UNIVERSITY OF NAIROBI

SCHOOL OF PUBLIC HEALTH

Title

INCIDENCE OF MALARIA AND VECTOR CHARACTERISTICS IN A HIGH TRANSMISSION REGION IN RURAL WESTERN KENYA: IMPLICATIONS FOR DEVELOPMENT OF TARGETED MALARIA ELIMINATION STRATEGIES.

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A Thesis submitted in Fulfillment for the award of the Doctor of Philosophy degree in Public Health in the School of Public Health, University of Nairobi

JULY, 2017

DECLARATION

I declare that this is my original work in fulfillment of a doctor of Philosophy degree in Public Health at the University of Nairobi. It has not been done before by anyone else. Where other people's work or my own work has been used, this has properly been acknowledged and referenced in accordance with the University of Nairobi's requirements.

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DEDICATION

This thesis is dedicated to my brother Dr. Julius Kato for giving me an opportunity to get quality education by paying my fees all through my primary, high school and undergraduate University education.

To my late brother Joseph Waswa, you gave me the motivation to work hard in school and you always wanted the best for me. Thank you for everything.

To my mother Cecilia, for her prayers, support and offering a conducive environment for me to learn.

To my husband Ken for his patience and understanding while I committed most of my time to school work.

To my children; Cecilia, Diana and Kurt for always being around to give me joy after the hard work.

To Dr. Diana Menya for the mentorship and always offering supportive advice.

Lastly to the families in Webuye HDSS that opened their doors for us for a whole year and therefore made the study successful.

AKNOWLEDGEMENT

I would like to convey my gratitude to the following persons, without whom I would not have completed this thesis. First, to my supervisors Dr. Dismas Ongore, Dr. Ann Mwangi and Dr. John Vulule who have been guiding me in every step of the way.

I wish to specifically thank my mentors; Prof Wendy Prudhomme O' Meara and Dr. Andrew Obala with whom I consulted from time to time.

I would also like to extend my thanks to the families that we visited in the community. They welcomed us into their houses throughout the study for one full year.

I wish to thank my research assistants Edith Wamalwa and later Ibrahim Khaoya, Isaac Kunusia, Eznah Mukeli, Eric Nalianya, Lilian Nukewa, and Aggrey Wekesa for their commitment during the data collection process and throughout the study and without whom this study would not have been possible.

Above all, I would like to thank the Almighty God very much for enabling me to go through this course and overcoming the many hurdles that would have interfered with my studies.

FUNDING

This research study was partially supported by the following:

Consortium for Advanced Research in Africa (CARTA)

Malaria Eradication Scientific Alliance (MESA).

Disclaimer

The contents of this thesis are solely the responsibility of the author and do not necessarily represent the official views of any of the funders.

ACRONYMS AND ABBREVIATIONS

ACT: Artemisinin Combination Therapy

AMFm: Affordable Medicines Facility - malaria

AL: Artemether Lumefantrine

A.O.R: Adjusted Odds Ratio

CBS: Central Bureau Standards

CDC: Centers for Disease Control

DDT: Dichlorodiphenyltrichloroethane (DDT)

EIR: Entomological Inoculation Rate

ELISA: Enzyme - Linked Immunosorbent Assay

ERC: Ethical Review Committee

GEE: Generalised Estimating Equation

GPS: Global Positioning System

HDSS: Health and Demographic Surveillance Site

HRP2: Histidine Rich protein 2

IPTi: Intermitent Preventive Treatment for infants

IRB: Institutional Review Board

IREC: Institutional Research and Ethical Committee

IRS: Indoor Residual Spraying

ITN: Insecticide Treated Nets

KEMRI: Kenya Medical Research Institute

KNH: Kenyatta National Hospital

LLITNs: Long Lasting Insecticide Treated Nets

MCAR: Missing Completely at Random

MESA: Malaria Elimination Scientific Agency

MNAR: Missing Not At Random

MOH: Minstry of Health

MPHS: Minstry of Public Health and Sanitation

ODK: Open Data Kit

O.R: Odds Ratio

PDAs: Personal Data Assistant

PCR: Polymerase Chain Reaction

PSC: Pyrethrum Spray Catch

RA: Research Assistant

RDT: Rapid Diagnostic Tests

SOP: Standard Operating Procedures

UON: University of Nairobi

WET: Window Exit Trap

WHO: World Health Organisation

OPERATIONAL DEFINITIONS

Elimination: Cessation of transmission of malaria in a defined geographic area

Eradication: Global reduction of malaria incidence to zero

Fever Hotspots/Fever cold spots: Areas within the HDSS with higher than average fevers "fever hotspots" or lower than average fevers "fever cold spots."

Household: A group of people who eat from the same pot

Heterogeneity: variation in malaria risk/transmission in space and time.

Incidence of malaria: The risk of developing malaria within one year of study calculated in person months

Malaria control: Reducing the disease burden to a level at which it is no longer a public health problem.

Vector behavior: Feeding habits and resting habits of mosquitoes

Vector characteristics: These include the species, sex, composition, densities

Vectorial transmission capacity: The infectivity of the mosquito bites i.e the blood meal index and sporozoite rate.

ABSTRACT

Background and significance

Malaria is still the leading cause of morbidity and mortality especially among children below five years of age in Kenya. Although current reports indicate declining prevalence of malaria in some parts of Africa, some reports paint a grim and opposite picture in some of the areas. In Kenya, some of the regions such as Bungoma County continue to experience persistently high prevalence of malaria all year round despite the scale up of control measures. This can be attributed to various plausible reasons among them local spatial variation in malaria risk (heterogeneity). Heterogeneity in malaria risk and transmission has been previously documented even on a very small scale. Spatial analysis of self reported fevers in the HDSS indicates clustering of fevers in some villages. The main objective for this study was therefore to determine whether there were actual differences in transmission of malaria in villages with higher than average fevers (fever hotspots) and villages with lower than average fevers as well as the risk factors by measuring malaria transmission indices for a period of one year. The information can be used for designing strategies for targeting malaria control measures to the local situation with the final aim of elimination of malaria in this area.

Methodology: This was a prospective closed cohort study. The study was conducted in the Webuye Health and Demographic Surveillance Site (HDSS) located in Bungoma East Sub-County, a region which has had persistent and perennial malaria burden. Six villages (two in the fever coldspot and four in the fever hotspot) were selected for fixed entomological surveillance. One household was randomly selected in each of the villages to set up a window exit trap (WET) while two other households within the same village were selected for monthly mosquito monitoring using Pyrethrum Spray Catches (PSC). Parasitological surveillance was done for all household members in the same households where mosquito surveillance was set up as well as their immediate neighbours for a period of one year at quarterly intervals. A total of 400 participants in 72 households were followed up longitudinally and tested for malaria quarterly for the entire period. The person-month

incidence rate of malaria was computed for one year. Risk factors for malaria in the fever hotspots and coldspots were computed using multi-level mixed effects modelling. A t-test was used to compare vector densities in the fever coldspots and fever hotspots as well as the incidence of malaria. ANOVA was used to test if there were significant differences in malaria incidence among the villages. Generalised estimating equation (GEE) was used to model factors associated with asymtomatic status. Linear regression was used to show the correlation between the vector densities and the incidence of malaria.

Results: Although there was no statistically significant difference in the incidence of malaria infections between the fever hotspots and fever coldspots, those living in fever hotspots had almost one and half times increased risk of infection compared to those in the fever coldspots. There was marked and significant hetereogeneity in the incidence of malaria among the villages. Entomological risk factors such as increased larval sites and mosquito densities were mainly responsible for the observed differences in the incidence of malaria in both the fever hotspots and coldspots. Almost half (46.3%) of all the malaria infections were asymptomatic indicating a high prevelence of asymptomatic infections within the region. Malaria infections during the dry season (January) were less likely to be asymptomatic (A.O.R: 0.26, C.I: -2.289 - 0.400).

Conclusions and Recommendations

There is significant heterogeneity in the incidence of malaria among the villages correlating with entomological risk factors. There is need to target interventions based on the presenting local context in-order to increase their effectiveness. The high number of asymptomatic cases indicates the need to set up active malaria surveillance inorder to capture the asymptomatic individuals and treat them so as to reduce the parasite reservoir. Targeting the asymptomactic reservoir will reduce malaria infections further and therefore contribute towards the goal of elimination.

Key words

Malaria incidence, prevalence, hotspots, vector behavior, rural western Kenya.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%: Percent

R0 = baseline rate

Exp (Bo)

RR = Rate Ratio

tt = exposure length

Alpha = alpha level

PO =Subject allocation factor

1.0 CHAPTER ONE

1.1 Background

Malaria infection has plagued the African continent for many decades. It remains the leading cause of morbidity and mortality in Sub Saharan Africa with over 90% of malaria related deaths occurring among children below five years (World Health Organisation, 2012).

Prior to achieving the current elimination and eradication in most of the developed countries, malaria was endemic in most parts of the world in the mid-19th century affecting up to 90% of the global population. Global campaigns to eradicate malaria in the 1950s – 1970s were only able to eliminate malaria in about 37 out of 143 countries mainly in the western world. This was later put on hold because of numerous setbacks (Centers for Disease control and prevention, 2011; Nájera et al., 2011; World Health Organisation, 1969). This was followed by a period where little attention was given to malaria yet the disease continued to cause the highest morbidity and mortality especially among young children. Some countries such as Tunisia (1979), Maldives (1984), and the United Arab Emirates (2007) went ahead and eliminated malaria during this period (Wernsdorfer, 2008; World Health Organisation, 1969, 2016) .

The humanitarian crisis that followed the increased morbidity and mortality due to malaria especially in endemic regions led to the adoption of the Global Malaria Control Strategy in 1992 and to the creation, in 1998, of the Roll Back Malaria Partnership to coordinate global efforts in combating malaria (RBM, 2010; Wernsdorfer, 2008; World Health Organisation, 2016). In addition, controlling malaria was included among the millennium development goals and also african leaders affirmed their commitment to halving malaria by 2010 in the Abuja Declaration in 2000 (World Health Organisation, 2016).

The increased efforts led to the scale up of malaria control efforts by the international community in 2007 which has seen a decline in malaria infections in many African countries. However, reports

indicate that some of the countries in Sub-Saharan Africa and parts of Southeast Asia are still far from reaching the required targets of control (World Health Organisation, 2016).

The Kenyan Government adopted several strategies in controlling malaria. The global strategy of ensuring that all persons at risk of malaria sleep under a treated net was adopted in 2009 (Division of Malaria Control [Ministry of Public Health and Sanitation], 2011; Ministry of Health Kenya, 2006). The other malaria control interventions are the indoor residual spraying mainly used in epidemic prone regions as well as treatment with subsidized Artemisinin Combination Therapy (ACT) (Division of Malaria Control [Ministry of Public Health and Sanitation]Kenya National Bureau of Statistics, 2016). Despite adoption of these control measures, malaria still remains the leading cause of morbidity and mortality especially among children below five years of age in Kenya. Malaria also accounts for 30-50% of all outpatient attendance and 20% of all admissions to health facilities. It is also estimated to cause 20% of deaths in children under five years (Division of Malaria Control, 2015; Ministry of Health Kenya, 2006).

Although several factors are responsible for the consistent existence of malaria infection, vector characteristics play a key role in influencing the transmission dynamics of the disease (Elliott, 1972; Soleimani-Ahmadi et al., 2013; Trape et al., 1992). Characteristics and behavior of the vector affects malaria transmission by determining the degree, time, and place of human-vector contact. It also influences the possibility of interrupting transmission with anti-vector measures, by determining the times, places, and duration of vector-insecticide contact (Elliott, 1972; Fontenille et al., 1990; Oyewole et al., 2007). Malaria control strategies such as the ITNs and IRS work by killing the vector population consequently reducing the vector densities, thereby reducing human contact with vectors and overall reducing malaria infections. Consequently some studies have documented low transmission of malaria in regions with low vector densities and vice versa (Elliott, 1972; Pinto et al., 2000; Rahman et al., 1993; Trape et al., 1992). On the other hand, the reverse is increasingly becoming common with current reports documenting a high prevalence of malaria in areas where

low mosquito densities have been reported (Thomson et al., 1994). Some credible explanations to the current situation allude to the fact that low exposure to the malaria vectors would mean loss of partial immunity and therefore development of severe disease hence more people are likely to present with symptomatic malaria (Okiro et al., 2009). In Kenya, some of the regions in the western highlands which hardly recorded malaria infections previously currently experience outbreaks (Pascual et al., 2008). This has been attributed to the changing weather patterns with increasing temperatures at high altitudes that now support breeding of mosquitoes (Chaves et al., 2012).

Current data on malaria in Kenya shows declining trends in malaria vectors as well as malaria prevalence in previously endemic areas mainly attributed to scale up of malaria interventions especially ITNs and IRS (Division of Malaria Control (DOMC) & Minstry of Public Health and Sanitation, 2009; Mwangangi et al., 2013; O'Meara et al., 2008). Unfortunately, some regions continue to experience very high incidence of malaria infections throughout the year. Previous studies have shown that there exist differences in malaria transmission even within villages neighbouring each other (Greenwood, 1989; Ye et al., 2007). Small scale variations and temporal heterogeneity in mosquito densities could be responsible for the differences in transmission at village level hence presenting an opportunity for targeting control strategies (Greenwood, 1989; Woolhouse et al., 1997).

