009 (≈ 10%) and dip in January 2010 (-3%) and then a rise in February 2010 (≈ 14%). A decrease in antibody prevalence continued until March to the same levels as the U-shaped ecosystem (Figure 6).

In Iguhu *P. falciparum* sporozoites were detected in December 2009 and January 2010 and in Emutete February and March 2010. No sporozoites were detected in the V-shaped ecosystem (Fort Ternan and Marani). Increase in transmission indicated by the CSP_MSP antibody prevalence was detected in November 2009 in all sites. First detection of sporozoite transmission at Iguhu occurred in December 2009 while in Emutete it was not detected until February 2010.

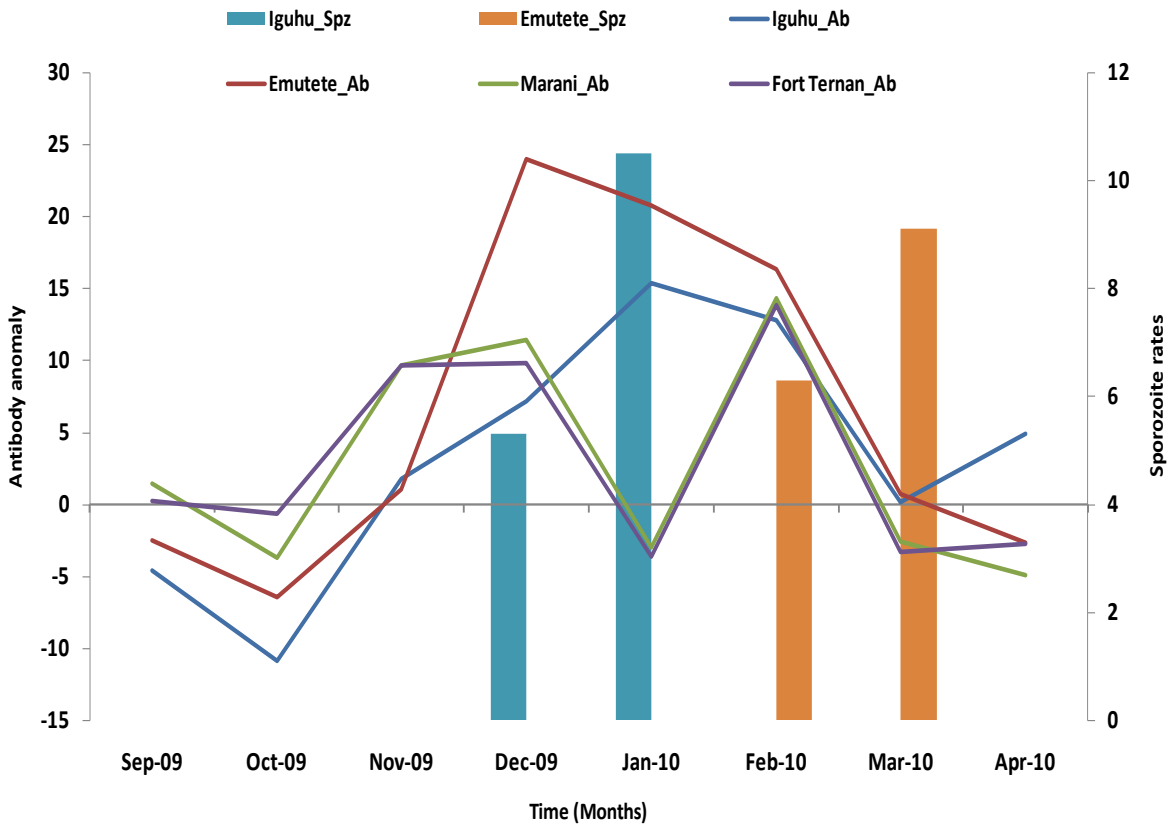


Figure 6: Comparisons between CSP_MSP antibodies and EIR

Table 4: The relationship between monthly indoor resting densities *of An. gambiae s.l* and the prevalence CSP_MSP antibodies in the human population

Site	r^2	p value
Iguhu*	0.567	0.031
Emutete	0.071	0.282
Fort Ternan*	0.570	0.030
Marani	0.159	0.204

There was a significant correlation between indoor resting densities of *An. gambiae* and CSP_MSP prevalence in Iguhu and in Fort Ternan as shown in Table 4.

