UNIVERSITY OF NAIROBI

A DESCRIPTIVE ENTOMOLOGICAL SURVEY OF MALARIA VECTORS IN BUSIA COUNTY

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DECLARATION

I declare that this dissertation is my original work under the guidance of the supervisors listed below and has not been submitted to the University of Nairobi or any other higher learning institution.

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My wife and daughter

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I am deeply grateful to the Kenya Medical Research Institute, Centre for Biotechnology staff for their technical support.

Above all, I thank God for this far

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ACT	Artemisinin Based Combination Therapy
An.	Anopheles
CDC	Center for Disease Control and Prevention
CS	Circumsporozoite.
DDSR	Division of Disease Surveillance and Response
DDT	dichlorodiphenyltrichloroethane
DfID	Department fo International Development
EIR	Entomological Inoculation Rate
ELISA	Enzyme Linked Immunosorbent Assay
GoK	Government of Kenya
GPIRM	Global Plan for Insecticid Resistance Management.
GPS	Global Positioning System
HBI	Human Blood Index
IDSR	Integrated Disease Surveillance and Response
IRD	Indoor Resting Density
IRS	Indoor Residual Spraying
ITN	Insecticide Treated Nets
IVM	Integrated Vector Management
KMIS	Kenya Malaria Indicator Survey
KNBS	Kenya National Bureau of Standards
LLIN	Long Lasting Insecticide Nets
MoH	Ministry of Health
NMCP	National Malaria Control Programme
PCR	Polymerase Chain Reaction
PMI	Presidential Malaria Initiative
RBM	Roll Back Malaria
RDT	Rapid Diagnostic Test
s.l	*senso lacto*
s.s	*senso stricto*
VC	Vectorial Capacity
WHO	World Health Organization

LIST OF FIGURES AND TABLES

ABSTRACT

Malaria is a leading cause of sickness and deaths in globally especially among children and pregnant women with majority (90%) of cases being in Africa. The prevalence of Malaria in Kenya is 8% with the highest prevalence (28%) being in Lake Endemic region (KMIS 2015). Successful reduction of malaria burden in an area requires knowledge of vector species present. The main objective of this study was to determine the entomological indicators of malaria in Busia County which is within the Lake endemic zone. Three sub-counties of Busia (Bunyala, Teso South and Matayos) were sampled and mosquitos were collected using light traps, Pyrethrum spray catches, Aspiration and human landing catches in 28 houses in each sub-county. Collected mosquitos were identified using taxonomical keys and through PCR and also analyzed for blood meal and sporozoites through ELISA. Results showed that *An.arabiensis* was the predominant species followed by *An.gambiae* with a heterogeneous distribution, P <0.001 (x^2 = 39.37, df = 6). The indoor resting density of anopheles mosquitoes was 9 mosquitoes/house/day while the human biting rate was 1 mosquito/person/day. The main source of blood meal was human (27%) and bovine (12%), p <0.001 (x^2=47.656, df=9). The sporozoite rate was 7% and annual EIR, 13 infective bites/person/year meaning a person is likely to get 13 episodes of malaria in a year. The main breeding habitat for Anopheles was small water pools. In conclusion, the primary malaria vectors in Busia are *An.arabiensis* and *An.gambiae s.s*. The indoor resting density is high and reduction can be achieved through indoor residual spraying. The presence of sporozoite and *Anopheles* species fed on human blood underscore the malaria endemicity status of Busia County. The findings of this study provide important information for control of malaria in Busia County.

CHAPTER 1: INTRODUCTION

Malaria is a major infectious disease in the tropics with 90% of cases being reported in Africa. Each year about half a billion cases of malaria are reported of which about a million die. However this trend has been shown to decrease due to improved funding especially on vector control interventions (Long Lasting Insecticide Nets-LLINs and Indoor Residual Spraying- IRS), diagnosis and treatment. These interventions have been scaled up especially in malaria endemic countries in the world. A major threat to the interventions is insecticide resistance and drug resistance. To overcome this, there is need to carry out routine surveillance of both the parasite and the vector. This will allow early detections of resistance and alos provide information on the entomological and parasitological indicators of malaria.

According to the last Kenya Malaria Indicator survey (KMIS, 2015) the prevalenc of malaria in the country is 8%. This is a decline form the results of KMIS 2010 where the prevalence was 11%. The Lake endemic region recorded a prevalcnec of 27% while the Coast endemic region recorded a prevalence of 8% which was an increase from 4% (MoH 2015, MoH 2010). This clearly shows there has been a decline in malaria cases due to the up of intervention aiming both at the parasite and the vector. Scale up of LLINs by partners such as Presidential Malaria Initiative (PMI) has been the main contributing factor

Vector control mainly relies on availability of information regarding vector biology. In malaria, information on mosquito biology is important when making decision on the appropriate vector control intervention. Therefore entomological surveillance forms the basis for success of vector control programs. In South Africa, the upsurge of malaria cases due to resistance of *Anopheles funestus* after use of pyrethroids rather than DDT was explained by vector surveillance. The applicability of Larval Source Management (LSM) and Integrated Vector Management (IVM) strangely, solely relies on availability of entomological data.

1.1 Problem Statement and Justification

Busia partly lies within epidemic prone and endemic zone of malaria transmission in Kenya. According to data from Disease surveillance and response unit weekly bulletin, the average malaria positivity rate in Busia was 54.4% in 2015. This county has been receiving malaria control interventions inform of LLINs, Intermittent Preventive Therapy (IPT) and Artemesinin Combination Therapy (ACT). Again, this county borders Uganda where malaria is prevalent and Siaya County which is an endemic zone for malaria in Kenya and therefore is at high risk of malaria epidemic. Early epidemic warnings and effective malaria control requires entomological data. Currently data on entomological indicators in Busia County is scanty and can barely provide guidance to policy makers on malaria prevention. In order to reduce mortality and morbidity due to malaria in Busia County there is need to provide evidence based data on entomological indicators. The aim of this study was to provide information on malaria vectors in Busia County. This information will act as tool towards effective control of malaria in Busia county and Kenya at large.

1.2 General Objectives

To determine entomological indicators of malaria in Busia county

1.3 Specific Objectives

1. To determine malaria vectors present in Busia county
2. To determine feeding and resting densities of malaria vectors in Busia county
3. To determine the breeding sites of malaria vectors in Busia county
4. To determine the sporozoite/infectivity rate of malaria vectors in Busia county
5. To determine entomological inoculation rate (EIR) in Busia County

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

Malaria is a parasitic infection caused by Plasmodium parasites which are transmitted to human by Anopheles mosquitos. There are five plasmodium species that cause malaria in human; *Plasmodium falciparum, P. ovale, P. vivax* and *P. malariae.* One species known as *P.knowlesi* is known to cause malaria in monkey. Severe malaria especially in Africa (90 to 98 %) is caused by *P.falciparum* (RBM, 2010) Species of *P.vivax* have been shown dominates the West Africa region. This species forms dormant stages called hypnozoites hence survives for a long time causing malaria relapse (Howes et al., 2015)

There are about ninety seven (97) countries in the world at risk of malaria with an estimated 3.3 billion people. WHO report of 2013 recorded an estimated 198 million cases of malaria with half a million deaths. Majority of the cases and deaths were mainly in Africa with 95% being children under the age of five and pregnant mothers due to their weak and developing immune system In Africa, a child can suffer up to six (6) episodes of malaria annually. Among the school going children, malaria has led to school absenteeism and poor academic performance especially in endemic zones. On the other hand pregnant women develop complications such as severe anemia, miscarriage and in severe malaria cases maternal death. In addition low birth weight is common to children born to mothers suffering from malaria. Despite this, there has been a significant reduction of the cases since 2000. For example, malaria cases and deaths decreased by 670 million and 4.3 million respectively between 2000 and 2013 in the whole world (WHO, 2014, WHO 2015).

2.2 Kenya malaria situation

There are four malaria epidemiological zones in Kenya, lake and coast endemic zone, epidemic prone zone, seasonal/arid zones and low risk zone. Malaria transmission in these zones varies due to factors such as rainfall, temperature and humidity. Other factors such as human activities and behavior contribute to this variation. The lake endemic zone is in Nyanza region where attitude is between 0 to 1300 meters. Malaria transmission in this zone is perennial. The high temperatures in this zone favors the sporogonic cycle while the rainfall creates the breeding sites for malaria vectors. Busia (the study site) is in this zone. Similar conditions are found in Coast endemic zone though humidity is high.

The epidemic prone zone lies in western region (Kakamega, Vihiga etc) and highlands parts of Rift Valley such as Nandi and Kericho. Malaria epidemic in this zone occurs whenever there is a temperature increase above18^{o}C (Reiter 2008, World Report, 2006). Above this temperature, the sporogonic cycle occurs faster and below this temperature the cycle doesn't get to completion. This area receives rainfall almost throughout the year. Therefore malaria transmission is determined by temperature increase since the breeding habitats are readily available. The low risk zone includes areas in Central Kenya and Nairobi characterized by temperature. The Seasonal transmission zones are those mainly in North Eastern region (Mandera, Marsbit, Wajir). Temperatures in this zone favor sporogonic cycle but there isn't rainfall which creates breeding habitats. However, whenever it rainfalls there is an upsurge of malaria cases Therefore malaria transmission in this is controlled mainly by presence or absence of rainfall (KMS, 2015)

2.2.1 Burden of the Disease

It is estimated that about 30% and 3% to 5 % of outpatient and inpatient attendance respectively in Kenya is due to malaria. Reports from ministry of health show that Kenya loses about 170 million working annually due to malaria. According to KMIS results of 2015, malaria prevalence in Kenya is 8% (KMIS, 2015).This is decline from 2010 results which reported a malaria prevalence of 11% The lake endemic zone recorded a prevalence of 27% down from 32% in 2010. There was an increase in malaria prevalence in Coast endemic zone from 4% in 2010 to 8% in 2015 (MoH, 2010). The low risk zone and seasonal/ arid zones recorded a prevalence of less than one. A significant reduction in malaria cases and deaths has been reported in Kenya due to increased coverage and use of interventions such as LLINs and ACTs (Okiro et al., 2007).