Bungoma East Sub-county located within Western Kenya is one of the regions with a persistently high prevalence of malaria (Hamel et al., 2001). The Health and Demographic Surveillance Site (HDSS) is a mapped out geographically homogenous area within the Bungoma East Sub-County which is predominantly rural and the population is quite homogenous in terms of their characteristics. Spatial analysis of longitudinal collection of self reported fevers in the HDSS has shown some areas of higher than average fevers (relative risk of more than 1 for fever episodes) referred to as “fever hotspots” and areas of lower than average fevers referred to as“fever cold spots”

(relative risk for fever episodes of less than 1) (O'Meara et al., 2014). This study therefore sought to determine if there was actual heterogeneity in malaria transmission in the fever hotspots and fever cold spots and whether this was related to mosquito characteristics and behavior as well as ITN and human behavioural characteristics. This information is important for targeting interventions based on the presenting local context.

1.2 Problem statement

Efforts by the international community in scaling up malaria prevention and control measures has met considerable success in many parts of Africa and given renewed hope that elimination and eventually eradication of malaria is possible. However, the success reports are not uniform in all the affected regions, some regions are still experiencing very high incidence of malaria (Zhou et al., 2011). In addition, some of the regions that had already achieved substantial control of malaria are currently beginning to experience a resurgence in new cases (Cohen et al., 2012; Division of Malaria Control [Ministry of Public Health and Sanitation]Kenya National Bureau of Statistics, 2016). This threatens to reverse some of the gains already made such as reduction in child mortality (Hamel et al., 2011). Cohen et al. (2012) attributes the current persistence in malaria infections to a possible breakdown in malaria control measures.

Although there has been an extensive scale up of malaria interventions in Kenya, malaria infections persist at unacceptably high levels in some of the regions especially in Western Kenya (Division of Malaria Control (DOMC) & Minstry of Public Health and Sanitation, 2009). In recently published work, human and mosquito behavior were found to be major threats to ITN efficacy (Obala et al., 2015). However, these may be heterogeneous and vary on a fine scale within an area. Heteroegeneity is therefore a possible and likely explanation to the current persistence of malaria in this region. Existence of micro-epidemiological differences in transmission (heterogeneity) of malaria and other infectious agents has been described before (Greenwood, 1989; Woolhouse et al.,

1997). In addition, previous studies have also shown that this heterogeneity in transmission is responsible for reduction in the efficacy of malaria control measures (Bejon et al., 2010) which could lead to this persistence in malaria infections.

The prevalence of malaria within Bungoma County remains unacceptably high (Bungoma District Health Management Team, 1996; Hamel et al., 2001; Ministry of Health Kenya & Health Policy Project, 2015). Malaria is currently the number one cause of morbidity and mortality within this County (Bungoma District Health Management Team, 1996). Specifically, Bungoma East Sub-County located within Bungoma County has got persistent, all year round malaria infections despite malaria intervention measures that have already been put in place. An increase in ITN ownership in the sub-county of more than 70% has not yielded the expected impact, and morbidity and mortality from malaria still remains very high (Ministry of Health Kenya & Health Policy Project, 2015). Reports show that over half of the more than 400 children admitted in the Webuye district hospital (main referral hospital which serves residents of Bungoma East Sub-County) every month suffer from malaria (HDSS, 2009). Recent community based surveys showed that 73% of children are asymptomatically infected (HDSS, 2009). Over the same period, admissions to the paediatric ward at the Webuye Sub-County hospital remained quite high. In the first six months of 2012, 1450 children were hospitalized with malaria. In addition, data from rural health facilities in the district show hundreds of laboratory-confirmed malaria cases per month (HDSS, 2009) .

Longitudinal surveys on self reported fevers within the HDSS are carried out half yearly. These have shown clustering of fevers in some villages (higher than average clusters of fevers) while other villages had less than average fevers (lower than average fever clusters). This could be an indicator of possible spatial heterogenity in transmission of malaria in the HDSS even though it's within a geographically homogeneous area and therefore needs to be investigated. In addition, there is a

paucity of information on heterogeneity of malaria risk especially in endemic/high transmission settings. There is need to identify actual malaria hotspots and risk factors within this high transmission region with the view of presenting the local situation for targeting of interventions and eventually elimination.

1.3 Rationale

As malaria infections begin to decline in many parts of the country, transmission becomes more heterogeneous. This brings in the need to identify, investigate and respond to malaria hot spots in order to reduce transmission in the affected area as well as prevent transmission to the neighboring areas.

Although Kenya has made significant advances in controlling malaria in the last decade, the disease still remains the leading cause of child morbidity and mortality. Routine distribution of ITNs to vulnerable groups and mass distribution to all people in endemic or epidemic prone regions has led to an almost 10 fold increase in the ITNs (Division of Malaria Control [Ministry of Public Health and Sanitation], 2011; Division of Malaria Control [Ministry of Public Health and Sanitation]Kenya National Bureau of Statistics, 2016). Additionally, the recommended first-line treatment regimen changed from sulphadoxine/pyrimethamine to artemisinin-based combination therapy which has been freely available in all public health facilities across the country since 2006. As a result of this scale up of interventions, there has been a notable reduction in the burden of disease as well as declining mosquito populations in some areas especially at the Coast (Mwangangi et al., 2013; O'Meara et al., 2008; Okiro et al., 2007). Unfortunately, this has not been the case for all regions that were previously endemic for malaria in Kenya. Current reports show persistently high prevalence of malaria in some of the areas despite the scale up of the control measures (Hamel et al., 2011; Zhou et al., 2011).

The Health and Demographic Surveillance site located within Bungoma East sub-county is an example of an area where incidence of malaria has not declined despite scale up of control efforts. In the past few years, there has been a notable increase in ITN coverage within the district from 25% to 67% (O'Meara et al., 2011). However, this has not translated to any substantial reduction in malaria infections in this region. Instead, the number of reported malaria infections continues to rise with more than 300 children getting admitted every month in the Webuye Sub-County Hospital which is the main referral facility in their catchment area. (HDSS, 2009).

Further, there is a clustering of self reported fevers in some of the villages forming fever hotspots which may be an indication of heterogeneity in malaria transmission (based on the assumption that fevers could be malaria) within a relatively geographically homogenous area hence the key question on what is likely to be causing the differences in malaria transmission (if actual) within the same area?

Although the concept of heterogeneity has been documented previously, most of the studies investigating heterogeneity in malaria risk have mostly been limited by their cross-sectional study design. Additionally most of these studies have been conducted in low transmission settings. Information on heterogeneity in high transmission settings remains scant.

This study therefore set out to investigate malaria transmission indices (parasite surveys and vector densities) and associated risk factors in fever hotspots and coldspots in a high transmission setting. Behavioural factors such as ITN use and malaria prevention behaviour between the fever cold spots and fever hot spots were also included.

The longitudinal design for follow up of community members for malaria instead of health facilities was chosen as it was more likely to give a true picture of the actual incidence of malaria given that many people who are infected by malaria do not present with symptoms and therefore don’t go to health facilities to seek for treatment.

1.4 Null Hypothesis

1. There are no differences in knowledge and behaviour related to malaria between the fever hotspots and fever coldspots.
2. There is no difference in the incidence of malaria between fever hotspots and fever coldspots in high transmission settings.
3. There is no difference in the prevalence of asymptomatic malaria cases and associated factors between fever hotspots and fever coldspots.
4. Risk factors for malaria are not different in the fever hotspots and fever coldspots
5. There is no difference in malaria vector characteristics between the fever hotspots and fever coldspots within the HDSS.

1.5.1 Research Question

1. Is there an actual difference (heterogeneity) in malaria transmission indices (prevalence, incidence and mosquito characteristics) in villages with higher than average fevers "fever hotspots" compared to those with lower than average fevers "fever cold spots?" within the HDSS in Bungoma East Sub-County?

1.5.2 Sub - Research Questions

1. Is there a difference in Knowledge and behavioural factors related to malaria among the fever hotspots and fever coldspots?
2. Is there a difference in the incidence of malaria infections between the fever hotspots and fever coldspots within the HDSS?
3. What is the prevalence of asymptomatic infections and associated risk factors in the fever hotspots and fever coldspots?
4. What are the risk factors for incidence of malaria in both the hot and cold fever spots within the HDSS?

5. Is there a difference in malaria vector characteristics between the hot and cold fever spots within the HDSS?

1.6.1 Broad Objective

1. Determine whether there is an actual difference (heterogeneity) in malaria transmission indices (prevalence,incidence and mosquito characteristics) in villages with higher than average fevers “fever hotspots” compared to villages with lower than average fevers “fever coldspots” within the HDSS in Bungoma East Sub-County.

1.6.2 Specific Objectives

1. Determine knowledge and behaviour relating to malaria in fever hotspots and fever coldspots.
2. Determine the burden of malaria infections (prevalence and incidence) in the fever hotspots and fever coldspots.
3. Determine the prevalence of asymptomatic malaria and associated factors among those who test positive for malaria in the fever hotspots and fever coldspots.
4. Determine risk factors for incidence of malaria in both the fever hotspots and fever coldspots.
5. Determine and compare changes in the incidence of malaria with mosquito characteristics in both the fever hotspots and fever coldspots.

1.7 Expected outcomes

The main expectation from this study was to determine whether there were actual differences in malaria transmission indices (prevalence and incidence of malaria as well as mosquito characteristics) between the fever hotspots and the fever coldspots and whether there is some form of stability in transmission between the various seasons throughout the year.

The second expectation was to identify factors related to individual behavior and mosquito characteristics that could be responsible for the differences observed in malaria incidence in the fever hotspots and fever coldspots within the HDSS.

2.0 CHAPTER 2: LITERATURE REVIEW

2.1 Background on malaria, Elimination and Eradication Efforts

Malaria has plagued the world and particularly the African continent for many decades. The disease is endemic in most countries in sub-Saharan Africa with at least 106 countries affected. Globally the number of deaths from malaria fell from an estimated 839 000 in 2000 (range: 653 000–1.1 million), to 438 000 in 2015 (range: 236 000–635 000), a decline of 48%. However, sub-saharan Africa bore more than 90% of these deaths in 2015 (World Health Organization, 2015). Although there has been a general decline in the number of infections and deaths related to malaria in some of the regions, some countries have made much slower gains in reduction of malaria. Currently, it is estimated that 15 countries account for 80% of malaria cases and 78% of deaths (World Health Organization, 2015). Further to this, more than half of this global burden of malaria is shouldered by about five countries mainly Nigeria, Democratic Republic of Congo, Ethiopia, United Republic of Tanzania and Kenya (Fact sheet on global burden of malaria - UN Millenium project 2005).

Recent studies on the burden of malaria in Africa, show declining trends in malaria incidence in most countries which is mainly attributed to the current scale up of interventions (O'Meara et al., 2010). However, current reports show some areas exhibiting persistently high prevalence of malaria as well as resurgence in other regions which had already started experiencing a decline in malaria (Division of Malaria Control [Ministry of Public Health and Sanitation]Kenya National Bureau of Statistics, 2016; Hamel et al., 2011; Zhou et al., 2011).

Elimination and eventually eradication of malaria is currently firmly back on the global agenda for malaria. Early global campaigns to eradicate malaria in the 1950s – 1970s succeeded in eliminating malaria in 37 out of 143 countries mainly in the western world but these efforts were later put on hold because of numerous setbacks (Centers for Disease control and prevention, 2011; World Health Organisation, 1969). This was followed by a period when malaria received very little attention in

terms of international funding yet the disease continued to increase morbidity and mortality around the world and especially in sub-Saharan Africa.

In 2007, the goal of malaria elimination and eventually eradication was brought back to the global table by Bill and Melinda Gates, and this was later endorsed by the WHO and the Roll Back Malaria Partnership (Greenwood, 2009; RBM, 2010; World Health Organisation, 2016). The international community thereafter stepped up efforts to prevent and control malaria around the world. These measures have yielded considerable success in many parts of the world. As a result, there is renewed hope that elimination of malaria is eventually an achievable goal (Fenton & Anthony, 2009; World Health Organisation, 2016).

Elimination is “the reduction to zero of the incidence of locally transmitted malaria infection in a defined geographical area as a result of deliberate efforts. Continued intervention measures are required to prevent re-establishment of transmission" (Alonso et al., 2011). Given the current successes, the debate on elimination is not far fetched. However, the goal on eradication will only be possible in the long term with improved strategies and newer tools to fight malaria. In order for elimination efforts to be successful, there is need for each country to put into place and have stable political, social, financial, operational and technical factors. Further to this, each country should be able to make an assessment of their own situation and come up with their own strategies for elimination (Feachem et al., 2010). Key to the discussions on elimination is the issue of addressing the malaria vector. In a commentary,Ferguson et al. (2010) argues that elimination of malaria has remained quite elusive due to a failure to grasp the biological characteristics responsible for the vector’s evasion of malaria interventions.

In Kenya, malaria remains an important public health problem with more than 70% of the population living in malaria risk areas (National Malaria Control Programme (NMCP) et al., 2016). Malaria is

still the leading cause of death among children below five years in Kenya (Division of Malaria Control, 2015). The road to elimination for Kenya will require concerted efforts from all stakeholders to address the current issues in regions where malaria remains persistent and those where there is resurgence. This may require that in some of the areas, specific targeted strategies be put into place.