4.2 DISCUSSION

This study found that there were very few vectors collected in the study area. This contributed to the rapid diagnostic kit being more sensitive in the measuring of transmission compared to the entomological parameters. Rainfall and temperature patterns played a major role in the number of vectors captured during the study. Prevalence of the CSP_MSP antibodies was high through out the study even during the times when there were no infectious mosquitoes.

4.2.1 Rainfall and Temperature

The rainfall and temperature patterns in the study sites show that at the beginning of the study, there was a lot of rainfall in the months of August and September when the study began. Rainfall and temperature patterns were important in this study due to the creation of vector breeding sites. Rainfall has been known to be a great factor that affects the distribution of the *An. gambiae* complex as *Anopheles gambiae* is a species that is adapted to regions that have moist climate (Minakawa *et al.*, 2002). Development rates of both parasites and the vectors depend on the temperatures (Githeko 2009). There were no vectors caught in September which could have been due to washing away of larvae from the larval habitats due to the constant high rainfall and no dry spell in between to give the mosquitoes time to develop. In November there was very little precipitation across all the study sites. An increase in precipitation in the month of December showed an increase in vectors in all the study sites, which was steady in the months of January and February after the rainy season. Based on findings from a study in the Usambara Mountains, Bodker *et al*. (2003) suggested that unexpected rains at the beginning of the warm season which is the dry season in a normal year, is an important generator of highland malaria epidemics. Low vector abundance appears to be one of the major factors contributing to low malaria transmission in the highlands of western Kenya (Minakawa *et al*., 2006). Different studies have also shown that multiyear cycles of the outbreak of disease patterns have been affected by the coupling of the malaria disease and an increase in rainfall (Pascual *et al.*, 2008).

4.2.2 Land Use and Valley Shape

The number of female mosquitoes collected varied according to the shape of the valley where the sites in the U-shaped valleys had the highest number of *Anopheles gambiae* collected compared to the V-shaped valleys. The presence of a high proportion of vectors in this region resulted from the creation of breeding sites due to reclamation of natural swamps for agricultural use (Minakawa *et al*., 2004). The small number of *An. funestus* vectors collected could be as a

result of clearing of swamps and creating agricultural channels which negatively affect the abundance of *An. funestus* and at the same time favour the development of *An gambiae s.s.* due to an *An. gambiae* shorter generation time in aquatic habitats (Githeko *et al*., 2006). Malaria in the highlands of western Kenya is characterized by unstable transmission which is closely related the ecosystem type and weather variability. The two ecosystems (U and V-shaped) have different vulnerabilities to malaria epidemics.

The houses that were near the swamps and other larval habitats were seen to contain more vectors than the houses which were further from the habitats. In the V shaped ecosystem good drainage patterns discouraged the development of larval habitats and therefore only a few vectors were captured. Many houses that were sampled for vectors were located further from the streams as many people preferred building their houses away from the streams. In Fort Ternan, *An. gambiae* was not the most abundant vector which denotes the changing vectorial patterns in the highlands of western Kenya. *An arabiensis* had not been detected in the highlands (Minakawa *et al.,* 2002). Thus differences in rainfall cannot explain the differences in the proportion of *An. arabiensis* in the two sites. More over the Fort Ternan and Marani have similar coverage of insecticide treated bed nets (70%, Githeko unpublished data).

4.2.3 Vector dynamics

Generally very few vectors were collected and this could be due to the use of the pyrethrum spray collection method in houses with ITNs as the mosquitoes could have left the houses during the night or before the spraying was done (Fillinger *et al*, 2009). Koenraadt *et al.,* (2006) found very few larval habitats of the *An. gambiae* which led to the very few vectors that were caught in an indoor vector collection experiment they carried out.

Current interventions using long lasting insecticide treated nets (LLIN) and in some sites indoor residual spraying (IRS) is thought to be suppressing vector populations and also selecting for the less efficient species the, *An. Arabiensis* (Bayoh *et al.,* 2010). Due to the reduced abundance of *An. gambiae* and dominance of the less efficient vector species, there is a risk of there being more individuals without immunity to malaria. This is because of the reduced exposure to malaria parasites (Noland *et al.,* 2008). Subsequently, this increases vulnerability to epidemics and severe malaria. These changes in transmission will require careful monitoring and surveillance as part of early detection of transmission risks and the evolution of epidemics.

Kristan *et al*. (2008) shows that routine entomological surveillance cannot be used for prediction or monitoring of malaria transmission in regions where there is low transmission.