2.3 Malaria Vectors

Malaria transmitting vectors, *Anopheles spp* are the most studied mosquitos in the world. There are about 537 species of *Anopheles* mosquitos. Among these, 20 are species complexes with about 115 sibling species. The sibling species look similar morphologically but have different genetic make-up. As such they can only be differentiated by molecular techniques (Manguin, n.d., 2013). The most efficient species of malaria are found in Africa attributing to high mortality and morbidity rate. There are two main complexes in Africa, *An. funestus* and *An. gambiae* complex. In America, the main complexes are *An. alibamaus, An. albitarsis and An. darling. An.gambiae* complex found in Africa has eight sibling species namely*; An. gambiae* s.s, *An .bwambae, An. arabiensis, An. quadriannulatus, An. melas, An. amharicus, An. merus* and *An.*

coluzzii (Sinka, Rubio-palis, et al. 2010, Coetzee et al. 2013). In this complex, *An. gambiae s.s* and *An. arabiensies* are effective vectors of malaria. The other species are considered as secondary vectors. *An. arabiensis* usually inhabits arid and semi-arid environments and breeds in shallow sun lit water pools. *An. gambiae s.s* inhabits savannah and forest zones where humidity is high. On the other hand, *An.merus* and *An.melas* are found in salty and brackish water. Their distribution varies widely in that *An.melas* is found in West African coast while *An.merus* in East African Coast. Species of *An.bwambae* are mainly found in Uganda where there are geothermal springs (Gillies M. T., 1987).

There are nine siblings of *An.funestus* complex namely *An. funestus* s.s, *An. rivulorum* (West Africa), *An. confuses* (East Africa), *An. leesoni* (West-Eastern Africa), *An. fuscivenosus* (Zimbabwe), *An. vaneedeni* (Nothern parts of South Africa), *An. brucei* (Nigeria), *An. parensis* (East Africa) and *An. aruni* (Zanzibar*). An. funestus s.s* has a wide distribution and is the main malaria vector while the others are secondary vectors especially *An. rivulorum* (Sinka, Bangs, & Manguin, 2010). Unlike *An.gambiae, An. funetsus* breeds in large water bodies that are shaded (Service, 2014)

In Kenya, malaria vectors are within the two complexes found in Africa*; An.gambiae* and *An.funestus.* Previous studies have shown that *An.gambiae s.l* is mainly distributed in Nyanza, Western and Coastal region as well as arid and semi-arid areas. *An arabiensis* is the main species reported in arid areas of Turkana, Mandera. *An. funestsus* occurs in Coastal region, highland areas close to Lake victoria (Okara et al., 2010). Areas in western Kenya especially those with water hyacinths ahev been found to harbor *An. rivorulum* which is a secondary vector. (Minakawa, Dida, Sonye, Futami, & Njenga, 2012).

2.4 Malaria Vector Behaviour

Malaria vectors have different behaviors in relation to feeding and resting. This defines their ability to transmit malaria in different settings. In addition this defines the vector control interventions. Anopheles mosquitoes are known to bite at evenings, nights and early in the morning (crepuscular). The main malaria vector, *An. gambiae,* is known to have a high preference for human blood according to studies conducted in Kenya, Equitorial Guinea (Mbogo et al., 1993) (Pappa, Reddy, Overgaard, Abaga, & Caccone, 2011). *An. gambiae s.s* is known to feed indoors (endophagic) and rest indoors (Service, 2014) (Faye et al., 1997). Studies have

shown that the peak biting time for *An. gambie*. is between 2100hrs and 0000hrs with increase at 0300hrs, the peak decreases and picks again at around 0500hrs (Service, 2014). Recent studies conducted in Kenya and Ethiopia have shown variation in biting time with much biting activity being experienced between 1800hrs and 2200hrs (Asale et al. 2016, Cooke et al., 2015). The endophagic, exophagic and anthropophagic nature of *An. gambiae s.s*, makes it an effective vector of malaria especially in Africa. Among, *An. gambiae* complex, *An. arabiaensis* has been shown to have variation in its feeding and resting behavior. This sibling species has a low human blood index due to its high preference for cattle as source of blood meal as shown by studies conducted in Tanzania, Kenya (Kweka et al. 2007, Mwangangi et al., 2013). In addition it rests outdoors (exopphilic) and feeds outdoors (exophagic). Like *An. gambiae s.s*, *An. funestus* is endophagic and endophilic (Sinka, Bangs, Manguin, et al., 2010).

2.5 Malaria Transmission Entomological Indices

There are a number of entomological indices that are used to define a vector of malaria as well as show relationship between vector, parasite and human. These indices are mainly calculated as proportions based on species of mosquitos collected, feeding behavior, density of mosquitoes, resting behavior, infectivity of mosquitoes and life span of a mosquito. They include

1. Malaria Vector Density – this is a proportion of malaria vectors collected in an area divided by number of house structures where the mosquitoes are collected. This indicator provides information in relation to number of malaria vectors per house in an area to allow monitoring of vector interventions. It is expressed as Number of mosquitos/house

$$Malaria\ Vector\ Density = \frac{Number\ of\ malaria\ vectors\ collected}{Number\ of\ houses\ surveyed}$$

2. Indoor Resting density – this refers to number of mosquitos that rest indoors at any given time. This index allows monitoring of effectiveness of indoor residual spraying as it shows variation in numbers of resting mosquitos based on residual effect of the insecticide. Calculation of this index requires number of mosquitos only collected using Pyrethrum Spray Catches (PSC)

   It is expressed as Number of Mosquitos/house/night

$$Indoor\ Resting\ Density = \frac{Number\ of\ mosquitos\ collected\ using\ PSC}{Number\ of\ houses\ surveyed\ using\ PSC}$$

3. Man/human biting Rate

This refers to the number of mosquitos likely to bite human in a given unit time (hour, day). There are two approaches in calculation of this rate. Direct calculation can be made from mosquitoes collected using human landing catches (HLC) as these mosquitos are presumed to have been caught when about to bite. An indirect method involves calculating proportion of mosquitos from light trap collection that fed on human. This is achieved by calculating the human blood index and multiplying it with number of mosquitoes collected using light trap. This is expressed as Number of mosquito bites/person/day

$$= \frac{Number\ of\ mosquitos\ \ collected\ using\ light\ traps}{Total\ Number\ of\ sleepers} \times HBI$$

4. Human Blood Index (HBI)

   Mosquitoes have different preference for blood. There are those that feed mainly on human, birds, cattle, reptiles etc. Human blood index is calculated in reference to mosquitos that feed on human. This is calculated as follows.

$$HBI = \frac{Number\ of\ Female\ mosquitos\ positive\ for\ human\ blood}{Total\ Number\ of\ mosquitos\ analysed}$$

5. Sporozoite rate

   This refers to the number of mosquitos that are infected with the sporozoite. It is assumed that such mosquitos are ready to transmit malaria. This is calculated as

$$Sporozoite\ Rate = \frac{Number\ of\ mosquitoes\ with\ sporozoites}{Number\ of\ mosquitoes\ analysed}$$

6. Entomological Innoculation Rate (EIR)

   This is a measure of malaria transmission in an area. It refers to the number of infective bites a person receives per unit time (day, month, year). It is a product of sporozoite rate and man/human biting rate

$$EIR = \ Sporozoite\ rate\ \times man\ biting\ rate$$

2.6 Malaria Vector control

There are three main malaria vector control interventions namely, LLINs, IRS and larval source management. These interventions mainly rely on use of chemicals/insecticides. There are four main classes of insecticides used in LLINs and IRS; organochlorides, organophosphates, carbamates and pyrethroids. Pyrethroids are the only insecticides used in LLINs. This is because human toxicity is low and at the same time they are effective against mosquitos. The choice of insecticide to use in IRS depends on the residual effect, human toxicity and effectiveness of insecticide on mosquitos. In the past DDT was used for IRS in Kenya but due to insecticide resistance and its bioaccumulation nature is no longer used (IPEP, 2006). Larval Source Management involves reduction or disruption of breeding habitats either by use of chemicals (larviciding) or environmental modification or manipulation. Larviciding is done in areas where the breeding habitats are few and findable. The chemicals used must be environmentally friendly in that they are safe both to flora and fauna (GPIRM, 2012). In Kenya *Bacillus thuringiensis israelensis* (Bti) has been used in larval control (Fillinger, Knols, & Becker, 2003).

There has been a notable reduction in malaria cases due to scale of these interventions as recorded elsewhere in this document. In Kenya, LLINs are distributed in malaria endemic areas through mass net distribution campaigns done after every three years. However, distribution can be done in targeted areas especially when there is an upsurge of malaria cases. IRS on the other hand is done in targeted areas especially those prone to epidemics. . The cost of IRS prohibits its wide use in many areas (KMS, 2015)

2.7 Insecticide Resistance

Insecticide resistance is the main threat to vector control interventions in Kenya and Africa at large. Resistance to insecticides arises due to selection pressure (Nkya et al., 2014, Brooke *et al*., 2002). Increased use of insecticide leads to selection of susceptible strains while the mutant strain increase in population. Use of agricultural pesticides has mainly contributed to this as these insecticides are washed into the water bodies where mosquitoes breed (Nkya et al., 2014). Two main mechanisms of insecticide resistance have been described; metabolic resistance and target site resistance. Metabolic resistance occurs when there is mutation in the genes coding for enzymes that detoxify insecticide in the insect. These enzymes are oxidases, esterases and S-glutathione transferases. Mutation on these enzymes is in such a way that there is rapid detoxification of the insecticide hence reducing the lethal dose. Target site mutation involves

modification of receptors (acetylcholinesterase- AChE, sodium channels - kdr and Gamma Ammino Butalic Acid-GABA receptors) that bind insecticides. The receptors become less sensitive to insecticide. This type resistance mainly associated with pyrethroid and organochlorides. Another form of insecticide resistance due to changes in mosquito behaviour occurs. In this case, mosquitos change their resting and feeding behavior to adopt or overcome the effects of the insecticide used. For example, mosquitoes that rest outdoors change their behavior and stars resting outdoors making it difficult to control (Nan-Nan, Fang, Qiang, Pridgeon, & Xi-Wu, 2006).