2.2 Malaria resurgence/persistence.

Recent reports document a general decline in the incidence of malaria in many countries across Africa (Feachem et al., 2010; Nahum et al., 2010; Okiro et al., 2007). Unfortunately this success story cannot be celebrated in all regions that are affected by malaria. Further evidence from some studies shows that transmission still remains high especially in areas where it has always been high (Zhou et al., 2011). Current studies have shown that some of the areas that had already started recording a decline in the incidence of malaria are currently experiencing a resurgence hence reversing the gains that had already been made in controlling malaria (Division of Malaria Control [Ministry of Public Health and Sanitation]Kenya National Bureau of Statistics, 2016; Hamel et al., 2011; Trape et al., 2011; Zhou et al., 2011).

A review of the factors responsible for resurgence of malaria classifies them into three: weakening of malaria programmes, increase in malaria potential, and technical problems such as malaria vector resistance to the pyrethroids (Nanaan et al., 2006; Ranson et al., 2011; Trape et al., 2011).

Kenya is among the five countries in Africa that bear the greatest brunt of malaria. Although some of the reports from previously malaria endemic regions within the country document a decline in the incidence, other reports paint a grim and opposite picture. Quite disturbing is the fact that some of these regions with high malaria transmission have had a serious scale up of malaria interventions recently (Hamel et al., 2011; HDSS, 2009; Zhou et al., 2011)

Malaria resurgence and persistence in some of the regions poses a great threat to the current talks on elimination. On the other hand, it also serves to alert the stakeholders in the fight against malaria to change their strategy. Countries will need to put up active surveillance systems to enable them detect any changes in the incidence of malaria and therefore act promptly and appropriately.

The real cause of the present malaria persistence despite intervention measures in some regions while in others there is an evident decline creates a need for further investigations. There are many possible plausible reasons including the issue of heterogenous transmission of malaria that may not be currently addressed by the control measures.

2.3. Malaria control measures and their impact on Incidence of malaria

The current decline in the incidence of malaria is mainly attributed to the recent scale-up in malaria interventions. This forecloses a larger discussion on why malaria has resurged in recent years. Prior to the recent scale up, malaria interventions such as ITNs and Indoor Residual spraying (IRS) were in place as early as the 80's. However, ITN use was limited to protection of specific population groups mainly children below five years and pregnant mothers (RBM, 2010; World Health Organisation, 2016).

Dichlorodiphenyltrichloroethane (DDT) was the earliest insecticide used to eliminate malaria in some countries especially in Europe, North America, the Caribbean and parts of Asia and South-Central America in the early 1950s. The use of DDT drew a lot of controversies regarding its safety. It was later abandoned due to numerous challenges and controversies (Donald et al., 1997).

ITNs were introduced and have been in use as early as the early 80's, although, this was initially limited to protection of vulnerable populations mainly children below five years and pregnant mothers (RBM, 2010). Currently, there are newer and more improved interventions such as the: *Long-Lasting Insecticidal Nets (LLINs)* which are designed to repel, disable, or kill malaria-bearing mosquitoes and are effective without re-treatment for the entire life of the net (around 3-5 years),

Indoor Residual Spraying (IRS) which employs insecticides to prevent malaria by killing mosquitoes that might bear the disease parasites. Finally *Artemisinin-Combination Therapies (ACTs)* which are currently the most effective form of treatment against malaria. *Intermittent Preventive Treatment (IPT)* in pregnancy provides antenatal malaria treatment to pregnant women, who are particularly vulnerable to the disease (RBM, 2010; USAID et al., 2015).

Kenya has adopted all the above malaria intervention strategies progressively. In the last 10 years, there has been an increase in donor funding towards malaria control measures in Kenya. Ownership of insecticide-treated bednets (ITNs) has increased from 6% to 48% (Division of Malaria Control [Ministry of Public Health and Sanitation]Kenya National Bureau of Statistics, 2016). Artemether Lumefantrine/ACTs have been available at no cost to patients in public facilities since 2006 and available at highly subsidized prices in the retail sector since the introduction of Affordable Medicines Facility- malaria (AMFm) in 2010.

Large scale coverage and appropriate use of ITNs reduces the density, feeding frequency and survival of mosquitoes by killing the mosquitoes using insecticides which protects all those using the nets and those not using the nets (Gimnig et al., 2003; Lindsay et al., 1991). Current reports document a reduction in the burden of disease as well as declining mosquito populations which correspond temporally with reduced malaria morbidity and mortality (O'Meara et al., 2008; Okiro et al., 2009). Although many areas continue to record a decline in malaria, there are some regions in Kenya where malaria remains stubbornly high despite the scale up of interventions (Hamel et al., 2011; Zhou et al., 2011).

The fact that malaria infections continue to increase in some areas despite the scale up of malaria control interventions, casts a spell of doubt on the future of the positive reports.

2.4 Behavioral Characteristics Predisposing to Malaria

Human behavior influences utilization of malaria interventions such as ITNs despite their availability. The scaling up and rolling out of ITNs to all community members was based on the assumption that they would sleep under mosquito nets and therefore protect themselves from the mosquito bites (Division of Malaria Control [Ministry of Public Health and Sanitation], 2011). It is however evident from current reports that this may not be the case (Obala et al., 2015).

Human behavior is complex and difficult to understand. Ownership of ITN does not necessarily translate to utilization. There is a complex interplay of factors that may affect this utilization and therefore such factors need to be addressed before uptake of an intervention (Obol et al., 2013). Many studies have reported a disconnect between the availability of the interventions such as ITNs and their subsequent use (Obol et al., 2013; Souleymane et al., 2014; Sunday et al., 2014). In most of these studies, there has been an upscaling of interventions yet the utilization and impact does not match the effort.

Engagement in risky behavior for example staying up late into the night may predispose individuals to more bites of the mosquito compared to those who go to bed early and sleep under the net (Geissbuhler et al., 2007). Most of the mosquitoes begin to bite at dusk, so when individuals stay out long, they are likely to be bitten before they go to bed to sleep under the mosquito nets. These bites can still cause malaria infection if the mosquito was infected.

Behavioral factors therefore may provide an alternative explanation to the increasing incidence of malaria despite a high coverage with malaria control measures. This may provide a plausible explanation as to why there could be higher incidence of malaria in the fever hotspots compared to the fever coldspots. It is postulated that availability of interventions may not always translate to their use because human beings are complex in the way they perceive issues even those that concern them.

Some of the reports document a failure in the uptake of interventions which they attribute to behavioral factors (Ingrid et al., 2009; Jane et al., 2011; Sunday et al., 2014; Yvonne et al., 2007).

Knowledge and attitudes towards interventions play a great role as to whether those interventions are adopted or not (Hlongwana et al., 2009). Uptake of treatment for malaria may be hampered by the wrong attitudes and therefore contribute to local variations in the disease (Greenwood, 1989). Communities with appropriate health seeking behavior may therefore have an increased chance of lowering the incidence of malaria since most of those who get sick get treated thereby reducing the parasite reservoir.

In this study, behavioral factors were also included in the electronic questionnaire to provide an alternative explanation to the current malaria situation in that area.

2.5 Heterogeneity in Malaria Transmission: Formation of Hotspots of Intense Transmission

Recent studies have shown that as we move towards elimination, malaria transmission is becoming more heterogeneous and the risk of malaria transmission varies even across neighboring villages (Bousema et al., 2010; Ye et al., 2007). Hotspots arise when there are geographical clusters of higher than average parasites as well as malaria incidence in an area. Malaria transmission in hotspots is characterized by intense exposure to mosquitoes, and high levels of (asymptomatic) parasite carriers (Bousema et al., 2012). The hotspots maintain infections during the low transmission seasons and fuel the transmission during the high transmission seasons.

A summary of the main factors responsible for development of hot spots narrows down to two main factors: Genetic and environmental factors (Bousema et al., 2012; Clarke et al., 2002; Greenwood, 1989). Households within a village are mainly composed of people who are related by blood and in some cases family members inherit genetic abnormalities of the red blood cells or even immune response genes hence contribute to differences in the prevalence of malaria within a village or

between villages (Greenwood, 1989). Environmental factors such as the number of mosquito breeding sites within a village, house designs and the level at which members in a village use ITNs for protection plays a key role in explaining these differences (Clark et al., 2008; Staedke et al., 2003; Trape et al., 1992).

Although there is a lot of emphasis on the need to detect malaria hotspots in order to target malaria control measures, challenges still abound in defining hotspots as well as their stability. Infected individuals may move from one area to another propagating infection in the new area and therefore the clusters of higher than average incidence of malaria may vary from time to time (Bejon et al., 2010). In addition, studies have shown that hotspots from asymptomatic parasitemia are more stable compared to hotspots for febrile illnesses (Bejon et al., 2010).

In most studies, malaria hotspots and cold spots are mainly defined using parasitemia with or without ecological data, however in resource limited settings such as western Kenya, appropriate diagnostic tools for malaria may not be available.

2.6 Asymptomatic Parasitemia and malaria transmission

Asymptomatic parasitemia refers to the detection of asexual or sexual parasites and an absence of any acute clinical symptoms of malaria (usually fever) during a specified time frame. Asymptomatic carriers of the plasmodium parasite act as reservoirs for the malaria infection and fuel the infection during the high transmission season (Bejon et al., 2010; Lindblade et al., 2013).

Studies have shown that asymptomatic patients are able to infect mosquito vectors and also remain infectious longer than the treated symptomatic patients (Alves et al., 2005). Asymptomatic parasitemia mainly develops because individuals in highly endemic regions get exposed to malaria parasites frequently and as a result develop partial immunity which protects them from developing acute clinical symptoms of malaria (Lindblade et al., 2013). According to Greenwood (1989), attitudes to the treatment of a case of malaria may also contribute to local variations in asymptomatic

cases of malaria. Communities where individuals seek effective treatment promptly when sick are likely to have fewer cases of asymptomatic cases of malaria compared to a neighboring community where there is a much greater reliance on traditional medicines.

High prevalence of asymptomatic malaria has been shown to occur mainly in endemic regions (Bottius et al., 1996). This poses a great challenge to elimination efforts because individuals who don't have symptoms are not likely to seek treatment and yet they remain infectious (Alves et al., 2005). Most malaria programs have passive malaria surveillance where they mainly detect malaria cases at health facilities and they are put on treatment. The challenge arises with those who do not develop any symptoms and therefore do not present to health facilities for treatment or seek any other form of treatment. These remain as reservoirs of the parasite for along time and keep re-infecting the mosquitoes which infect the humans since they cannot be detected unless there is active surveillance of cases (Bousema et al., 2014). This cycle continues and therefore could actually contribute to the persistence of malaria in an area. In addition, individuals with asymptomatic infections may suffer other adverse effects on their health such as chronic anemia, increased tendency to get bacterial infections, slowing mental development among others (Chen et al., 2016).

2.7 Vector characteristics and formation of Malaria Hotspots

Malaria is a vector-borne disease and therefore vector characteristics play a key role in the epidemiology of the disease. The female anopheles mosquito is responsible for the transmission of malaria. There are four main types of malaria parasites namely: *Plasmodium vivax, Plasmodium malarie, Plasmodium ovale and Plasmodium falciparum* with the latter as the most common parasite responsible for malaria infections in sub-Saharan Africa (Guerra et al., 2008).

Vector characteristics and behavior have been documented to determine the degree and level of malaria transmission in an area (Elliott, 1972; Soleimani-Ahmadi et al., 2013; Trape et al., 1992). Vectorial capacity which is the daily rate at which future inoculations arise from a currently infective

case is a key characteristic important for malaria transmission. Studies have shown that Vectorial Capacity directly influences the incidence of malaria (Smith et al., 2005). According to Zhou et al. (2011), changes in vector characteristics and behavior are responsible for the current persistence of malaria in some regions.

It is widely believed that abundance of anopheles mosquitoes translates to a high transmission of malaria within an area (Molineaux, 1998; Rowton, 2009; Smith et al., 2012; Yazoumé et al., 2008). On the contrary, some researchers have found areas with high anopheles numbers that do not experience high malaria infections (Fantini, 1994). Further to this, although many reports attribute the low incidence of malaria in previously endemic regions to declining mosquito densities due to malaria control strategies (Mwangangi et al., 2013; O'Meara et al., 2008; Okiro et al., 2007), some studies report contrary findings. Meyrowitsch et al. (2011) studied mosquito densities in an area without organised malaria control strategies over the same period of time and also found declining mosquito densities over time. He therefore disagrees with the blanket argument that vector decline is mainly attributed to malaria interventions. Other factors such as climatic changes could be responsible for the current decline in mosquito densities besides the malaria control measures.

Most species of anopheles mosquitoes bite indoors during the night and rest indoors until they are fully gravid before exiting the house to find breeding sites. Some species bite indoors (endophagic) and rest outdoors (exophilic) and so exit the house after feeding to find resting places outside. Malaria control strategies mainly target this principle (Guerra et al., 2008). However, currently some of these mosquitoes have modified their biting behavior to evade the indoor anti- vector measures and therefore biting both indoors and outdoors and others are even able to bite during day time (Reddy et al., 2011; Russell et al., 2011). This poses a challenge to the current vector control strategies which target indoor vectors.