4.2.4 Vector biting frequencies

Plasmodium falciparum sporozoites were detected in December and January at Iguhu and February and March at Emutete all in the U-shaped valleys and none in the V-shaped ecosystems (Marani and Fort Ternan). This is because the vector numbers were very high during this period in the U shaped valleys compared to the V shaped valleys. The U shaped ecosystems favoured the development of larval habitats during the rainy season due to the poor drainage in the area. Very many swamps and little pools of water appear after the rains which are easily warmed up when the rain stops and temperatures increase as was the case in the rainfall and temperature patterns in the highlands.

There were no sporozoites detected in the most abundant species in Fort Ternan as the *An. arabiensies* is a more zoophilic and exophillic species. This vector rests indoors and prefers to bite animals instead of humans and if they feed on humans, they prefer to rest outdoors (Githeko *et al.,* 1994). This makes them a less efficient vector in the transmission of the malaria parasite, though in the Central Kenya highlands it has been recorded to be the vector that sustains transmission of the malaria parasite (Chen *et al.,* 2006).

4.2.5 P. falciparum and CSP-MSP antibodies dynamics

In November 2009, there were no *P. falciparum* parasites detected in Marani, while in December and January, the parasite prevalence for both Marani and Fort Ternan was very low. This shows that the transmission was very low. From the vector distribution patterns, these months had no vectors collected in Fort Ternan, and relatively few vectors in Marani which could back up the low transmission of parasites in these areas. In the U shaped ecosystem, the parasite prevalence was high throughout. However, in October 2009, there was a higher prevalence of antibodies in all the sites suggesting exposure to parasite transmission. In Emutete the peak of antibody prevalence was observed in December 2009 while sporozoites were first detected in February 2010. Analysis of the relationship between the indoor resting densities of female *An. gambiae* and the prevalence of antibodies revealed a significant correlation between the two variables at Iguhu and Fort Ternan but not at Emutete and Marani. This may have resulted from the few vectors collected in the former sites. The human population had a wider

dispersion compared to the houses sampled for vectors. These results show that there are very few vectors in the western Kenya highlands and at some points there are no parasites detected in the population living in these regions. However, transmission could still be going on though in very low levels. This is easily detected by the immunological profile of the population in the region as early as three months before the entomological parameters could detect any sporozoites in Iguhu and five months before detection of sporozoites in Emutete. This is very important in the surveillance of malaria transmission in the highlands as measures could be put in place early enough to prevent epidemics from occurring. Relying on the parasite detection by microcopy only, we could be misleading. This is because parasites are only detected in the blood stream when there is no treatment. Parasites could have still been in the early stages in the liver and not in the circulatory system. Antibodies can be detected as soon as there is any exposure to the parasite.

4.2.6 Relationship between EIR and CSP-MSP antibodies

The standard for assessing transmission, the entomological inoculation rate lacks precision because mosquito distributions are heterogeneous (Drakeley *et al.,* 2003 and Mbogo *et al.,* 1995) and sporozoite infection rates are low even in the most highly endemic areas. This is compounded in areas of low transmission by low absolute numbers of mosquitoes. This was shown during the study when Sporozoites were detected in Iguhu in the months of December and January and sporozoite rates were calculated at 2.8 while in Emutete, sporozoites were detected in the months of February and March and sporozoites rates were calculated to be 3.1. These sites were located in the U shaped valleys where the highest numbers of adult female mosquitoes were collected throughout the study period. This is because these are broad valleys and they contain several reclaimed swamps that create stable larval mosquito habitats (Ndenga *et al.,* 2006). Parasite levels were also higher in the U shaped valleys compared to the V shaped ecosystems. Similar studies showed that water accumulated in valley bottoms in the Usambara Mountains and this was associated with an increased risk in malaria transmission (Balls *et al.,* 2004). There were no sporozoites detected in Marani or Fort Ternan which is located in the V shaped valleys. This could be attributed to the fact that Marani's drainage is very efficient leading to unstable larval habitats (Ndenga *et al.,* 2006). Epidemics caused severe morbidity and mortality in the 1990's onwards and as a consequence interventions to control transmission and disease were initiated 2003-2006 through the use of insecticide impregnated bed nets, indoor residual spraying

(IRS) artemisinin combination therapies (ACTS) (Okiro *et al*., 2010). These interventions are thought to be the reason why few vectors were collected and amongst which there were very few blood fed mosquitoes. Fewer vectors that were blood fed and indoors could also mean that feeding was limited to the times when the population in the region had not slept under their bed nets. In some regions, the bad quality of the bed nets was not good and the mosquitoes could enter.