In Africa, insecticide resistance has been reported in different countries. For instance, resurgence of malaria in Kwa Zulu, North of South Africa, in 1999 was due to insecticide resistance by *An funestus* (Sinka, Bangs, Manguin, et al., 2010, Hargreaves K et.al, 2000). As stated earlier, pyrethroid is the only class of insecticide used in LLIN. Resistance to this class has been reported in East and West Africa in Burkina Faso and the Ivory Coast (WHO global malaria program, 2012, Djègbè et al., 2014, Ranson H et. al, 2004). Resistance to pyrethroids and DDT has been reported in Kenya in Western and Coastal regions with susceptibility to organophoshate and carbamates being as well reported (Ochomo et al., 2015). Studies conducted in Western region have shown resurgence of *An funestus* due insecticide resistance attributable to scale up of ITNS and IRS (Cooke et al., 2015, Ototo et al., 2015b). *An arabiensis* has been shown to exhibit behavioral resistance whereby it rests and feeds mainly outdoors and on animals respectively when compared to *An,gambiae s.s* (Kawada et al., 2011, Ochomo et al., 2015).

Management of insecticide resistance is critical in order to sustain the gains of vector control intervention. The Global Plan on Insecticide Resistance Management (GPIRM) which is an arm of WHO have been developing policies and strategies for insecticide resistance management. These strategies include mosaic spraying of insecticides, use of insecticide mixtures and combinations and rotation of insecticide based on vector control intervention among others (GPIRM, 2012)

2.8 Malaria Vaccine

The emergence of insecticide resistance and its wide spread in malaria endemic countries threatens malaria control activities. Advances in research have led to development of a malaria vaccine that if approved would supplement the vector control interventions. Currently promising

results have been reported in a malaria vaccine called RTS,S. The vaccine was tested in children 6 weeks to 17 months old and was shown to have an efficacy of 38.9% and 28.5% against clinical and severe malaria respectively (Mvi & Gsk, 2015). The vaccine is awaiting pilot implementation which will involve administration in a large population of children in malaria endemic areas in the world especially Africa.

CHAPTER 3: METHODOLOGY

3.1 Sampling Size determination for entomological survey

Entomological studies use household as the study unit since calculation of sample size using mosquito as a sample unit yield biased data. For example one cannot define how many mosquitos can be collected from one household due to unique distribution and abundance of mosquitos in different households and habitats. As result sampling of mosquitos was conducted based on existing entomological guidelines (WHO, 2012) and previous studies (Ndenga et al., 2016) (Ototo, Githeko, Wanjala, & Scott, 2011). According to these studies ten (10) to 15 houses per sentinel site have been used in entomological studies. To ensure a similar range and unbiased sample size was maintained in this study, two sentinel sites/villages were randomly selected in three randomly selected sub-counties of Busia using QGIS version 2.2.0 Random selection component. In each Sentinel site, 14 houses were randomly selected using Microsoft Excel 2010 RAND function from total number of houses in each village provided by community health workers using.

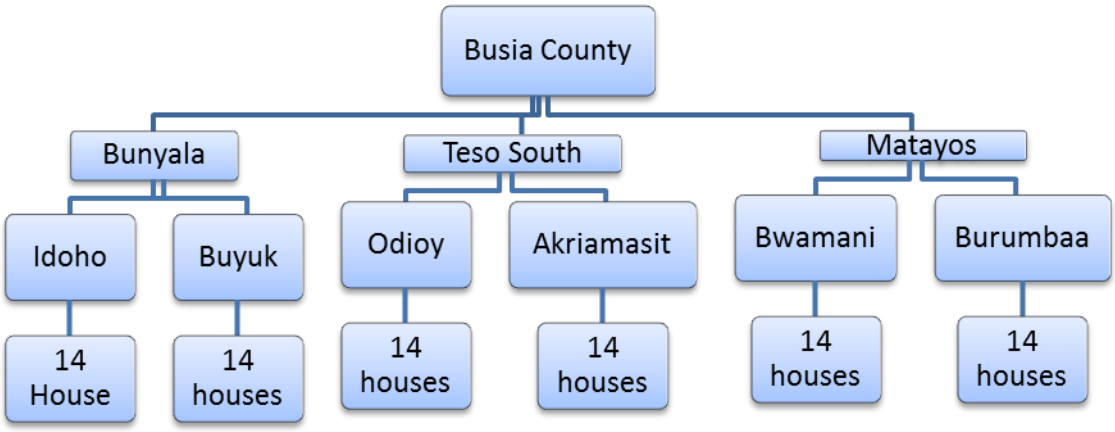

Figure 1: Sampling procedure

3.2 Study Area

The study area was Busia County. Busia County is in Western region of Kenya and borders Uganda on the West, Siaya County on the South and Kakamega on the East. Busia has 7 sub-

counties namely; Busia, Bunyala, Nambale, Butula, Teso North, Teso South and Samia. It lies within latitude of 0º and 0º 45 north and longitude 34º 25 east. Busia being in Western Region has tropical climate with a mean annual rainfall and temperatures between 900mm and 1,500mm and 24°C and 26°C respectively. Rainfall is bi-annual whereby long rains occur between March and June and short rains between September and October. The total population is about 736,300 people in 154,225 households with about 16% of the population living in urban areas (KNBS, 2009). The main economic activities are trade, fishing and agriculture. Trade mainly takes place in Busia town which borders Uganda while agriculture is mainly practiced in the rural areas with main cash crops being cassava, maize, beans, millet and sweet potatoes. Luhya is the main language.

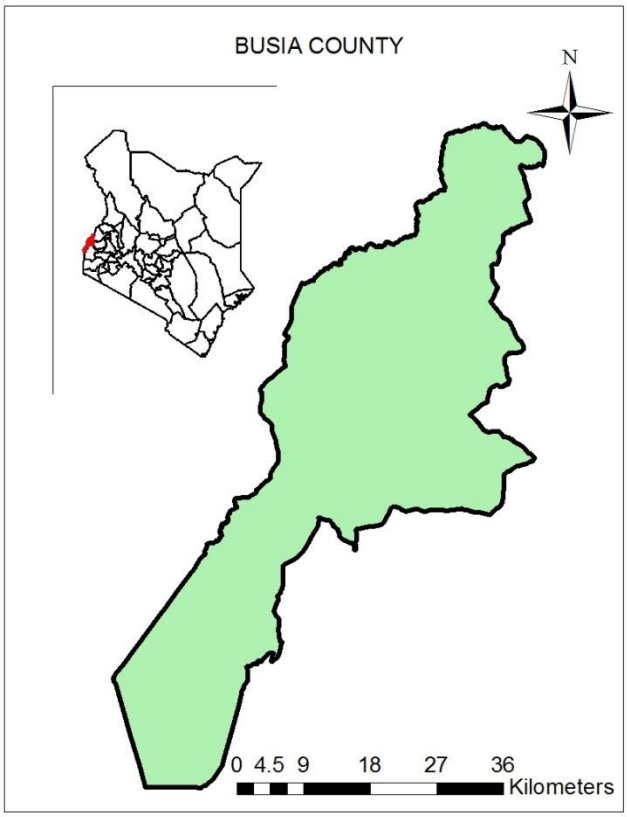

Figure 2: Map of the study Area

3.3 Mosquitoes Collection

Mosquitoes were collected using four (4) methods namely; CDC light traps, Pyrethrum Spray Catches (PSC), Aspiration (Hand Catches) and Human landing catches

3.3.1 CDC Light Traps

The light traps were set in twenty four houses in the study area. Traps were fixed between 1800hrs and 0600hrs and 1.5 meters above the ground next to a bed where a person would sleep. Traps were fixed only in bedrooms where a person/s would sleep under a net. They were removed the following morning by tying the collection cup and then disconnecting the batteries to avoid mosquitoes from escaping. The collected mosquitos were transferred into well labeled paper cups and placed in a cool box for transportation to the laboratory. Live mosquitos were killed by freezing.

3.3.2 Pyrethrum Spray Catches (PSC)

Indoor resting mosquitos were collected using this method in thirty (30) houses. Foodstuffs were removed from the house prior to spraying. White sheets (3m*3m) were spread in the house to cover about 75% of the house starting from the furthest corner to the door. Windows were closed and one person sprayed the house using a pyrethroid based insecticide from inside while the other from outside. The door was then closed for 15 minutes after which the white sheets were removed starting from those at the door to the furthest corner and spread outside the house. Knocked down mosquitoes were collected using forceps, transferred in well labeled petri dishes and placed in cool boxes for analysis in the lab.

3.3.3 Aspiration (Hand catches)

Mosquitos were searched in thirty (30) houses using this method. An aspirator with a mouth piece (polycarbonate) and a collecting piece (glass with sieve at the junction with mouth piece) was used. The collectors searched for mosquitos using a torch (Appendix 9, photo 5). Collected mosquitos were placed in well labeled paper cups and transported to the lab using a cool box. Live mosquitos were killed through freezing.

3.3.4 Human Landing Catches (HLC)

This was done in six houses by six different collectors. The collectors were the owners of the houses or those living in the houses. In cases where the owners of the houses did not accept to be mosquito collectors, the were requested to allow trained collectors from the same village to carry out the collections on their behalf. Each collector was provided with a torch, an aspirator and twelve (12) well labeled paper cups. One aspirator acted as bait by exposing the lower part of the leg from the knees while the other aspirated any mosquito landing on the exposed part of the leg.

They switched roles after every one hour. Mosquitoes were collected for twelve hours from 1800hrs to 0600hrs. Collected mosquitoes were placed in paper cups and transported to the lab the following morning. Live mosquitos were killed through freezing.