Given this kind of argument, this study sought to answer possible causes of heterogeneity in malaria transmission within an area where there has been an upscale of malaria control measures but malaria remains persistently high. This study focused on mosquito ecology and behaviour such as mosquito densities, composition, sex, sporozoite rates and how they corelate to the incidence of malaria over the same period of time. This was done for both the fever hot spots and coldspots.

The battle against malaria vectors especially in endemic regions needs to be re-strategized. This is because malaria vectors in areas where there has been a scale up of intervention measures have also adopted different survival mechanisms such as shifting their feeding habits from feeding on human blood to bovine blood (Mwangangi et al., 2013; Russell et al., 2011). In some of the cases, frequent use of the pyrethroids sprays on the farm has led to development of resistance by the mosquitoes (Jones et al., 2012; Nanaan et al., 2006). Given the emerging picture, there may be need to change tactic especially in the way of dealing with malaria vectors.

2.8 Conceptual Framework

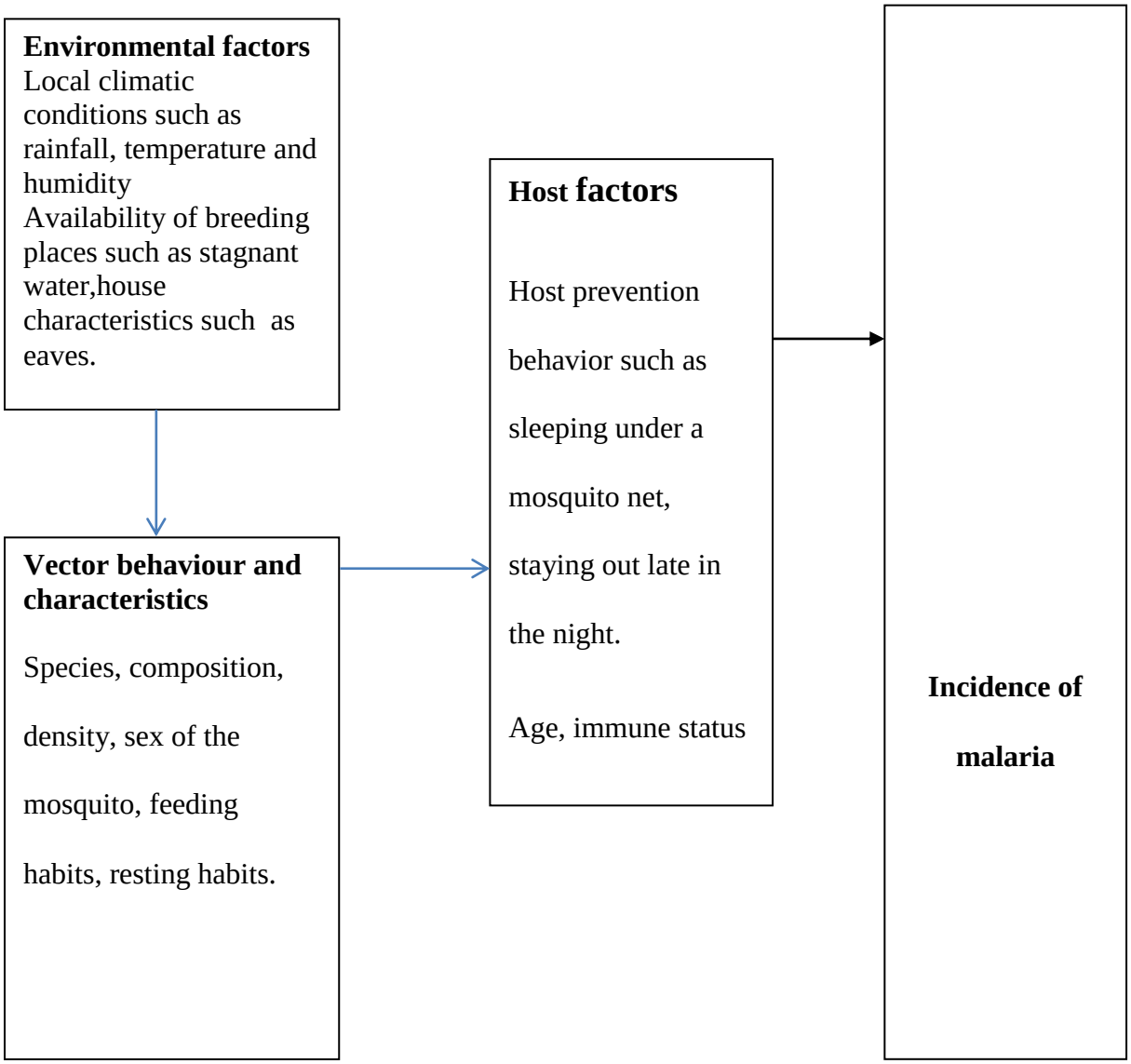

Figure 1: A summary of the Conceptual framework for possible factors responsible for malaria transmission adapted and modified (Yazoumé et al., 2008).

Vector: This is an organism that carries parasites to other organisms. In the case of malaria this is the anopheles mosquito.

Vector characteristics: Comprises the species, composition, density, sex of the mosquito - females of anopheles mosquito require a blood meal after mating to mature the egg, whether they are anthropophillic or zoophillic i.e. bite man or animals. Malaria vector characteristics determine the intensity of malaria transmission in an area (Ferguson et al., 2010).

Environment: The climatic conditions have to favor the maturity of the mosquito from larvae to the adult mosquito. Local climatic conditions affect the density of mosquitoes and the seasonality of transmission (Molineaux, 1998) .

Human host: Presence of a susceptible person who is bitten by the mosquito and hence finally develops the disease (Molineaux, 1998). The sick person becomes the source of the parasites to the mosquitoes hence maintaining the cycle from man to vector and vector to man (Yazoumé et al., 2008).

Malaria infection is mainly a combination of the host, vector and the environment epidemiological model (Yazoumé et al., 2008). The host and the vector can be compartmentalized into susceptible, infected and infectious groups mainly linked by a mosquito blood meal. The vector needs a blood meal immediately after mating to mature the eggs. It's during the blood meal that the mosquito injects the parasite into the human blood hence making the susceptible individual infected. The likelihood of the individual moving from susceptible to infected depends on prevention measures taken against mosquito bites such as sleeping under an ITN. An infected person develops the parasite and moves to the stage of infectious depending on their immune status and whether they had taken any prophylaxis. If a person is treated, they recover and become susceptible again for the next round of infection after the washout period for the treatment.

The adult mosquito lays eggs in breeding sites which hatch into larvae. A conducive environment such as a hot climate is required for the mosquito to develop into an adult. These concepts lay the foundation for the development of strategies to prevent and control malaria.

3.0 CHAPTER THREE: METHODS

3.1 Study site

This study was carried out in Webuye HDSS which is located in Bungoma East Sub-County (It is basically the entire sub-county with the exception of a very small urban strip) within Bungoma County (Figure 2).

Bungoma East Sub-County is located approximately 50 km east of the border with Uganda. It is bisected by the Nairobi-Uganda highway and has a small peri-urban center along the road. The Sub-County is bounded to the west and southeast by rivers. Commercial farming of sugarcane is the predominant economic activity and involves use of pyrethroid class of insecticides. Most families (>60%) live below the poverty line and very few households have electricity or access to municipal water. Webuye town lies at an elevation of about 1,500 m above sea level. Transmission of malaria is perennial with a seasonal peak following the main rains in May-June. Prior to scale-up of control efforts, entomological inoculation rate (EIR) was estimated to be 29 infectious bites per person per year (Shililu et al., 1998).

The Webuye Health and Demographic Surveillance Site (HDSS) was set up in 2007 and provides longitudinal follow-up of all families within four administrative locations of Bungoma East Sub-County (HDSS population 73,000 people) (Chrispinus et al., 2013). Webuye Sub-County hospital is the main referral hospital for families in the HDSS.

Malaria prevalence in this area remains persistent and very high with some of the studies putting it as high as 95% during the peak seasons and 71% during the dry/non-peak seasons (Hamel et al., 2001). The current MOH Bungoma County health policy project puts the prevalence a little lower at 60% (Ministry of Health Kenya & Health Policy Project, 2015). However, this is still much higher than other endemic regions such as Siaya County that now records a slightly lower prevalence of malaria at below 49% (Health Policy Project, 2015).

This is also evidenced by the local reports from the main referral hospital as well as the peripheral health facilities. Malaria transmission is perennial with the main peaks during the rainy season (May-June). Local reports indicate that of the 400 children admitted to the ward each month, between half and two-thirds of these are for malaria, depending on the season (HDSS, 2009). There are 12 other peripheral health facilities in the Bungoma East Sub-County – nine dispensaries, two health centres, and one sub-district hospital. Five of the 12 are located within the boundaries of the HDSS.

The Health and Demographic Surveillance Site (HDSS) therefore serves as a mapped out research area within the Bungoma East Sub-County (Chrispinus et al., 2013) whose population is followed up at regular intervals for both demographic and health related data. Data from the regular longitudinal surveys showed some spatial clustering of self reported fevers within the HDSS with areas of higher than average fevers (fever hotspots) and others with lower than average fevers (fever coldspots) indicating some spatial heterogeneity of these fevers. It is on this basis that the study was designed to identify the actual malaria transmission hotspots as well as their risk factors based on the malaria transmission indices.

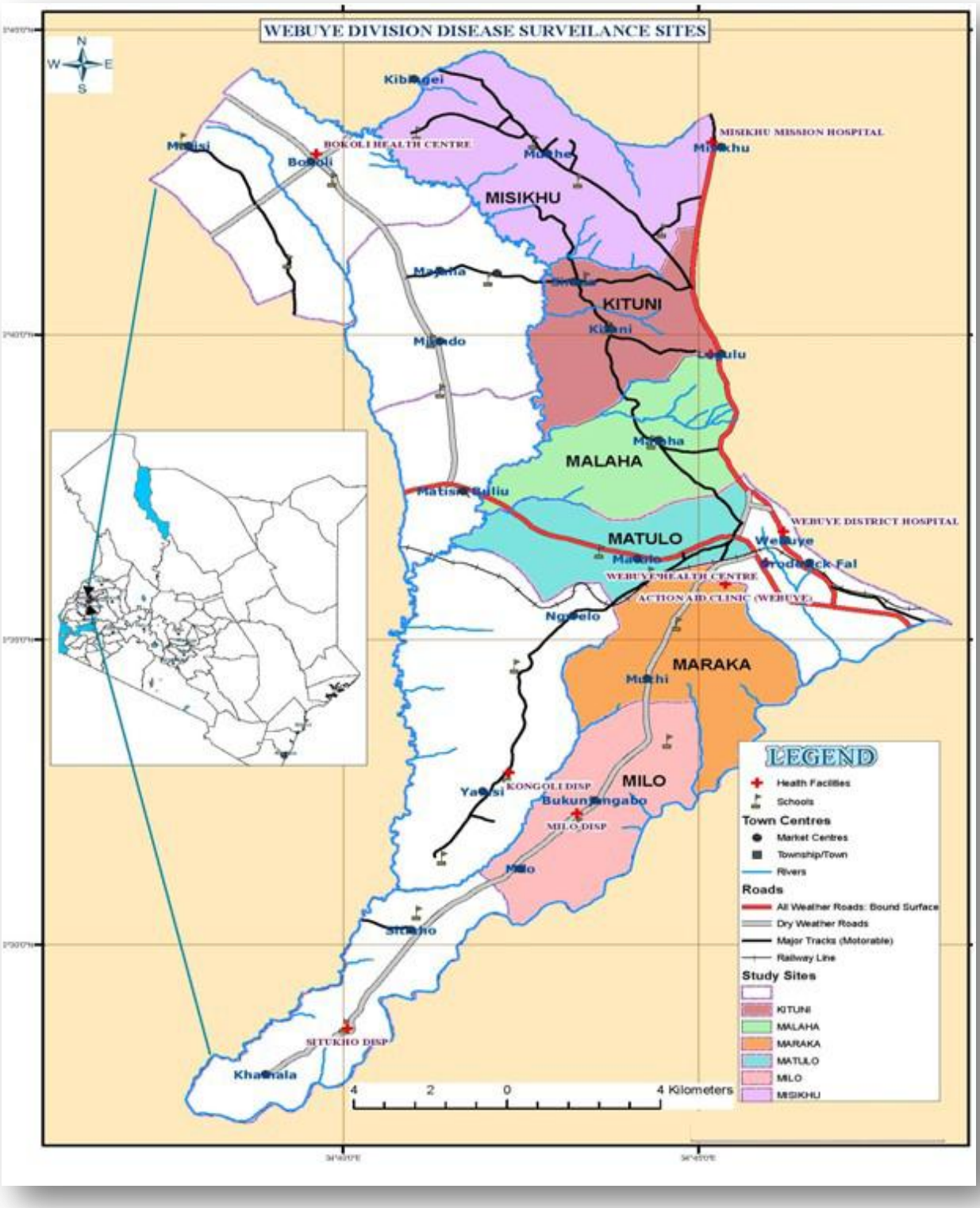

Figure 2: The Webuye Division Disease Surveillance Site Map (Chrispinus et al., 2013)

3.2. Study Design

This was a prospective closed cohort study. The choice of this research design was guided by both published and unpublished sources. This study sought to determine changes in malaria transmission indices (incidence) over time hence a prospective cohort design was the most appropriate. In addition, the choice of this design is supported by previous studies which have investigated incidence of malaria and or changes in mosquito characteristics that adopted a cohort/longitudinal design (Mwangangi et al., 2013; Nahum et al., 2010; Russell et al., 2013). The choice of Bungoma East Sub-County was informed by the fact that the Sub-County continues to experience persistently high malaria infection rates (prevalence of 60%) (Ministry of Health Kenya & Health Policy Project, 2015) despite a high coverage with ITNs and other malaria control interventions. The HDSS has been described in detail in 3.1 above.