An increase in Malaria transmission intensity increases the incidence of severe disease and death (Snow *et al*., 2002) and these cases of severe disease and deaths are usually estimated in hospitals. Such estimates are unreliable and always lead to over diagnosis (Trape *et al.,* 1987; Snow *et al.,* 1997; Schellenberg *et al.,* 2004) and miss large numbers of cases occurring in the community (Snow *et al.,* 2005). Direct measurement of malaria transmission within a community is thus essential, and this study derived samples from children in the community. The number of parasites found in human blood was influenced by acquired immunity which means that high levels of acquired immunity which is the presence of the CSP and MSP antibodies and rapid clearance of parasites from the circulation could lead to underestimation of transmission at high transmission intensity. In Marani and Fort Ternan, parasites were hardly detected during some periods of the study.

Our data shows that there were no parasites detected in Marani in the months of November 2009 and March 2010 and in Fort Ternan in the month of February 2010. Malaria antibodies were detected in the study population during these months. These antibodies could be an immune response created as in previous infections which had already been treated hence clearing the parasites from the blood system and leaving the antibodies. In these study sites, no sporozoites were detected in any of the female mosquitoes collected during the study period and the results showed no parasites detected in some of the months mentioned. However, transmission was still on and this was detected using the serologic markers of CSP and MSP in the rapid diagnostic kit. A study by Bousema *et al*., 2010 showed that serologic markers can be used to detect heterogeneity in malaria transmission in the Gebiley District of Somalia where malaria transmission occurred at levels too low to be detected by microscopy. From the data collected during the months when sporozoites were detected, there was the highest prevalence of the CSP and MSP antibodies in Iguhu and Emutete. There was also the highest number of mosquito

vectors collected during that time. The immunological profile shows that there was a continuous exposure to the parasite though there was no detection of the parasite itself in the circulatory system of the study population by use of microscopy. A comparison between the EIR and the prevalence of the CSP and MSP antibodies showed that there was a positive correlation between the Entomological inoculation rates and the prevalence of the CSP and MSP antibodies. This was seen in two sites of Emutete and Iguhu.

While the CSP-MSP antibody profiles in the human population were very similar in Marani and Fort Ternan, the vector profile was not similar with Fort Ternan indicating lower indoor resting densities. One possibility is that a large population of *An. arabiensis* females in Fort Ternan was not resting indoors and did not feed indoors. Correlation between the CSP-MSP profiles and the indoor resting densities showed a significant correlation in Iguhu and Fort Ternan. This shows that transmission could be detected by the use of CSP-MSP antibodies instead of the entomological parameters.

These results indicate that surveillance of CSP-MSP antibodies provided an earlier and more responsive indicator to malaria transmission than EIR. The rapid diagnostic test kit (RDT) is cheap and easier to use than the entomological inoculation rates because a large human population can be assessed in a very short time and results are available immediately. In the use of the entomological inoculation rates, more time is needed to collect the indoor resting densities and analysis of the data collected takes days and a lot of data is needed. The rapid diagnostic test provides a new opportunity for early detection of hyper-transmission and may complement results of the climate based early malaria epidemic prediction models (Githeko and Ndegwa 2001). The current work indicates that antibodies to malaria, in the human population CSP-MSP may be more sensitive to changes in transmission than the traditional EIR and that they have the potential to detect epidemic threats. From the results of the study, an upsurge of antibodies was detected at least three months earlier than the detection of the sporozoites. The changing vectorial system which is because of the inadequate vector control measures that do not cover all aspects of the vector developmental stages, may favour *An. arabiensis* which may pose challenges in vector surveillance due to its zoophilic and exophilic behaviour. A more integrated approach in vector control needs to be put in place that will also curb the *An. arabiensis* species which is becoming more dominant.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The following conclusions can be made from the study

i. Biting frequencies were very low in the study sites with no sporozoites detected in two of the study sites with a changing vectoral system. This may favour *Anopheles arabiensis* that would pose challenges to vector surveillance due to its zoophillic and exophillic nature.

ii. CSP-MSP antibodies were detected throughout the study in all the study sites shows an on-going transmission however low it was.

iii. There was a correlation between CSP-MSP antibodies and the entomological inoculation rate and the CSP-MSP antibodies detected transmission 2-3 months earlier. This shows that the CSP-MSP based kit is more sensitive and has the potential to detect early epidemic threats

5.2 RECOMMENDATIONS

- Different methods such as the CDC light traps should be incorporated into the study.
- Blood meal analysis should be done to ascertain the feeding habits of the vectors that are collected in the indoor resting vector population.

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