Mosquitos are attracted to human by odour and gases such as carbon dioxide. This will not be considered as it requires time to determine the person attracted to more mosquitos than the other and as such may lead to bias.

3.4 Larval and pupae Collection

Immature stages of mosquitoes (larvae and pupae) were searched near the houses. Collection of larvae in large water bodies such as swamp, river etc. was done using a standard dipper (500ml). The dipper was lowered slowly to the water body and collected water placed in a white tray for visibility of larvae and pupae. The larvae were transferred into a whirl pack using a pipette and transported to the lab for identification. A pippete was used to collect water from small breeding habitats such as hoof prints and tyre tracks.

3.5 Ethical Consideration

This is study was cleared by Kenyatta National Hospital/ UoN Ethical Review Committee. The heads of households surveyed were informed that participation in the study was absolutely voluntary and that even after consenting they would be free to withdraw from the study at any time.

3.6 Data Analysis

Data was entered in Microsoft Excel ® 2010 where proportions were calculated using the in-built Microsoft Excel ® 2010 calculator. Statistical analyses was conducted using IBM SPSS® Version 20. Proportions of mosquito present were calculated from the total number of collected. Statistical significance on mean of *Anopheles gambiae s.l* species was calculated by ANOVA. Cross tabulation on blood meal analysis and sporozoite rate was done and calculated by chi-square. The human biting rate was calculated as the product of light trap densities and human blood index. The product of human biting rate and sporozoite rate gave daily the EIR which was multiplied by 30 days and 365 days to get monthly and annual EIR respectively.

CHAPTER 4: RESULTS

4.1 Net Usage

During the study, eighty four (84) houses were visited in three sub counties of Busia as shown in table 1. In these households, 107 nets and 244 sleepers were enumerated. The overall net usage is 44% with 49%, 38% and 43% usage being recorded in Bunyala, Matayos and Teso South respectively.

Table 1: Number of Houses, Sleepers and Net Coverage

Sub-county	Viallges	Number of Houses	Number of Sleepers	Number of LLIN used the previous night	% Net usage	% Overall Net usage
Bunyala	Buyuk	14	50	24	48	
	Idoho	14	51	25	49	49
Matayos	Burumbaa	14	34	14	41	
	Bwamani	14	34	12	35	38
Teso South	Akriamasit	14	37	14	38	
	Odioy	14	38	18	47	43
Total		84	244	107	44	

4.2 Malaria vector indices

4.2.1: Vector Composition

A total of 4293 adult mosquitoes were collected in the three sub-counties of Busia of which 76% (3242) were culicine and 28% (1182) anopheline (Figure 6). The highest number of anopheline mosquitoes was in Bunyala sub county (718) followed by Teso South sub-county (390) (Figure 3). The mean number of anopheline collected in the three sub-counties were significantly different, $P<0.001$ ($F=11.568$ ANOVA). Means between Bunyala and Matayos were significantly different and also between Teso South and Matayos ($P <0.001$).

Table 2: Number of *Anopheles gambiae* mosquitos collected by different methods

Method of collection	Sub-county			Totals	Percentage collection per trap
	Bunyala	Teso South	Matayos		
Light Trap	4	99	24	127	11%
PSC	9	88	9	412	35%
Aspiration	288	164	18	470	40%
HLC	111	39	23	173	15%
Totals	718	390	74	1182	

The mean of Anopheline collected were not significantly different between methods of collections P=0.250 (ANOVA)

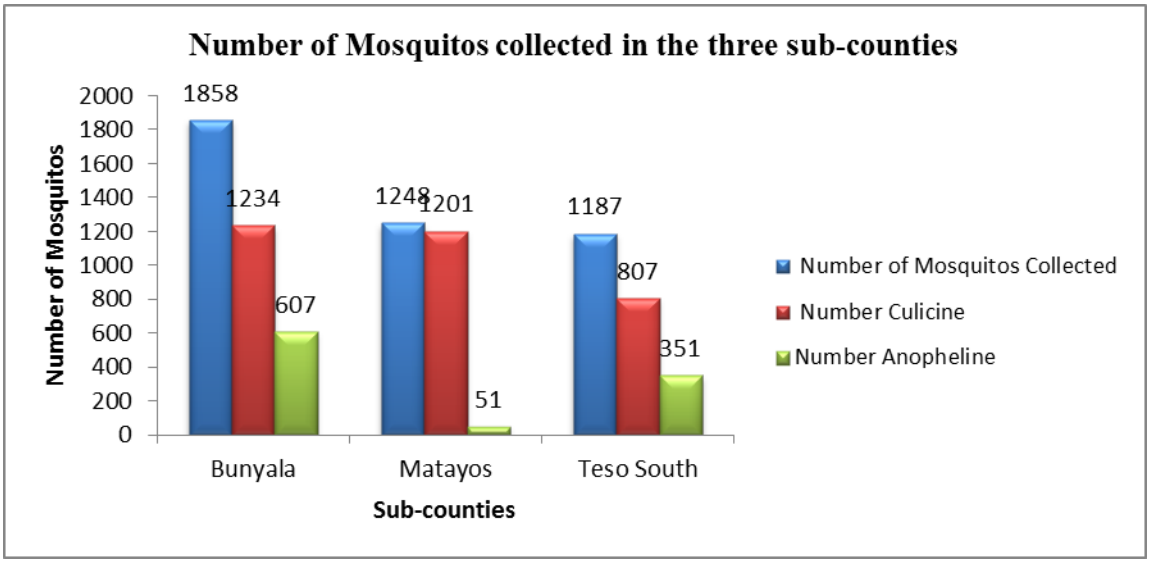

Figure 3: Number of mosquitos collected in the three sub-counties

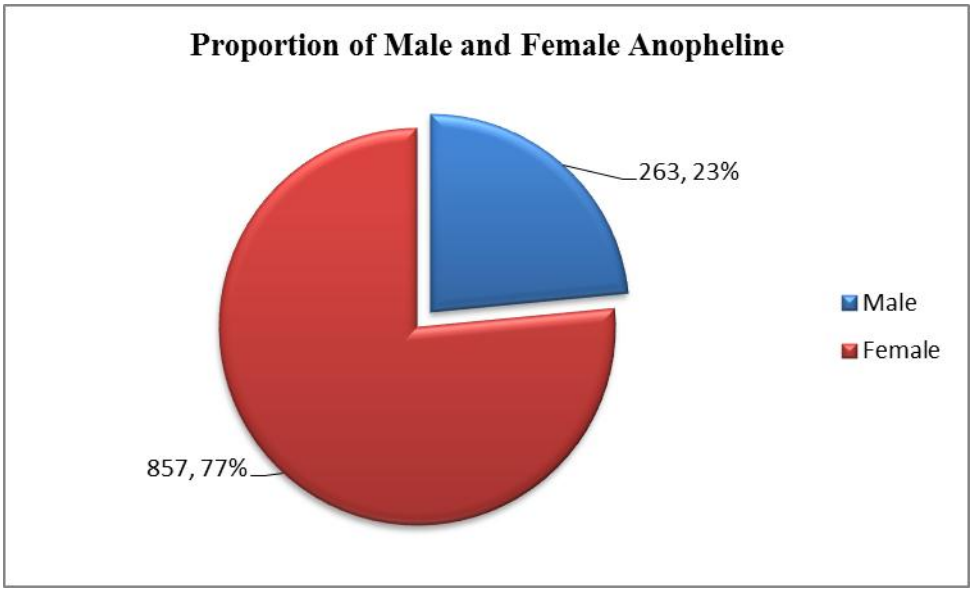

Figure 4: Proportion of Anopheline male and female mosquitoes collected

The proportion of Anopheline female and males was 77% and 23% respectively. Among Anopheline the main vectors of malaria identified using taxonomical keys was *Anopheles gambiae* (852) and *Anopheles funestus* (3) (Figure 4).

Malaria vector Density is therefore calculated as:

$$Malaria\ Vector\ Density = \frac{Number\ of\ malaria\ vectors}{Number\ of\ houses\ samples}$$

$$= \frac{855}{84}$$

= 10 Anopheles Mosquitoes per house

Among the 855 female anopheles mosquitoes, 233 *Anopheles gambiae s.l* were analyzed by PCR for species identification. Results indicated that the predominant species were *Anopheles arabiensis s.s* (39%) followed by *Anopheles gambiae s.s* (28%), 39% of mosquitoes analyzed could not amplify (Figure 5).

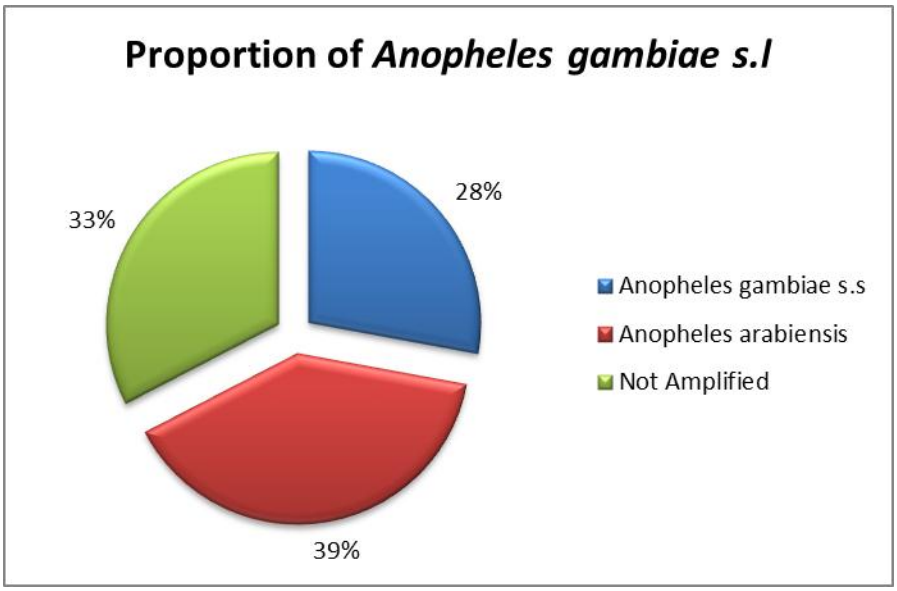

Figure 5: Proportion of *Anopheles gambiae s.l* identified by PCR

There was a statistical difference in distribution of *Anopheles gambie* s.l sibling species in the three sub-counties P <0.001 (x^2 = 39.37, df = 6). More *An.arabiensis* in Bunyala (71.7%) than in Teso South (27.2%) and Matayos (1.1%). *Anopheles gambiae* s.s was predominant in Teso South (44.6%) when compared to Bunyala (43.1%) and Matayos (12.3%) (Table 3)