Villages with higher than average fevers (fever hotspots) and those with lower than average fevers (fever coldspots) were identified first and thereafter subjects from the selected households were followed up for a period of one year.

The parasitological surveillance was nested in the sentinel entomology surveillance in both the fever hotspots and fever coldspots. Subjects were tested for malaria parasites using RDT (quarterly) for a period of one year in order to determine the incidence of malaria in this cohort.

3.3 Study population

The study participants were all household members irrespective of age in the randomly selected households where sentinel entomology surveillance was conducted as well as their immediate neighbors within both the fever coldspots and fever hotspots (as determined earlier) in the HDSS.

3.4 Sample Size Calculation

Sample size was calculated using SAS macro based on the poisson regression model used for calculations of sample size for recurrent events (Kuolung Hu, 2008).

The primary outcome in this study is the incidence of malaria in a subject followed up for a period of one year.

The following information is required for the calculation:

Program: Sample Size evaluation for Poisson Regression Model

R0 = baseline rate, Exp (Bo);

RR = Rate Ratio, denoted as exp (B1)/exp (BO)

tt = exposure length

Alpha = alpha level

Side =1 or 2

PO = Subject allocation factor for the compared (B1), the range of values is (0, 1)

Given the following information:

Baseline incidence of malaria: 1.2 episodes per person per year (O'Meara et al., 2014).

Risk ratio (relative risk for those in the fever hotspots developing malaria compared to those in the low fever spots): 1.5 (O'Meara et al., 2014)

Exposure length: 1 year

Side: 2 sided

Power (80%)

Subject allocation factor: 0.67 (2 subjects in the fever hotspots and 1 subject in the fever coldspots because the number of fever hotspots was twice the number of fever coldspots).

Total sample size = 174 participants

Adjusting the sample size for loss to follow up or dropout rate of 20% (1.2): (174)1.2 = 209

Adjustment for correlation within the households (design effect) of 1.7: (209)1.7 = 356

There are approximately 73,000 people in the HDSS with about 13,000 households (Chrispinus et al., 2013). To get the average number of people per household, there is need to divide the total number of people by the number of households (see below).

Number of persons per household = 73,000/13,000

= 5.6

Approximately 5 people per household.

Each household in the HDSS has approximately 5.6 people (total population in the HDSS divided by the number of households). Since we cannot have five and half people, the lower number which was 5 people per household was chosen as opposed to 6 people because this would increase the sample size in terms of number of households. Ultimately, the larger the sample size, the better the power of the study.

To get the actual number of households that would participate in the study, the total sample size (356) was divided by the approximate number of persons per household

Total number of households for interview = 356/5

= 72 households

The ratio of enrollment of households was two households in the fever hotspots to one household in the fever coldspots within the HDSS. This is because the number of hotspot villages was twice that of the coldpot villages.

The total number of households for interview in the fever hotspots was 48 households and 24 households in the fever coldspots.

In the actual execution of the study, the total sample size was 400 participants because these were all the people found in the 72 households.

3.5 Inclusion / Eligibility criteria

Fever Hot spots:

Permanent residence within the Webuye HDSS or at least having lived in the HDSS for the last one year and intend to live there for the next one year.

All household members residing in the selected households irrespective of their age

Head of household consents for the children and household to be included in study

Fever cold spots:

Permanent residence within the Webuye HDSS or at least having lived in the HDSS for the last one year and intends to live there for the next one year.

All household members residing in the selected households irrespective of their age

Head of household consents for the children and household to be included in study

3.6. Sampling procedure

Areas of higher than average incidence of fevers and those with lower than average incidence of fevers were selected based on previous information from longitudinal data on clustering of self-reported fevers in the HDSS. Villages that had "higher than average fevers" were referred to as "fever hot spots" and those that had "lower than average fevers" were referred to as "fever cold spots." These formed the larger sub-units/clusters from which the actual sampling was done. A summary of the spatial distribution of hot and cold fever spots is shown in the map in figure 3.

Multistage sampling was done. At the first level, six villages were selected purposvely based on the distribution of clusters of self reported fevers in the HDSS. Four villages in the fever hot spots namely; Kinesamo, Wakulinda, Nangili and Sitabicha and two in the fever cold spots namely; Lukhuna, and Maruti were selected for inclusion into this study (figure 3). Each village has approximately 80 households listed in the household register in the HDSS. This register served as the main sampling frame for the random selection of households that participated in the study. One household was randomly selected from each of the villages for fixing of a window exit trap (WET)

while two other households within the same village were also selected for Pyrethrum Spray Catch (PSC).

The parasitological surveillance was nested in households where mosquito sentinel surveillance was set up as well as their immediate neighbors. Three immediate neighbours within the closest GPS points to the entomology households were included for the parasitological surveillance. This is based on the fact that the mosquito flies through an average distance of of 0.5 to 1km (Charlwood et al., 1998; Takken et al., 1998; Trape et al., 1992) and hence hotspots are likely to form within this radius. A total number of seventy two households were selected in all the six villages for parasitological surveillance of household members for a period of one year at intervals of every three months. Forty eight households were selected in the fever hotspots and twenty four households for the fever cold spots (allocation ratio of 2:1).

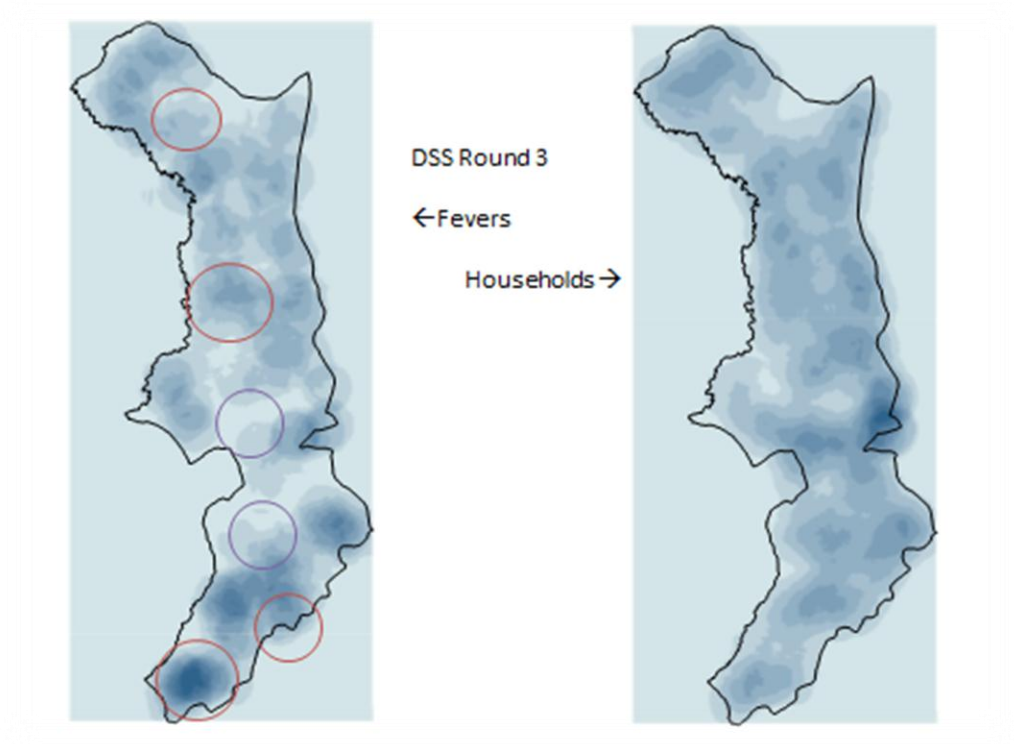

Figure 3 a) Round 3 Spatial Distribution of Fever Hotspots and Coldspots (fever hotspots in red circles and fever coldspots in purple circles) b) Map showing household densities for comparison

3.6.1 Entomology surveillance procedure.

Sentinel surveillance for mosquitoes was set up in six villages as stated in section 3.6 (four in the fever hotspots and two in the fever cold spots). One household in each of the six villages was randomly selected for fixing of a window exit trap. Two other households within the same village were also selected for monitoring of mosquitoes using Pyrethrum Spray Catches (PSC). In total, 18 households (12 in the fever hotspots and 6 in the fever cold spots) were put under monthly mosquito surveillance for a period of one year.

Informed consent was sought from the head of the household before fixing window exit traps (WET) which remained fixed in the house for a period of one year. Mosquitoes were collected from the window exit traps every day at 7 am for one week, during the first week of each month. During the collection week, traps were emptied in the morning. Live mosquitoes were collected from the WET by aspiration into paper caps and transported back to Webuye offices where they were frozen to death before being sorted by type (either anopheles or culex) and then counted.

Pyrethrum spray catches were done once every month for a period of one year. For PSC, the mosquitoes collected were mostly dead and therefore were transported directly to Webuye for sorting and counting. Abdominal status was checked and recorded, and then the mosquitoes were packaged in eppendorf tubes and labelled. The tubes were put in ziplocks before transportation to KEMRI Kisumu laboratories for grading, species identification and sporozoite rates (detailed procedure under data collection 3.6.3).

3.6.2 Parasitological Surveillance procedure

Quarterly malaria parasite surveys were nested in the mosquito surveillance study. Since it was a closed cohort study, we followed up the same subjects for a period of one year. The sampling procedure is already described in section 3.6. A total of 400 participants in 72 households from both the fever hotspots and fever coldspots were selected for participation in the study.

In each of the selected households, the head of the household was approached by the research assistant and requested for written informed consent to access the household. Thereafter, each of the household members was consented (those above 18 years) and assent taken from those above eight years to allow for a rapid diagnosis of malaria which involves a finger prick. Histidine-rich protien2 (HRP2)-based RDTs were used since they are able to detect both recent and current infection (Katharine et al., 2011). The use of RDT for testing of all suspected malaria cases before treatment is currently a recommended practice by the WHO (Services, 2010; World Health Organization, 2010). Members of the family who were older than 18 years were also required to answer some questions on knowledge and behaviour related to malaria.

Those who were found positive on RDT-test (irrespective of whether they symptomatic or asymptomatic) were given Artemether lumefantrine (AL) based on the Ministry of Health guidelines and asked to get to the nearest health facility for further management incase symptoms persisted (detailed procedure is explained under data collection in section 3.6.3c). Participants were informed that research assistants would visit their households every three months to test for malaria.

3.6.3 Data Collection procedure

a) Training of Research Assistants

Six research assistants with prior experience having worked in previous malaria studies were recruited to assist with the data collection process. They were re-trained on data collection of mosquitoes and larvae in the households as well as collection of parasitological data. The training was done for two days since they were already familiar with the malaria study. They were taken through the various parts of the questionnaire and how to complete it using the Open Data Kit (ODK) platform on the Android phones. A Standard Operating Procedure (SOP) was developed and was used to guide the performance of the procedure. They also had a simulation exercise on testing for malaria using RDT.

After the training, a pilot study was conducted to test the data collection tools. The responses were analysed and the tool was further refined to increase clarity before the actual data collection commenced. During each quarterly round of data collection, the enumerators were always taken through the electronic questionnaire and informed of any changes before going to the field.

b) Community Entry Process

Although the study had been nested in the main MESA study, it was still necessary to inform the community through its leadership that an additional study would be done in their area. Chiefs and village elders from the six villages were invited to a meeting at the Webuye HDSS office. They were taken through the details of the research that was to begin including the possible benefits to the community. The village elders were then requested to inform all the community members that the research team would be visiting them every three months and that there was need for their full co-operation and participation.

This step was necessary for the success of the study. The village elders informed the community members in their villages about the study. This worked to minimise refusals since the community were aware about the study and therefore cooperated very well. The study team was welcomed into nearly all the households visited for the entire one year period.

c. Field Work Procedures

i) Entomology Data Collection Process

Data collection started in July, 2014 and was concluded in July, 2015. Trained research assistants visited the selected households once every month and requested the head of the household for permission to spray the household or if it was the window exit trap, to empty it and remove the mosquitoes. Once the head of the household had given their consent, the research assistant would go ahead with the procedure.

The window exit trap figure (4a) was fixed once and remained at the window for the whole period of one year. Mosquitoes were collected from the window exit traps every day for one week, during the first week of each month. Collection of the mosquitoes was done early morning because that's when most mosquitoes exit the house after having had a blood meal in the night. Live mosquitoes were collected by aspiration into paper caps and transported to Webuye office. They were then frozen to kill them before sorting was done.