Table 3: Number of sibling species of *Anopheles gambiae* s.l in each sub-county

Sub county	Number *An.gambiae s.s*	Number *An.arabiensis*	Not Amplified	Totals
Bunyala	28	66	57	151
Tesosouth	29	25	19	73
Matayos	8	1	0	9
Total	65	92	76	233

4.2.2 Indoor Resting Density

Majority of the *An. gambiae* mosquitoes were collected by aspiration 40% (470) and PSC 35% (412). Among the 412 mosquitoes collected by PSC 295 were female *An. gambiae* collected in 34 houses. Therefore indoor resting density can therefore be calculated as

$$Indoor\ Resting\ Density = \frac{Number\ of\ Female\ Anopheles\ gambiae\ collected\ by\ PSC}{Number\ of\ houses\ sampled\ with\ PSC}$$

$$= \frac{295}{34}$$

= 9 *Anopheles gambiae* mosquitoes per house per day

4.2.3 Source of Blood meal

There were 206 female *An. gambiae* mosquitoes which were fed. Among these, 139 were analyzed for blood meal source. Results showed that the main source of blood meal was human (27%, n=38) and bovine (12%, n=16) and 57% from unknown source which could be reptiles or birds (Table 4). More blood fed mosquitoes were found in Teso South (50.4%) than Bunyala (38.3%) and Matayos (11.3%) p <5 (x^2=15.86, df =6). There was a statistical difference in source of blood meal within the species and within the source of blood meal p <0.001 (x^2=47.656, df=9). More *An.gambiae s.s* (23.5%) fed on humans than *An. arabiensis* (20%).

Table 4: Source of blood meal for *An.gambiae s.l*

Species	Blood Meal Source				Totals
	Human	Bovine	Cat	Unknown Source	
An.gambiae s.s	23	3	0	34	60
An .arabiensis	15	13	1	46	75
Totals	38	16	1	80	135

Therefore human blood index (HBI) calculated as

Therefore human blood index (HBI) calculated as

$$HBI = \frac{Number\ of\ Female\ Anopheles\ positive\ for\ human\ blood}{Number\ of\ Anopheles\ analysed}$$

HBI for *An gambiae s.s* $= \frac{23}{60} = 0.38$

HBI for *An arabiensis*$= \frac{15}{75} = 0.2$

Overall HBI for *An gambiae s.l* $= \frac{38}{135} = 0.28$

4.2.4 Human biting rate

This is the product of density of mosquitos collected using light traps and human blood index

$$Mosquito\ Density\ by\ Light\ trap$$

$$= \frac{Number\ of\ An.gambiae\ \ collected\ using\ light\ traps}{Total\ Number\ of\ sleepers} \times HBI$$

$$= \frac{93}{51} \times 0.27$$

= 0.5 Anopheles mosquitoes/person/day

4.2.5 Biting Rhythm of *Anopheles gambiae*

About 173 *An. gambiae* mosquitoes were collected by human landing catches for one day. There was a gradual increase in biting time from 2100hrs to 0000hrs with a peak again between 0200hrs and 0300hrs and another peak at 0600hrs (Figure 6).

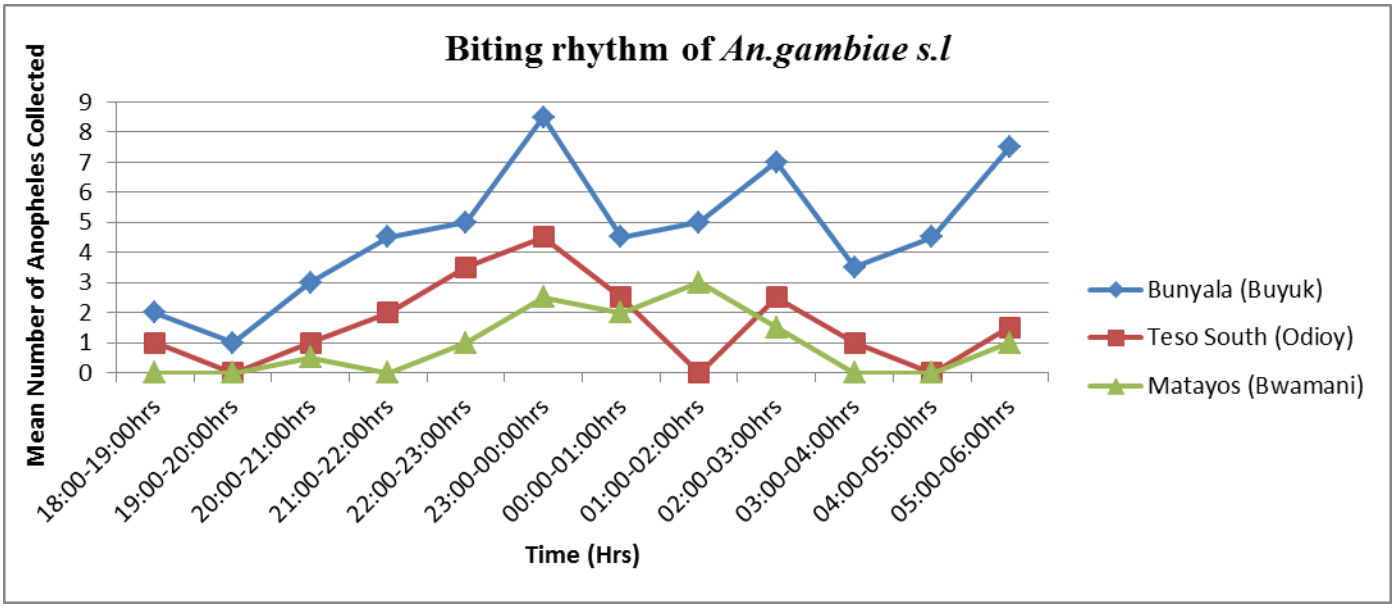

Figure 6: Biting rhythm of *An.gambiae s.l* in Bunyala, Teso South and Matayos

4.2.6 Sporozoite Analysis (ELISA)

Results generated from CS ELISA showed that 7% (n=32) of the *An. gambiae* analyzed were positive for sporozoite (Figure 7). There was a statistical difference in sporozoite rate in the two species whereby *An.gambiae s.s* (27/32) had more sporozoite than *An. arabiensis* (5/32) $p<0.001$ (x^2=142.995, df=5). Statistical significance was also noted in number of *An.gambiae s.l* with

sporozoite in each sub county p<0.05 (x^2=7.696, df =2). Bunyala (48.5. % n=32) had more *An.gambiae s.l* with sporozoite than Teso South (22.6%, n=8) and Bunyala (29.0%, n=9)

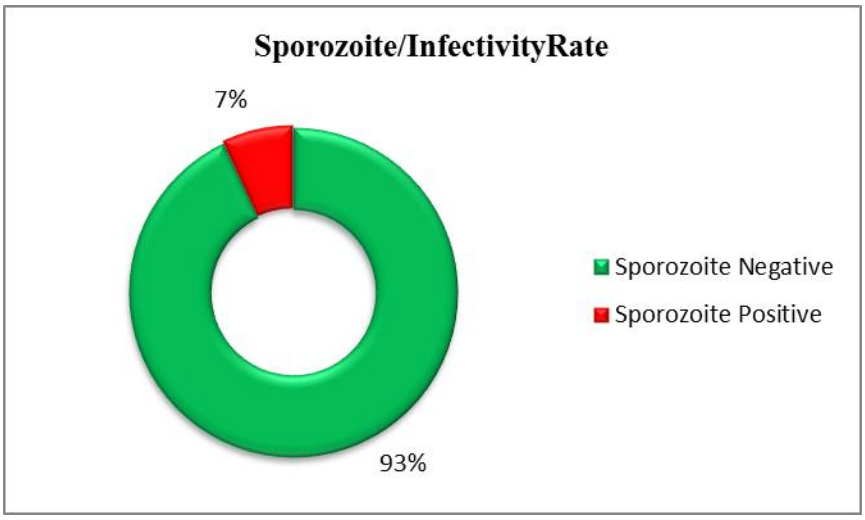

Figure 7: Proportion of *An. gambiae s.l* with Sporozoite

4.2.7 Entomological Inoculations Rate (EIR)

EIR shows the number of infective bites per person. This can be calculated as a product of sporozoite rate and human biting rate

EIR = Sporozoite rate × Human Biting rate

0.07× 0.5 = 0.035 infective bites /person/ night and 1.05 infective bites/person/month (0.035 × 30).

The Annual EIR is 13 infective bites/person/year (0.035× 365)

4.3 Mosquito breeding sites

About 85 breeding habitats were surveyed in the three sub-counties. All the 85 sampled sites, were positive for larvae with 66 (78%) being positive for anopheline larvae and 54 (64%) culicine. The main breeding habitat for Anopheles larvae were shallow pools (15 habitats) (Figure11). In Bunyala, Matayos and Teso South the main breeding habitats were Shallow water pools, ditches and large water pools respectively (Figure 8).