For the pyrethrum spray catches (PSC), the research assistant would visit the households before 7am in the morning. The research assistant would make a phone call the previous day before coming the following morning. This was to alert the household members and also ensure there was someone in the household to receive them. The research assistant would seek permission from the head of the household to allow for the procedure. He/she would request the household to keep away all food and cover it well to prevent the spray from getting to the food. After this, he would close all windows and doors, followed by knocking all surfaces to distabilise the mosquitoes. He would then cover the floor of the house with white sheets and after removing all food items, spray inside the house and all round outside through eaves (Figure 4b). The chemical used for spraying was a mixture of pyrethrum and butoxide. After spraying, mosquitoes were knocked down by the spray and were collected from the sheets into petri dishes lined with whatmann No 5 filter paper (Figure 4c and 4d). No live mosquitoes were collected by PSC, because of the chemical, the few that were alive died almost immediately after collection. The mosquitoes were then sorted, counted and their abdominal status recorded. The culex mosquitoes and male anopheles were disposed off. After sorting, each female anopheline mosquito was put in an Eppendorf tube and was well labelled as per the household Id and the unique individual mosquito Id. The tubes were then put in ziplock bags which had silica gel to reduce moisture. Finally the well packaged mosquitoes were transported to KEMRI Kisumu laboratories for grading, species identification, and sporozoite rate. Mosquito species were

identified morphologically using a microscope and a morphological identification key while the sibling species were identified using PCR. ELISA was used measure the sporozoite rate.

Entomology data was entered into the open data platform kit (ODK) using android phones (Appendix 4a and b). Entomology procedures are demonstrated in the figures below (Figure 4a, 4b, 5a and 5b).

Figure 4a): Window Exit Trap

Figure 4b): Pyrethrum Spray Catch (PSC)

Figure 5a): RA picking Mosquitoes after PSC

Figure 5b): Mosquitoes on petri-dish

ii) Parasitology Surveys

The research assistants visited the selected households for parasitological surveillance every three months (quarterly). These visits coincided with the main seasons of that area. The head of the household was requested for written consent to gain entry into his/her household (Apendix 1a & b, 2a & b). All members of the household were also requested to give a written informed consent before a malaria test was carried out at each of the visits (Appendix 3a & b). In addition, each of the household members above 18 years was required to complete some of the questions on the questionnaire.

On the day of the survey, the research assistants called some of the households (those that had telephone numbers) and informed them that they would be visiting the next day. This would ensure that as many people as possible would be found at home. The research assistant (RA) would then request for consent and then test each individual in the household. Each research assistant carried an SOP (Appendix 6) that would guide them through the entire procedure.

After testing, members of the household who were found positive by RDT for malaria were treated using Artemether Lumefantrine using the MOH guidelines. Pregnant mothers, babies below six months and those found to be very sick (those vomiting and with very high fever) were referred to the nearest health facilities for appropriate treatment. Those on treatment were asked to observe for signs of worsening of their condition and report this to the nearest facility. Each of those found with malaria was also given a small note detailing the drug given and the dosage and the test results. Members of the household were also given a hotline whereby they could call at any time in-case one of them developed fever within the two months preceding the next round of testing. The research asistants returned to the same households every three months to test the same individuals for malaria. Each individual in the household was given a unique identifier as well as the household.

In some of the cases where some of the household members were missing, the researchers would return to the household until they found him/her and tested them. This was done for all the five rounds.

A structured questionnaire (Appendix 5a and 5b) was programmed into the Android phones using the open data platform kit (ODK). The questionnaire was structured to collect data in four major areas: Household details/demographic data for individual household members, Information on ITN use, RDT information for each household member and finally malaria related knowledge and behavior questions. Global Positioning System (GPS) coordinates were taken for each of the households that were visited using the Android phone GPS system (Figure 6d). The research assistants entered data directly into their android phones (figure 6c). The data was extracted by the data manager after every round of data collection. Some of the data related to malaria risk behavior such as time to sleep, eating location, resting location and the general knowledge questions on malaria were only collected during the first round only. This is because previous reports have shown that behavior is a complex phenomenon and it does not necessarily change within very short intervals of a few months especially if there are no major behavior change communication campaigns (Central Office of Information, 2009; Michie et al., 2008).

Information on ITN use and RDT testing was collected every three months. For the ITN information, household members were asked about possession, number of ITNs versus sleeping spaces, the use of ITNs, maintenance and also the research assitants checked the integrity of the various ITNs (figure 6b) i.e number of holes, size of the holes and generally how they put it up when going to sleep. Travel history was also taken during every round of data collection. During each visit, the researchers asked whether any of the household members had been sick in the last three months. Those who had been sick and confirmed by the doctor (a hospital card was requested to confirm the diagnosis), this was taken as one episode of malaria for that individual in that quarter. If

the same individual was still positive at the time of the visit, the episode would be censored since it had already been included in the previous episode.

The investigator of the study acted as the main quality assurance officer for this study. She was always in the field alternating everyday with the research teams that either went to the hotspots or to the coldspots. All the individual electronic entries were checked rigorously after each round of data collection to ensure completeness. Any missing information was clarified before the next round. If the forms were poorly entered or had some mis-information, the research team called the particular household since we had all their phone numbers or their nearest neighbor. Some of the procedures done during the fieldwork are represented in figures 6a, 6b, 6c and 6d below.

Figure 6a): RA conducting RDT

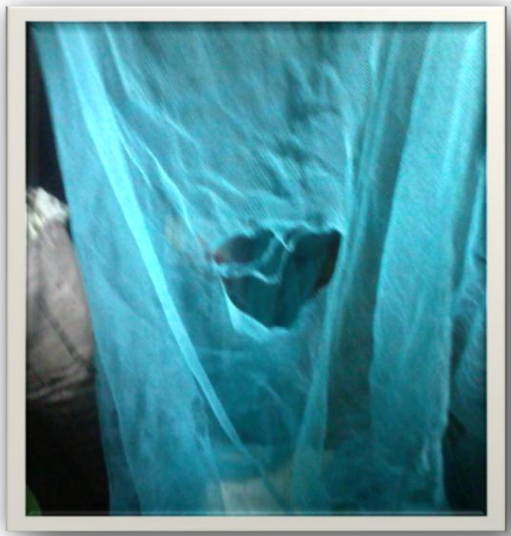

Figure 6b): Torn ITN

Figure 6c): RA entering data in Android phone

Figure 6d): RA taking GPS

3.7 Data Management and Quality Assurance

3.7.1 Data management

Two separate databases were created. One database was for the entomology data while the other was for the parasitology data. Entomology data was extracted from the android phones every month while the parasitology data was extracted after every three months. It was then cleaned and all the missing information was clarified before the following round of data collection. Data cleaning was done by checking for duplications, completeness, correctness of the information entered into the electronic questionnaire. For incomplete entries, the research assistants were asked to go back to the field and fill the missing information. The dublicated entries were dropped and for the incorrect information, research assistants were asked to get back to the field and get the correct information. If the person could not be completely traced, the entry would be dropped.

In the parasitology surveys, five rounds of data were collected. After the final round of data collection, all the five rounds of data were merged. Thereafter, cleaning of the whole merged data set (as described above) was done by the investigator and the data manager before embarking on data

analysis. The datasets were password protected and only the investigator and data manager would access the information when needed.

3.8. Statistical analysis plan

Analysis of the data was based on a clearly laid down analysis plan to address the objectives of the study.

Descriptive analysis for the demographic/household variables was done and presentation was mainly done by tables, graphs and charts. Descriptive statistics were also used to describe the vector densities. Chi square test was used to check if there was any relationship between the knowledge variables and the region as well as the behavior variables and the region. Fishers' exact test was used where more than 20% of the cells had an expected frequency/value of less than 5. This is because the Fishers' exact gives a better approximation than the chi-square when the sample size is smaller. Incidence of malaria at the household level, village level and the two regions was also computed using survival analysis. The cumulative prevalence at village, regional level as well as based on the five visits was also calaculated. Cumulative prevalence of asymptomatic infections was computed at household level, village level and the two regions (fever hotspots and fever coldspots). For both, a t-test was used to compare the incidence of asymptomatic infections between the two regions (hotspot and coldspt) while ANOVA was used to compare the incidence of malaria in the various villages. A t- test was used to compare vector densities in the cold and hot fever spots. Linear regression was used to compare the incidence of malaria and the densities of mosquitoes. Generalised Estimating Equations (GEE) was used to model factors associated with asymptomatic status.

Multi-level mixed effects modelling was used to model risk factors for malaria in the fever hotspots and fever coldspots. Linear regression was used to show the correlation between the vector densities and the incidence of malaria.

3.9. Ethical Considerations

Ethical approval was given at the Institutional Research and Ethics Committee (IREC) at Eldoret via an amendment made to the original MESA protocol (IREC approval No. 000778 - Appendix 9a). Ethical approval was also sought and given by the UON/KNH IRB since this was a requirement by the University (Ref No: KNH-ERC/A/298 – Appendix 9b).

Permission was further sought from the head of the HDSS, Moi University to allow for the study to be conducted within the HDSS. Additionally, we sought written informed consent from the head of each household before interviewing them during every round of data collection (Appendix 1a & b and Appendix 2a & b). Further, each of the household members above the age of 18 years was asked to sign a separate consent form (RDT consent form - appendix3a&b) to allow for a malaria test. Minors above the age of eight years (8) signed an assent form and their parents consented for them after their assent. Individuals were allowed to withdraw any time from the study without any form of victimization.

All participants were provided with ethical review hotline numbers where individuals would call and complain if they were unhappy about the process or needed to report any form of misconduct, adverse effects or just seeking assistance.

Finally, all the participants were also provided with a study number which was operating 24 hours and they could voice their concerns or ask any questions related to the study directly from the investigator.

All patients who were RDT positive (whether symptomatic or asymptomatic) were provided with the first line treatment (Artemether Lumefantrine).

4.0 CHAPTER FOUR: RESULTS

4.1 Introduction

A total of 400 participants (all age-groups) from 72 households were observed for a period of one year. There were a total of about 1900 repeated observations after observing each household member for 12 months.

At the beginning of the study, there were 400 participants but by the end of the study, about 16 (7.9%) had been completely lost to follow up leaving 384 participants. The main cause for the permanent loss to follow up was household members who had got jobs outside Bungoma County necessitating them to move out of their village house together with their families or in most cases only the head of the household moved to the new town because of securing a new job.

4.2 Demographic/Household Characteristics of the participants

a) Age Distribution

There were no major variations with age distribution within the fever coldspots and fever hotspots. Majority of the participants were children between the ages of 6 - 10 years (Table 1).

Table 1: Age Distribution of the participants By Region (Frequencies and Percentages)

Age Category	Fever Coldspot	Fever Hotspot	Total
0 – 1	5 (3.6)	6 (2.4)	11 (2.9)
2 - 5	26 (18.8)	38 (15.4)	64 (16.7)
6 – 10	27 (19.6)	52 (21.1)	79 (20.6)
11 -14	14 (10.1)	26 (10.6)	40 (10.4)
15 -21	19 (13.8)	37 (15.0)	56 (14.6)
22 -30	19 (13.8)	22 (8.9)	41 (10.7)
31-40	11 (8.0)	29 (11.8)	40 (10.4)
41-50	8 (5.8)	15 (6.1)	23 (6.0)
>50	9 (6.5)	21 (8.5)	30 (7.8)
Total	138 (100)	246 (100)	384 (100.0)

* Percentage in parenthesis

b) Education of the participants

This applies to older children and the adults only. Majority of the participants had primary school education level or none. There were no major differences in the proportions denoting education between the fever hotspots and fever coldspots (Table 2).

Table 2: Education Level of the Participants by Region (Frequencies and Percentages)

Education status	**Fever Coldspot**	**Fever Hotspot**	**Total**
None	1 (1.03)	6 (3.4)	7 (2.6)
Not Applicable	1 (1.03)	0 (0.0)	1 (0.4)
Pre-primary	1 (1.03)	0 (0.0)	1 (0.4)
Primary-some	61 (62.9)	113 (66.1)	174 (64.2)
Primary-finished	10 (10.3)	26 (15.0)	36 (13.3)
Secondary-some	9 (9.3)	14 (8.0)	23 (8.5)
Secondary-finished	8 (8.2)	10 (5.7)	18 (6.6)
postsecondary_some	1 (1.0)	1 (0.6)	2 (0.7)
Post-secondary_ finished	5 (5.2)	4 (2.3)	9 (3.3)
Total	97 (100)	174 (100)	271

*Percentage in Parenthesis

c) Employment Status of the participants

This question was only asked to participants above the age of 18 years. Most of the particiapants were not employed.