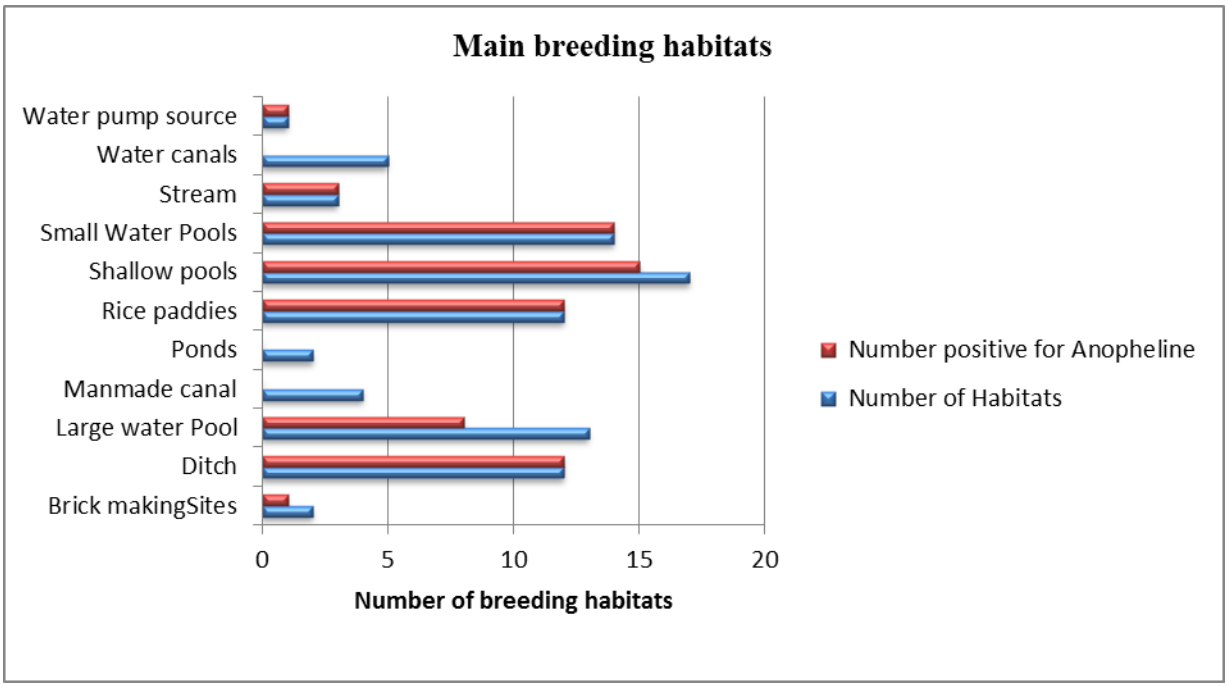

Figure 8: Main breeding sites

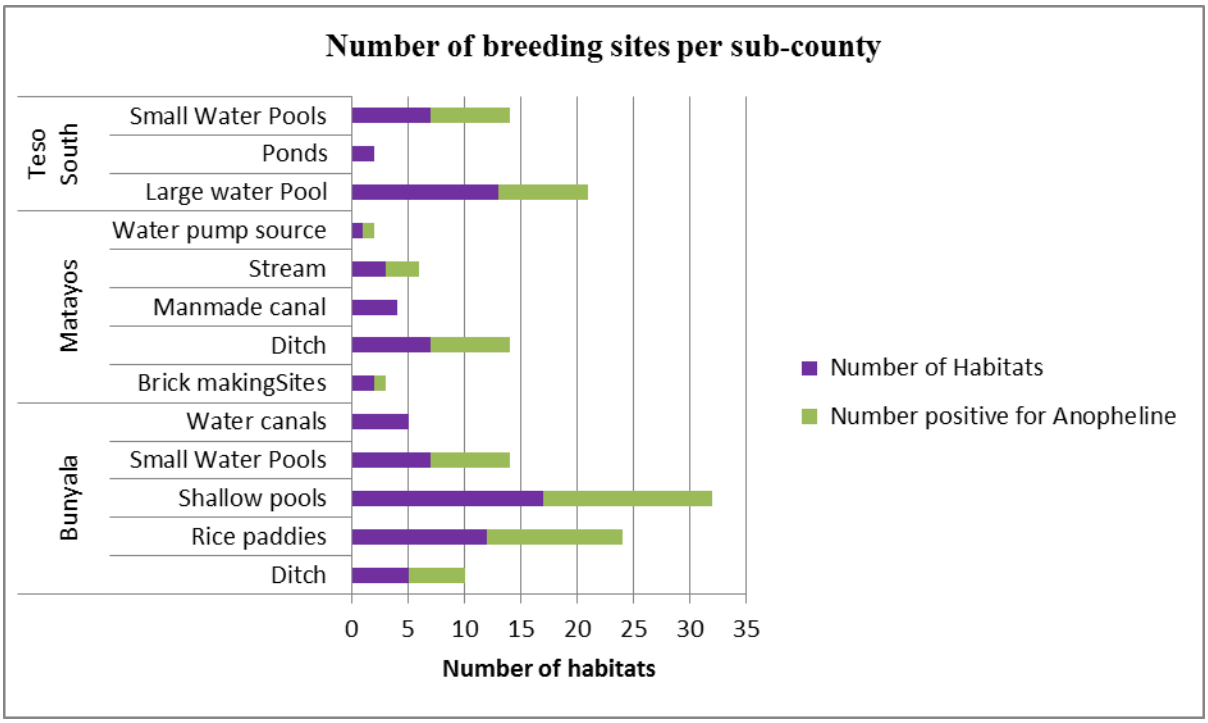

Figure 9: Breeding sites by county

CHAPTER 5: DISCUSSION, LIMITATION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The study was conducted in Busia County which lies within endemic zone of malaria transmission. Results from this survey showed that the net usage in Busia county was 44%. This area received mass net distribution in 2012 and December 2015. According to KMIS 2015, the overall net usage in the country was 48% and 67% in Lake endemic zone and Busia lies within this zone.

There are three main malaria vectors in Kenya, *An. gambiae* s.s, *An. arabianesis* and *An. funestus*. In this study the main malaria vectors that were collected were *An. arabiensis* (58.6 %), *An. gambiae s.s* (41.4%). The statistical difference in distribution *of An. arabiensis* and *An. gambie s.s* in the three sub-counties shows their distribution is heterogeneous even in close localities. A recent study conducted in Western Kenya reported that the main vectors of malaria were *An.gambiae s.s* (49%) and *An. arabiensis* (51%) (Ototo et al., 2015b) and this varied from one place to another. A study conducted in Lake endemic region in Kenya showed that the main vectors of malaria are *An. gambiae s.s, An. arabianesis* and *An. funestus* (Minakawa et al., 2012) Another study conducted in Western Kenya to determine knockdown resistance showed that the predominant species was *An. arabiensis* (96%) followed by *An.gambiae s.s* (27.3%) (Kawada et al., 2011). Similar findings have been recorded in study conducted in Rusinga Island, Western Kenya where 92.3% of *An. gambiae* mosquitos analyzed were *An. arabiensis* and *7.7% An.gambiae s.s* (Olanga, Okombo, Irungu, & Mukabana, 2015).Therefore *An. arabiensis* is the predominant malaria species in Busia County according to this study.

The indoor resting density of Anopheles mosquitos in Busia County was 4 mosquitos per house per night and a range of 0-8 mosquitos per house per day with highest being Bunyala Sub-county (8) and lowest Matayos (0) .The mean indoor resting density of *An.gambiae* according to a study conducted by Atieli et al. (2009) was shown to be 2 mosquitos per house per day. A study conducted in Ethiopia to evaluate the indoor resting denstities of *An. arabiensis* reported a mean IRD of 3 mosquitos per house per day (Animut, Balkew, & Lindtjørn, 2013). The IRD results from this study are therefore consistent with existing studies conducted in similar setup. Similar findings were reported in Nigeria during the peak seasons (March to August) where the Indoor

resting density of *An.gambiae* ranged from 2-4 mosquitos per house per day. The indoor resting density of *An. gambiae* mosquitos has been shown to increase significantly after the rains with a peak between May and July in Kenya (Ndenga et al., 2006). This study was conducted during the peak period and this could explain the high indoor resting density. In addition indoor residual spraying has not been carried out in the area since 2012. The indoor resting density (IRD) results from this study support the endophilic nature of *An. gambiae s.l* (Paaijmans & Thomas, 2011)

The biting activity of *An. gambiae* from human landing catches started at 1800hrs and stopped at 0600hrs with peaks at 2200hrs, 0000hrs, 0300hrs and 0500hrs. *An. gambiae* is known to start biting at 1800 and end at 0600 (Service 2014, Ototo et al. 2015). At 1800hrs when biting begins majority of the people are outdoors or not under an LLIN, therefore malaria transmission may occur during this period and also at 0600hrs when people are awake. During the peak biting time, majority of people are asleep and the most appropriate vector intervention would be use of LLIN. Biting rhythm therefore provides malaria control programs with important information on the appropriate vector control interventions. Research has shown that mosquitos are attracted to people based on odour and carbon dioxide (Service, 2014). During this study this was not considered when choosing human landing catches collectors due to time and methodology involved. Therefore this would have resulted in biased results

The host preference for blood meal was humans and bovines, 28% and 12% respectively. The human blood index was 0.28 with the highest being in Bunyala sub-county where most fed *An.gambiae s.l* were collected. The HBI for *An.gambie s.s* and *An.arabiensis* was 0.38 and 0.2 respectively. Similar results were observed in Coast Kenya where high degree of human feeding was recorded in *An.gambie s.s* than *An.arabiensis* and also in Equatorial Guinea (Mbogo et al. 1993, Pappa et al. 2011). A study conducted by Muriu et al (2008) reported a HBI range of 0.4-0.7 in *An.gambiae s.l.* The results of this study support the anthropophagic nature of *An. gambiae s.l.* However preference for blood meals has been shown to vary in that the *Anopheles* species adapts to available host in absence of human host or presence of other hosts. A study conducted in Mwea reported reduced anthropophagy and increases zoophagy of *An .arabiensis* (Muriu et al., 2008). This is comparable with the results of this study whereby *An. arabiensis* had a high preference for bovine than *An. gambiae s.s.* The preference of *An. arabiensis* to cattle has led to the phenomenon of zooprophylaxis as a vector control intervention (Mahande et al., 2007). The

presence of fed mosquitos on human blood is an indication of absence or ineffective use of vector control intervention aimed at preventing human-vector contact such as LLIN. The net usage in Busia as recorded elsewhere in this document is 44% which is below the Kenya Malaria Policy of having 80% of individuals in malaria endemic zones using appropriate vector control intervention (KMS, 2015). This could explain the high HBI reported.