Table 3: Employment status of Respondents by Region

Employment status	**Fever Coldspot**	**Fever Hotspots**	**Total**
Not-Applicable	14 (17.9)	21 (17.6)	35 (17.8)
Employed	6 (7.7)	11 (9.2)	17 (8.6)
Self-employed	18 (23.1)	19 (16.0)	37 (18.8)
Skilled Manual laborer	0 (0.0)	1 (0.8)	1 (0.5)
Unskilled manual laborer	9 (11.5)	13 (10.9)	22 (11.2)
Unemployed	28 (35.9)	50 (42.0)	78 (39.6)
Retired	3 (3.8)	4 (3.4)	7 (3.6)
Total	78 (100)	119 (100)	197 (100)

*percentages in parenthesis

4.3 Household Characteristics

a) Household Members

A total of 400 particpants from 72 households (48 in the fever hotspots and 24 in the fever coldspots) participated in the study for a period of one year.

b) ITN ownership

Every household that was visited in both the fever hotspot and fever coldpsots had atleast one ITN and therefore this translates to 100% ownership in terms of each household having atleast one ITN. This however does not imply that each individual has access to a net as this is only household ownership. There were a total of 167 sleeping spaces, of which 125 (74.8%) had a bed net while 42 (25.2%) did not have.

Most of the ITNs (68%) were acquired free of charge during the mass ITN campaign in the area that was conducted in 2011. In addition, some families were also able to acquire more ITNs freely during pregnancy when they visited the ANC and when they took their babies to child welfare clinics during the postnatal period. About 9% of the participants admitted having bought some more ITNs at an approximate cost of between Ksh.50 and Ksh.150. In some of the cases, some people sold the same ITNs that they had been given freely at a small cost of only ksh.50. All the ITNs acquired during the campaigns were LLINs and were therefore already treated at the time they were acquired. Despite the fact that the nets were already treated, 7% of the respondents tried to retreat their nets even though it was not really necessary because the chemical in the ITN should last for at least five years yet these nets were mostly acquired in a mass campaign in 2011.

At least half of all the bednets in both the fever hotspots and fever coldspots were torn (had some holes). About 55.6 % of all the torn bednets had less than five holes, 25% of the ITNs had 5 to 10 holes and 19.4% had more than 10 holes. The holes were of varying sizes, 36.1% were smaller than a coin, 38.9% of the holes were larger than a coin and 25% were larger than a fist (Figures 7a, b, c and d).

Fig.7a) ITN hole-smaller than a coin

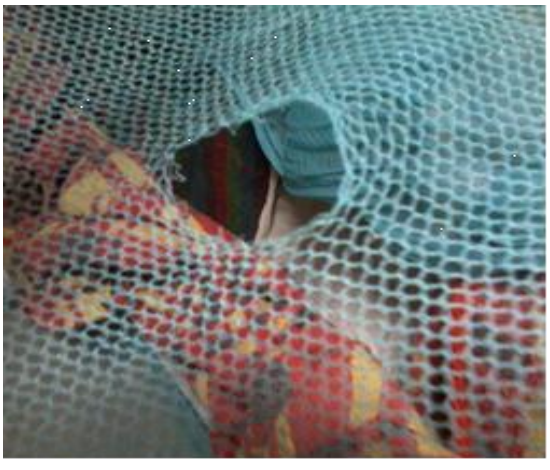

Fig.7b) ITN hole-slighlty smaller than a fist

Fig. 7c) ITN hole-larger than a fist

Fig. 7d) ITN with two holes-larger than a coin

b) Spraying of the Interior walls

All the households in both the fever hotspots and fever coldspots did not use insecticides to spray their households against malaria vectors and neither was there any on going indoor residual spraying of households at the time. Households that were currently under PSC monitoring of mosquitoes were the only ones that were sprayed on a monthly basis.

4.4. Objective One: Knowledge and Behaviour Related to Malaria in the fever Hotspots and Coldspots

4.4.1 Knowledge Related to Malaria by Region

The questions in this section included knowledge on the causative agent, mode of spread, breeding sites for vectors, common biting time for vectors, measures to prevent malaria and medications used for malaria. A chi-square test was used to check if there was a relationship between the knowledge variables and region (Fever hotspot or coldspot). Fishers' exact was used where the expected value/frequency in atleast 20% of the cells was less than 5.

Although knowledge on the causative agent for malaria was 100% in both the fever hotspots and fever coldspots, the responses on the mode of spread of malaria were very varied with some citing dirty water, unhygienic conditions and bites from flies (table 4a).

There was no statistically significant difference in the knowledge regarding mode of spread of malaria, breeding sites for vectors, mosquito biting time and malaria prevention between the fever coldspot and the fever hotspots. However, there was a statistically significant difference in knowledge regarding medications used for malaria between the coldspots and hotspots, p = 8.77 (χ^2= 0.033) (Table 4a).

Table 4a): Relationship between Knowledge Related variables and Region

Mode of spread	**Fever cold spot**	**Fever hotspot**	**Statistical test**
Mosquito Bite	40 (83.3%)	79 (89.8%)	
Dirty water	4 (8.3%)	1 (1.1%)	Fishers Exact
Unhygienic conditions	2 (4.2%)	2 (2.3%)	
Don't Know	2 (4.2%)	6 (6.8%)	P = 0.13
Mosquito Breeding Sites			
Standing Dirty Water	39 (79.6%)	62 (71.3%)	
Garbage	1 (2.0%)	0 (0.0%)	
Vegetation	6 (6.9%)	18 (20.7%)	χ^2= 14.8,P = 0.09
Don't Know	3 (3.4%)	7 (8.0%)	
Biting Time			
Night	19 (38.8%)	37 (42.5%)	
Dawn	4 (8.2%)	5 (5.7%)	
Dusk	24 (49.0%)	41 (47.1%)	χ^2 =1.9, p = 0.98
Sunrise Sunset	2 (4.1%)	4 (4.6%)	
Prevention Measures			

Use of ITNs	34 (69.4%)	65 (73.9%)	
Drain Stagnant water	6 (12.2%)	9 (10.2%)	
Use of Mosquito Coil	4 (8.2%)	5 (5.7%)	χ^2=6.6,P=0.09
Others	5 (10.2%)	9 (10.2%)	
Malaria Therapy			
First line Therapy	21 (42.9%)	47(53.4 %)	
Old Therapy	10 (20.4%)	4 (4.6 %)	
Other	11 (22.5%)	21 (23.9%)	χ^2= 8.8,P=0.033
Don't know	7 (14.3%)	16 (18.2%)	

4.4.2 Behavioural Characteristics predisposing to malaria Risk by Region

Respondents above 18 years of age were asked questions related to behavior that could predispose individuals to malaria inorder to establish if they had behaved in a manner that could actually put them at risk of contracting malaria.

Individuals were asked about the time and place they take their dinner, their resting location after dinner, the time they go to bed, whether they had travelled in the last three months, whether they kept open containers with water, if they slept under the net the previous night, how often they use the ITN in a week and what prompted them to use the ITN (Table 4b and 4c). Questions on dinner time, resting location after dinner, time to bed were asked only once in the first round of data collection. However, questions regarding their travel history in the last three months and net use were asked during every round.

There were no significant differences in most of the variables that denoted behavior related to malaria risk in both the fever hotspots and the fever coldpspots. However, ITN use was significantly associated with the region in the second and fourth quarters of data collection, P <= 0.0001 (χ^2 = 16.827) (Table 4d).

Table 4b: Relationship between Behavioural Factors and Region

Dinner Time	**Fever Cold spot**	**Fever Hotspot**	**Statistical Test**
Before 7pm	8 (16.3%)	9 (10.2%)	
Between 7pm and 8.30pm	40 (81.6%)	75 (85.2%)	Fishers Exact
After 8.30pm	1 (2.0%)	3 (3.4%)	P = 0.63
Others	0 (0.0%)	1 (1.1%)	
Resting Location after Dinner			
At 8pm	42 (85.7%)	67 (76.1%)	
At 10pm	6 (12.2%)	11 (12.5%)	χ^2 = 10.2,P = 0.12
At 12midnight	1 (2.0%)	9 (10.2%)	
Between 9&midnight	0 (0.0%)	1 (1.1%)	
Regular ITN use (Self-reported)			
Daily	40 (81.6%)	65 (73.9%)	
Sometimes/Occasionally	3 (6.1%)	12 (13.6%)	
Never	5 (10.2%)	11 (12.5%)	χ^2 = 4.21,P =0.38
Other	1 (2.0%)	0 (0.0%)	
Keeps Open Containers			
Yes	96 (71.1%)	187 (78.9%)	
No	39 (28.9%)	50 (21.1%)	χ^2= 2.87,P = 0.09

ii) Association of Travel History with Region

Respondents from both fever hotspots and fever coldspots were asked if they had travelled outside Bungoma County to other endemic regions during the two months preceding the survey. Throughout the one year of study, only a handful of people (11.5%) had travelled outside Bungoma County. A chi-square test/Fishers exact test was used to check whether there was an association between the travel history and the region (fever hotspots and coldspots) during each survey. The results indicated that there was no relationship between region and travel history of the particiapants. There was no relationship between the travelling history and region i.e fever hotspots and fever coldspots in any of the five quarters (Table 4c).

Table 4c: Association of Travel History with Region

Travel History	**Fever Cold spot**	**Fever Hotspot**	**Statistical Test**
Visit One			Fishers Exact
Yes	129 (97.7%)	230 (98.3%)	p = 0.05
No	3 (2.3%)	4 (1.7%)	
Visit Two			
Yes	130 (98.5%)	237 (97.5%)	P = 0.71
No	2 (1.5%)	6 (2.5%)	
Visit Three			
Yes	136 (98.5%)	242 (96.8%)	P = 0.68
No	2 (1.5%)	8 (3.2%)	
Visit Four			
Yes	136 (97.8%)	225 (95.3%)	P = 0.745
No	3(2.2%)	11 (4.7%)	
Visit Five			
Yes	135 (98.5%)	234 (97.9%)	P= 0.663
No	2 (1.5%)	5 (2.1%)	

iii) Association between ITN Use and Region by Visit Number

In the first, third and fifth visits, there was no significant association between ITN use and whether one was in the fever hotspots or coldspots. However, in the second and fourth visits, there was a significant association between region and ITN use which may imply possible differences in the use of ITNs in the fever hotspots and coldspots during particular seasons (Table 4d).

Table 4d: Association between ITN Use and Region by Visit Number

Sleeps under the Net	**Fever Cold spot**	**Fever Hotspot**	**Statistical Test**
Visit One			
Daily	54 (40.9%)	118 (50.4%)	
Sometimes	67 (50.8%)	105 (44.9%)	χ^2= 4.1
None	11 (8.3%)	11 (4.7%)	P = 0.13
Visit Two			
Daily	40 (30.3%)	124 (51.0%)	
Sometimes	88 (66.7%)	86 (35.4%)	χ^2= 36.1
None	4 (3.0%)	33 (13.6%)	P <= 0.001
Visit Three			
Daily	60 (43.5%)	104 (42.3%)	
Sometimes	62 (44.9%)	115 (46.8%)	χ^2 = 0.1
None	16 (11.6%)	27 (11.0%)	P = 0.94
Visit Four			
Daily	39 (36.1%)	97 (40.9%)	
Sometimes	100 (71.9%)	118 (49.8%)	χ^2 = 16.8
None	0 (0.0%)	22 (9.3%)	P <=0.001
Visit Five			
Daily	59 (43.1%)	82 (34.3%)	
Sometimes	68 (49.6%)	143 (59.8%)	χ^2 = 3.7
None	10 (7.3%)	14 (5.9%)	P = 0.16

4.5 Objective Two: Incidence and Prevalence of Malaria in Fever Hotspots and Fever Coldspots

There were 321 malaria infections over the one year period of observing this cohort. The incidence of malaria was 45 per 1000 person months in the fever hotspots and 34 per 1000 person months in the fever coldspots (Figure 9a and b). Parasite prevalence by RDT in the fever cold spot was was:17.3%, 9.6%, 13.6%, 13.9% and 22.8% per survey respectively while in the fever hotspots, parasite prevalence was: 20.3%, 7.5%, 9.4%, 11.9% and 35.7% per survey respectively. Prevalence of malaria infections also varied among the individual villages by season/visit number. Highest malaria infection prevalence recorded was 45.7% in Sitabicha village during the final quarter in July and the lowest was 0.9% in Lukhuna village during the fourth quarter.

Overall the mean prevalence of malaria infections for the whole year was slightly higher in the fever hotspots compared to the fever coldpots. Prevalence per visit in the two regions was highest in the fever hotspots during the first and the last visits which were both conducted in July one year apart. However, during the second, third and fourth visits, the coldspots registered a higher prevalence of malaria compared to the hotspots (Figure 8). The visits were made quarterly in the months of July, October, January, April and July the following year. These visits also overlapped with the usual seasons in Western Kenya. Prevalence of malaria infections peaked during the rainy season and was lowest during the dry season. The month of July comes immediately after the main rainy season (the rains begin in April, May and June).