The current study showed an overall *P. falciparum* sporozoite rate of 7 %. This was a slightly higher sporozoite rate as *An.gambiae* is known to have sporozoite rate of 1-5% (Service, 2014). This could be due to the peak malaria transmission period (May-July) when the survey was conducted. Comparable results were recorded in a study conducted in Western region where sporozoite rate of *An.gambiae* was 6.3% (Shililu, Maier, Seitz, & Orago, 1998). Another study conducted in Kopere, Western Kenya reported a sporozoite rate of 6% in *An.gambiae* (Obala et al., 2012). Consistency in high sporozoite rate in these studies clearly shows that this is an endemic zone for malaria and vector control intervention needs to be improved. However variation in sporozoite rate occurs in different malaria epidemiological zone. For example in Baringo County, Kenya the sporozoite rate of *An. arabiensis* was 0.02% according to a study conducted by Albert et al (2011). Busia being in lake endemic zone has a malaria prevalence of 27% compared with Baringo which is an epidemic prone zone with a prevalence of 3% (KMIS, 2015). The presence of high levels of parasite in blood and favorable environmental factors for sporogonic cycle in Busia County, explains the difference in sporozoite rate.

The daily EIR was 0.035 infective bites/person/day meaning that the chances of getting malaria in a day in Busia is 0.035 and 13 times likelihood of being infected by malaria in an year. High sporozoite rate has been recorded in Western region in the past where the EIR of *An. gambiae* was 0.08 infective bites/person/day and an Annual EIR of 29.2 infective bites/person/year (Shililu et al., 1998). A high annual EIR of 61.79 infective bites/person/year has been recorded in Fort Ternan, Busia County (Imbahale, Mukabana, Orindi, Githeko, & Takken, 2012). Very high EIR (121 infected bites per annum) has been reported in Africa in a study which involved 159 sites (Hay, Rogers, Toomer, & Snow, 2011). Variations in EIR observed are influenced by vector feeding rates and densities which are partly influenced by vector control interventions. The impact of LLIN could be the reason for the reduced EIR in this region when compared with other studies. However a longitudinal study would give better information in this case. Weather

conditions especially temperatures also influence EIR. High temperatures of between 25^{o}C-30^{o}C fasten the sporogonic cycle while temperatures of below 18^{o}C hinder the sporogonic cycle.

This study demonstrated that the main breeding habitats of *An. gambiae* larvae were small and shallow water pools. *An. gambiae* is known to breed in shallow and small sunlit water pools, puddles, hoof print, tyre tracks, rice paddies (Service, 2014). A study conducted in Western Kenya reported *An.gambiae* main breeding habitats as shallow water habitats (Gimnig et al., 2001) and this concurs with the findings of this study. Studies done in Africa have also pointed pools as breeding sites of *An.gambiae*. However the season, of larvae collection determines the breeding sites. Water pools are mainly temporary as they arise after the rains. This study was conducted after the long rains and this could justify the reason why the main breeding habitats are shallow and small water pools. These pools are usually a challenge in management because they are temporary and not easy to map.

5.2 Limitations of the study

Due to high numbers of Anopheles mosquitos that were collected, only a proportion was subjected to blood meal and sporozoite ELISA and PCR for species identification due to time factor. The specimens were well preserved for future identification. In addition, the larvae collected were not analyzed for species identification due to time constraint and financial resources. The outdoor mosquito collections were not performed due to financial constraints. In addition, malaria parasitological surveys were not conducted alongside the entomological data collection for comparison purposes due to limited time and finances. The blood meal ELISA was only conducted for Human, bovine and cat and not for reptiles and birds due to unavailability of reptile and birds anti-serum during the analysis.

5.3 Conclusion

The main malaria vectors in the study area are *An .arabiensis* and *An. gambiae s.s.* The indoor resting density of these vectors is high (9 mosquitoes per house per day). The malaria vectors in the area mainly feed on human and bovine. The sporozoite rate is high when compared with other study showing that the potential for malaria transmission is high when net usage is at 44%. The yearly EIR shows that a person living in the study area is likely to get 13 episodes of malaria which means malaria transmission is perennial in the study area. Small pools and puddles are the main breeding habitats for Anopheles mosquitos in the area. These breeding habitats usually occur immediately after the rains and hence are difficult to control and map. The high indoor resting density, high sporozoite rate and presence water pools in the study are useful factors to be considered in the choice and implementation of vector control intervention in the area.

5.4 Recommendations

1. Indoor Residual spraying should be implemented in the area to reduce high indoor resting density recorded in this study
2. Routine entomological surveillance to should be conducted to monitor vector densities and behavior for appropriate vector interventions
3. Longitudinal studies should be conducted in the area to monitor changes in vector behavior over a period of time and impact of LLIN on vector densities
4. Larval source management should be implemented in the area especially where breeding habitats are few and findable

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APPENDICES

Appendix 1: Informed consent form.

Respondent study Number: ---------- **Date: ----------**

Study Title: **A Descriptive Entomological Survey of Malaria vectors in Busia County**

Investigator: Lenson Kariuki, MSc Tropical and Infectious diseases, University of Nairobi,

Supervisors: 1. Dr. Mukoko Dunstan, Medical Microbiology, University of Nairobi

2. Dr. David Odongo, Biological Sciences, University of Nairobi

Investigator's statement:

The purpose of this consent form is to provide you with the information required to help you make a decision on whether to participate in this study or not

Introduction:

Malaria is a vector borne disease transmitted by a female *Anopheles* mosquito. In Kenya malaria has a prevalence of 8% and mainly occurs in Lake and Coast endemic zones. Mosquito control for malaria involves use of treated nets (LLINs), indoor spraying. To achieve effective control, information regarding mosquitos biology such as breeding site, densities, feeing behavior and resting behavior among others is required. Thus this study seeks to determine entomological indicators of malaria in Busia County. As a result we will collect mosquito samples in your house different methods and this will take about 20 minutes and in some cases 12 hours when using some methods such light traps. The results obtained will be treated with utmost confidentiality.

Benefits:

The results of this study will be useful in informing government or policy maker on effective malaria control interventions in Busia County.

Risks:

There are no direct or indirect risks anticipated in this study.

Voluntariness:

Participation in the study will be fully voluntary. You are free to refuse to participate or withdraw from the study at any time. There will be no financial reward to you for participating in the study.

Appendix 2: Consent Certificate

Participant's statement:

I _____having received adequate information regarding the study research, risks, benefits hereby AGREE to participate in the study on **"Descriptive Entomological Survey of Malaria vectors in Busia County".** I understand that my participation is fully voluntary and that I am free to withdraw at any time. I have been given adequate opportunity to ask questions and seek clarification on the study and these have been addressed satisfactorily.

Respondent's Signature: _____ Date _____

Investigator Statement

I _____ declare that I have adequately explained to the above participant, the study procedure, risks, and benefits and given him /her time to ask questions and seek clarification regarding the study. I have answered all the questions raised to the best of my ability.

Interviewer's Signature _____ Date _____

Questions about the study:

If you ever have any questions regarding the study you can contact:

Principal Investigator Contacts
Lenson Kariuki
P.O Box 20750-00202
Nairobi Kenya
Phone No: 0721823308

Lead Supervisor contacts
Dr Dunstan Mukoko
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Kenyatta National Hospital/University of Nairobi - Ethics & Research Committee
P O BOX 20723-00202, Nairobi, Kenya.
Telephone number : +2542726300-19 Ext.44102
E-mail: knhuonerc@gmail.com Post address:

Appendix 3: Informed Consent Form (Kiswahili version)
Namba ya Mshiriki……Tarehe…….

Jina la Utafiti: Upelelezi wa mbu wanaoambukiza Malaria katika Kaunti ya Busia

Mpelelezi Mkuu

Lenson Kariuki, MSc Tropical and Infectious Diseases, Chuo Kikuu cha Nairobi,

Wasimamizi

1. Dk Mukoko Dunstan, Medical Microbiology, Chuo Kikuu cha Nairobi
2. Dk David Odongo, School of Biological Sciences, Chuo Kikuu cha Nairobi

Kauli la Mpelelezi

Ningependa kuomba kushiriki katika utafiti huu. Madhumuni ya fomu hii ya idhini ni kutoa maelezo unayohitaji kukusaidia kuamua kama utashiriki katika utafiti huu au la.

Kuanzishwa

Malaria ni sababu kuu ya vifo na magonjwa duniani huku asilimia tisini ya kesi zikiripotiwa kutoka Afrika. Wanaothirika zaidi ni watoto chini ya miaka mitano na wanawake wajawazito. Katika Kenya takribani asilimia sabini ya watu huishi kwenye maeneo yaliyo na malaria. Kwa uchache watoto 14,000 hulazwa hospitalini kila mwaka kwa ajili ya malaria huku kukiwa na wastani wa vifo 34,000. Njia mwafaka za kuzuia malaria huhitaji habari kuhusu mbu kama vile mahali mbu huzalia, wakati wa kuuma, mahali ambapo mbu hupumzika na mahali pakupata damu. Hivyo basi utafiti huu una lengo la kukusanya habari kuhusu mbu wa malaria katika Kaunti ya Busia. Utaulizwa maswali ambayo itachukua wastani dakika kumi na tano. Tutakusanya sampuli za mbu katika nyumba yako na pia nje ya nyumab kwa kutumia njia tofauti. Matokeo ya utafiti huu katika nyumba yako yatafichwa kwa usiri mkubwa.

Faida

Matokeo ya utafiti huu itakuwa muhimu kwa kuhabarisha serikali kuu ya kitaifa, Kitengo cha kuzuia malaria na serikali ya Kaunti ya Busia kuhusu njia mwafaka za kudhibiti malaria.

Hatari

Hakuna hatari yoyote utakapata kwa kushiriki katika utafiti huu. Kushiriki itakugarimu muda wako kama dakika kumi na tano. Hata hivyo, sisi tutajaribu kukutumikia haraka iwezekanavyo.

Kushiriki

Kushiriki katika utafiti itakuwa kwa hiari yako. Wewe ni huru kukataa kushiriki au kuondoka kutoka utafiti wakati wowote. Hakutakuwa na zawadi ya fedha kwa ajili ya kushiriki katika utafiti huu.

Usiri

Taarifia utakayotoa katifika utafiti huu itawekwa kwa usili mkubwa. Pia habari kuhusu njina lako na jamii yaki hazitatolewa kwa mtu yeyote au jukwaa bila idhini yako.

Appendix 4: Consent Certificate (Kiswahili version)
Cheti cha Ruhusa

Mimi ----------baada ya kupokea taarifa za kutosha kuhusu huu utafiti, hatari na faida katika Kaunti ya Busia, nimekubali kuhusika. Naelewa kwamba ushiriki ni kwa hiari yangu na kwamba niko huru kujiondoa wakati wowote. Mimi nimepewa fursa ya kutosha kuuliza maswali na ufafanuzi kuhusu utafiti huu na haya yananitozeresha .

Sahihi ya Mjibu……..Tarehe……

Kauli ya mpelelezi

Mimi…….ningepe nda kutangaza ya kwamba nimeelelezea vya kutosha mshiriki kuhusu, utaratibu wa huu utafiti, hatari na faida na nimempa muda wakutosha kuuliza maswali na kutafuta ufafanuzi kuhusu huu utafiti. Nimejibu maswali yote aliyouliza kwa kadri ya uwezo wangu.

Sahihi ya mpelelezi…… Tarehe ……

Maswali kuhusu utafiti

Kama una maswali yoyote kuhusu utafiti huu unaweza kuwasiliana na:

1. Mpelelezi mkuu: Lenson Kariuki
   Sanduku la Posta: 20750-00202, Nairobi Kenya
   Nambari ya simu ya mkononi: 0721823308
2. **Msimamizi: Dr Dunstan Mukoko**
   Idara ya Medical Microbiology, Chuo Kikuu cha Nairobi
   Sanduku la posta Nairobi, Kenya
   Nambari ya simu ya mkononi: 0722348188
3. **Kamati ya maadili ya Utafiti :**
   Hospitali la Kitaifa la Kenyatta / Chuo Kikuu cha Nairobi
   Namba ya simu ya: 25 42 72 63 00-19 Ext.44102
   Barua pepe: knhuonerc@gmail.com

Appendix 5: Adult Mosquito Data Form

County……	Sub county……	Village	Date

Site of Collection: Indoor ☐ Outdoor ☐	Method of collection

Household No	Number of Sleepers	Number of Nets	Number of mosquitos collected	No culicine		No of Anopheline		Anopheles species collected					
				Male	Female	Male	Female	*Anopheles gambiae complex*			*Anopheles funestus complex*		
								Fed	Unfed	Gravidity	Fed	Unfed	Gravidity

Appendix 6: Larvae Data form

County…… Sub-County Date……
Name of Collector

Village	Type of Breeding Habitat	No of Habitats	No positive	No positive for culicine	No positive for Anopheline	
					Anopheles gambiae complex	*Anopheles funestus complex*
Totals						

Appendix 7: Sporozoite ELISA Procedure (Doolan & Beier, n.d.)**.**

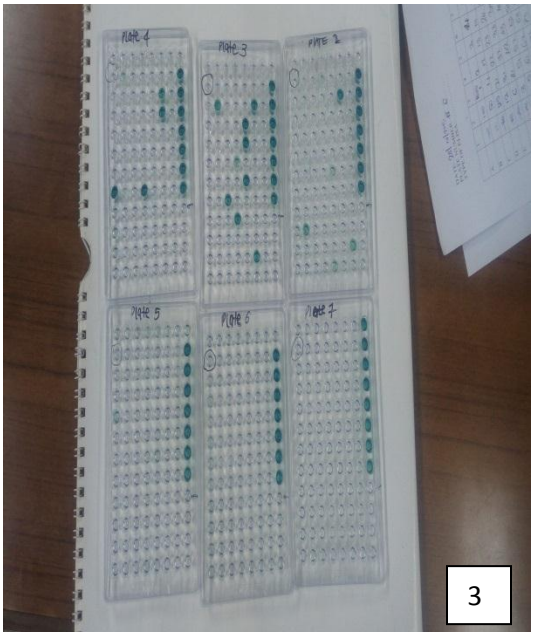
1. Prepare the mosquito sample for ELISA testing. Label sets of 1.8-mL tubes with the corresponding mosquito sample numbers. Add 50 μL of BB–NP-40 to each vial. Using a sharp clean surgical blade, cut the mosquito between the thorax and the abdomen (normally done on a filter paper). Transfer the head–thorax with forceps to the labeled tube, and transfer the abdomen to the corresponding tube for bloodmeal identification if the mosquito is blood-fed. If the mosquito is not blood-fed or no bloodmeal analysis is required, discard abdomen.
2. Grind the mosquito in the tube using a nonabsorbent glass rod or plastic pestle. Add 200 μL of the BB to bring the total sample volume to 250 μL. To avoid contamination, clean the pestle and wipe it dry before grinding the next sample. Repeat the procedure until all samples are prepared. Arrange samples in numbered order within storage boxes and keep samples in a freezer at –20 or –70°C until testing.
3. Coat number-coded ELISA plates with monoclonal antibody (MAb). In each well, add 50 μL of the diluted capture MAb. Cover the plates with another clean ELISA plate and incubate for 30 min at room temperature in subdued light.
4. Block the plates. Using an 8-channel manifold attached to a vacuum pump, aspirate the capture MAb from the microtiter plate. Bang the plate hard on an absorbent tissue paper or gauze to ensure complete dryness. Fill each well with BB using a manifold attached to a 60-mL syringe. Incubate for 1 h at room temperature in subdued light.
5. Load the plates with mosquito samples. Aspirate the blocking buffer from the wells using the manifold attached to a vacuum pump and bang plate to complete dryness. Place 50 μL of 100, 50, 25, 12, 6, 3, 1.5, 0 pg of positive control recombinant protein in the first column wells. Into the second column, add 50 μL per well of the negative controls; normally,field-collected male Anopheles mosquitoes or culicine mosquitoes are used as negative controls. Load 50 μL of each mosquito sample to the remaining wells of the plate, checking carefully that numbered mosquito samples are placed in the wells according to the completed ELISA data form. Cover the plate and incubate for 2 h at room temperature in subdued light.

6. Add peroxidase-conjugated monoclonal antibody. After 2 h, aspirate the triturate from the wells and wash the plate two times with PBS-Tw20. Add 50 μL of the peroxidase-labeled enzyme and incubate for 1 h at room temperature.
7. Add the substrate. Aspirate the enzyme conjugate from the wells and wash three times with PBS-Twn 20. Using a multichannel pipet, add 100μL of ABTS substrate and incubate for 30 min. Positive reactions, which appear green, can be determined by reading plates at 414 nm using an ELISA plate reader; absorbance values two times the mean of negative controls provides a valid cutoff for sample positivity.
8. Record results for each tested mosquito.

Appendix 8: Blood meal ELISA Procedure (Doolan & Beier, n.d.)

1. Prepare wild-caught half-gravid to freshly fed mosquitoes by cutting them transversely at the thorax between the first and third pairs of legs (under a dissecting microscope, ×10–20). In a labeled tube, place the posterior part of the mosquito containing the blood meal in 50 μL PBS and grind with a pestle or pipet repeatedly. Dilute sample 1:50 with PBS and freeze samples at –20°C until testing.
2. Load 96-well polyvinyl microtiter plates with mosquito blood meal samples by adding 50 μL of each sample per well. On the same plate, add 50 μL samples of positive control antisera for human and cow (diluted 1:500 in PBS), and four or more negative control unfed female mosquitoes or male mosquitoes obtained from the same field collections and handled as above. Cover and incubate at room temperature for 3 h (or overnight).
3. Wash each well twice with PBS-Tw20.
4. Add 50 μL of host-specific conjugate (anti-host IgG, H&L) diluted 1:2,000 (or as determined in control tests) in 0.5% BB containing 0.025% Tween-20, and incubate 1 h at room temperature.
5. Wash wells three times with PBS–Tw-20.
6. Add 100 μL of ABTS peroxidase substrate to each well.
7. After 30 min, read each well with an ELISA reader. Samples are considered positive if absorbance values exceed the mean plus three standard deviations of four negative control, unfed female, or male mosquitoes. The dark green positive reactions for peroxidase (or the dark yellow reactions for phosphatase) may also be determined visually

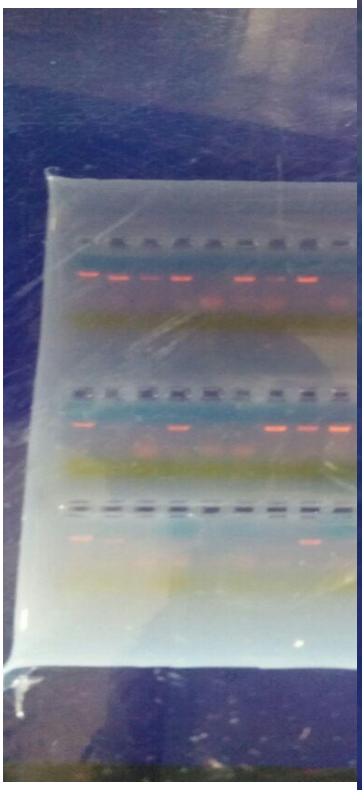
Appendix 9: Photos



5

6

7



8

(***Photo 1:*** *Larvae sampling*
habitat, ***Photo 3:*** *Sporozoi*
meal, ***Photo 5****: Mosquito A*
catches method, ***Photo 8****: In*