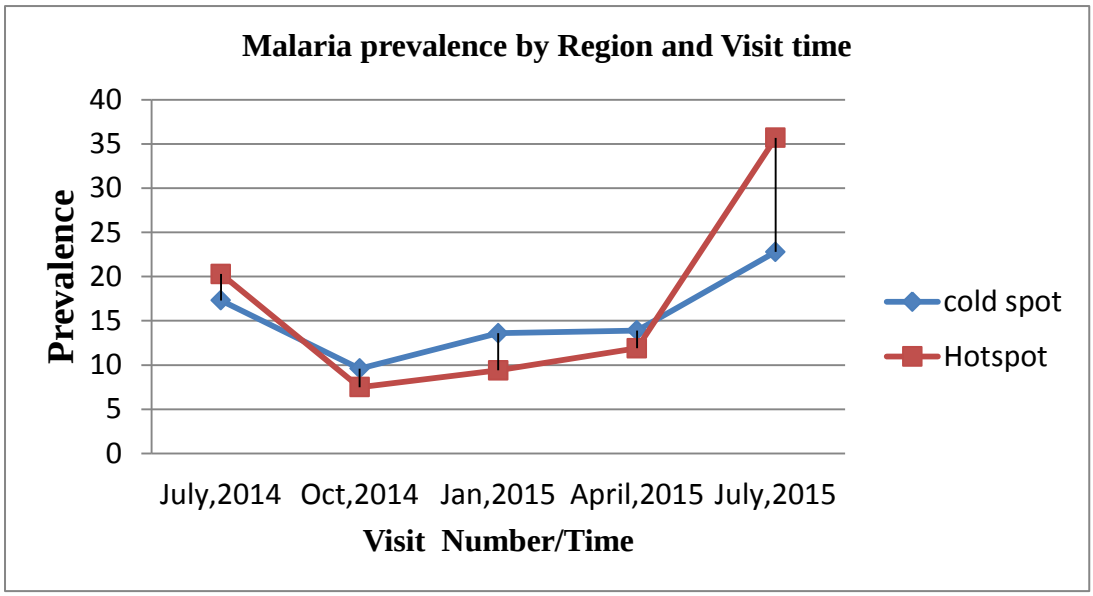

Figure 8: Malaria Infection Prevalence by Region and Visit Time

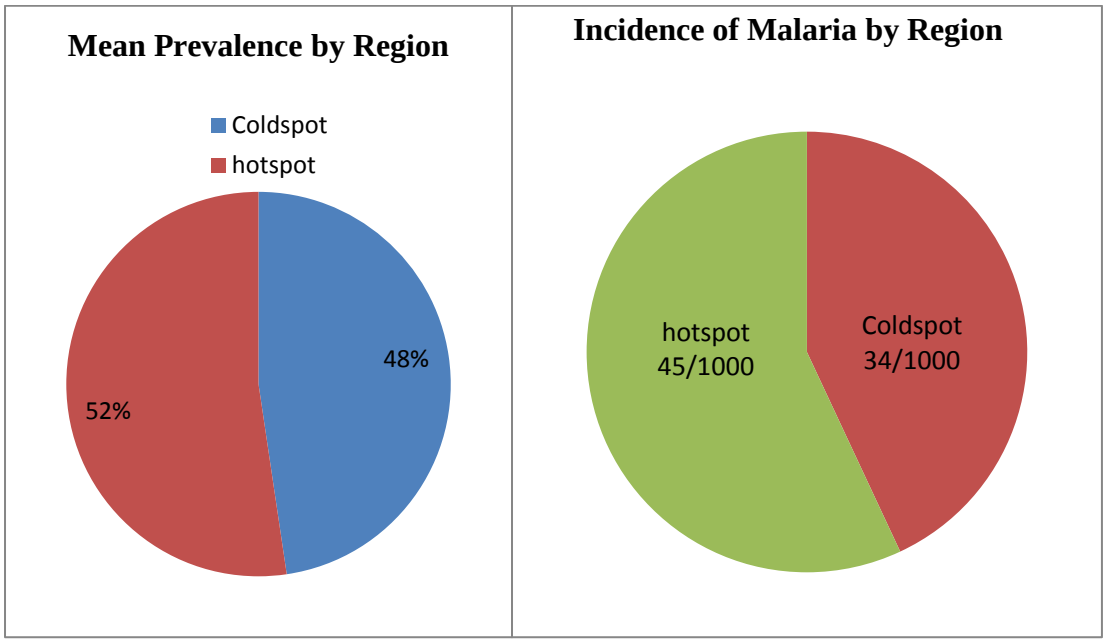

Figure 9a and b): Mean Prevalence and Incidence of Malaria Infections by Region

4.5.1 Prevalence of malaria per village by Visit time

Prevalence of malaria infections was highest cumulatively throughout the year in a hotspot village (Sitabicha) and lowest in a coldspot village (Lukhuna). Surprisingly, one village in the coldspot (Maruti Village) contributed to a high mean prevalence of malaria infections overall. There was an interesting pattern in the disease prevalence over time with the hotspots contributing to the highest prevalence during the first and last visits. However, one fever coldspot village (Maruti village) recorded very high mean prevalence for the whole year surpassing most of the fever hotspot villages (Figure 10).

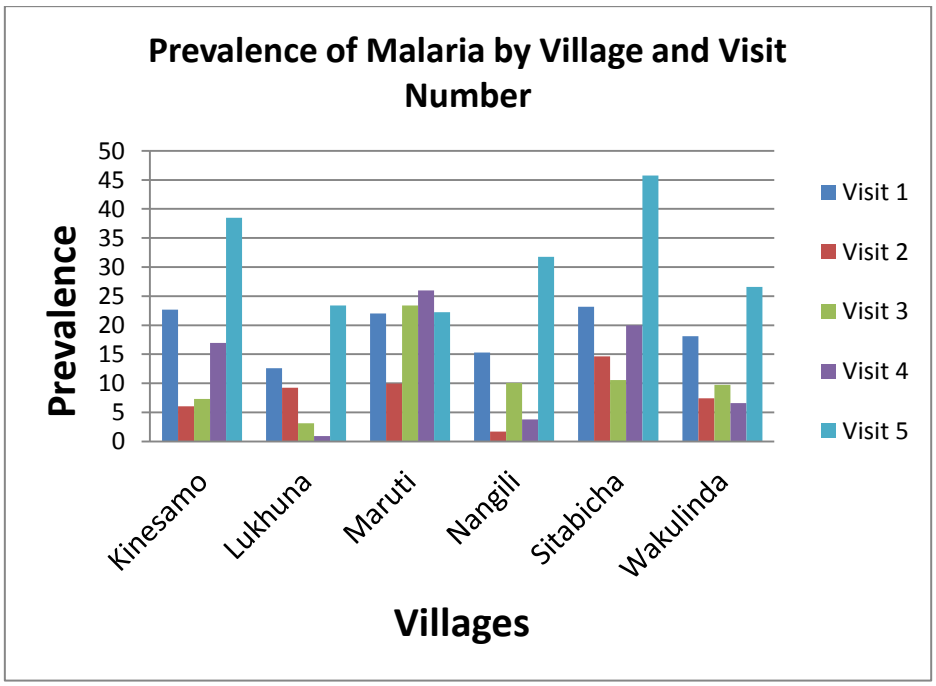

Fig 10: Prevalence of Malaria infections by Village and Visit Number

4.5.2 T-test for differences in incidence of malaria in the two Regions

A t-test was used to check whether the observed differences in the incidence of malaria infections were significantly different between the fever hotspots and fever coldspots. The dependent variable

was incidence of malaria infections and the independent variable was region (binary categorical - fever hotspots or coldspots) (Table 5).

Table 5: T-test for differences in Incidence of malaria infections in the two Regions

Group	Obs	Mean	Std. Err.	[95% Conf. Interval]
coldspot	122	15.4	2.13	11.20 19.66
hotspot	236	16.9	1.44	14.11 19.79
combined	358	16.4	1.19	14.08 18.79
diff		-1.5	2.52	- 6.48 3.45

Diff= mean (Coldspot – mean(Hotspot) t = -0.6015

Ho:diff=0 degrees of freedom = 356

Ha: diff < 0 Ha: diff != 0 Ha: diff > 0

P = 0.27 P = 0.55 (p associated with two tailed significance) P = 0.73

The group means are not significantly different as the p-value in the P under Ha: diff! = 0) is more than 0.05 (i.e based on a 2-tailed significance level). Therefore there are no statistically significant differences in the incidence of malaria between the fever hotspots and fever coldspots.

4.5.3 Anova test for differences in incidence between villages

Anova was used to test whether there were statistically significant differences in the incidence of malaria infections among the villages. There are statistically significant differences in the incidence of malaria infections between the villages as determined by Anova f (3.24), p = 0.0071 (Table 6).

Table 6: Anova test for differences in Incidence of malaria infections among Villages

Source	**df**	**F**	**Prob>F**
Model	5	3.24	0.0071
Village	5	3.24	0.0071
Residual	353		

4.6 Objective Three: Prevalence of Asymptomatic status and Associated Risk factors in Fever Hotspots and Coldspots

4.6.1 Asymptomatic Malaria Infections in Fever Hotspots and Coldspots

An asymptomatic malaria infection refers to the detection of asexual or sexual parasites in blood and an absence of any acute clinical symptoms (usually fever) during a specified time frame. In this study, asymptomatic malaria infection was defined as anybody who had confirmed parasitemia by RDT but had no recent history of symptoms and/or signs of malaria and had not taken antimalarial treatment in the last two weeks.

A total of 321 malaria infections were detected during the five cross-sectional surveys over the course of one year. Almost half (46.3%) of these were asymptomatic. Overall, a higher proportion of the asymptomatic infections (67%) were in households within fever hotspots. The proportion of infections that were asymptomatic in the fever coldspots were 73.1%, 31.8%, 13.3%, 55.6% and 48.2% during the first, second, third, fourth and fifth visits respectively. In the fever hotspots, the proportion of infections without symptoms was 47.7%, 48.5%, 35%, 41.3% and 47.5% during the first, second, third, fourth and fifth visits respectively.

The highest proportion of asymptomatic cases was between the ages of 6 to 14 years (Table 7). Aymptomatic infections varied across villages as well. Lukhuna village in the fever coldspot had no single asymptomatic case. All infected cases in this village presented with symptoms.

Table 7: Proportion of Asymptomatic Malaria infections by Age

Age Category	**% asymptomatic infections**
0-1 year	30
2-5 years	39.6
6-10 years	64.7
11-14 years	60.0
15-20 years	53.5
21-30 years	28.0
31-40 years	21.5
41-50 years	15.4
>50 years	8.4

4.6.2 (i) Asymptomatic Infections by Visit Number/Season

In general, the prevalence of asymptomatic infections was highest (peaked) during the first and last visit (Table 8). The two visits were conducted in July which coincides with the main rainy season. In July, the heavy rains have reduced and there is a lot of stagnant water where mosquitoes quickly breed and cause the heightened infections (both symptomatic and asymptomatic).

Table 8: Distribution of asymptomatic infections by visit number (Denominator-All Asymptomatic Infections within the two regions -row percentages add to 100%)

	Visit Number				
Asymptomatic infections	**1(July)**	**2(Oct)**	**3(Jan)**	**4(April)**	**5(July)**
Coldspot	19(33.3%)	8(14.0%)	7(12.3%)	10(17.5%)	13(22.8%)
Hotspot	17(17.5%)	19(19.6%)	15(15.5%)	11(11.3%)	35(36.1%)
Total	36(23.3%)	27(17.5%)	22(14.3%)	21(13.6%)	48(31.2%)

4.6.2 (ii) Asymptomatic Infections by Village

Maruti village had the highest incidence of asymptomatic cases (29.1 per 1000 person months) followed by Sitabicha village (22.2 per 1000 person months) and Kinesamo (14.3 per person months) (Table 9).

ANOVA test was used to check whether there were any statistically significant differences in the incidence of asymptomatic cases in the villages. The study did not find any statistically significant differences in asymptomatic infections between the villages (P = 0.68).

Table 9: Incidence of asymptomatic Malaria Infections by Village

Village	**Incidence Rate per 1000 person months**
Kinesamo	14.3
Lukhuna	0
Maruti	29.1
Nangili	0
Sitabicha	22.2
Wakulinda	8.4

4.6.3 Prevalence and Factors Associated with Asymptomatic Parasitemia in Fever coldspots and hotspots

A univariate Generalised estimating Equation model (GEE model) controlling for repeated measures and clustering was fitted to determine factors that were associated with asymptomatic parasitemia. Thereafter, a multi-variable adjusted GEE model was fitted to control for confounders and therefore determine factors associated with asymptomatic status in patients who had tested positive for malaria infections but did not present with any symptom.

The Univariate model identified the following factors: Village; People living in Lukhuna village (coldspots) were 30% less likely to be asymptomatic (O.R: 0.70, C.I; -1.178 - 0.484), region: People

living in the hotspots were 5% less likely to be asymptomatic (OR: 0.95, C.I-0.533 - 0.434), age in years: Children above five years were more than twice likely to be asymptomatic when they get a malaria infection (O.R: 2.66, C.I; 0.439 1.518) and the visit number/time: During the third visit which was conducted in January, people were 69% less likely to be asymptomatic (OR: 0.31, C.I; -2.008 - -0.311) (table 10).

Table 10: Univariate Logistic Regression GEE model for Factors Associated with Asymptomatic Parasitemia (Unadjusted model)

Variable	**Unadjusted Odds Ratio**	**P value**	**Unadjusted Odds Ratio (95% CI)**	
Village				
Kinesamo	1			
Lukhuna	0.70	0.413	-1.178	0.484
Maruti	1.37	0.368	-0.377	1.019
Nangili	0.65	0.379	-1.389	0.528
Sitabicha	1.68	0.126	-0.146	1.193
Wakulinda	0.56	0.158	-1.378	0.224
Region				
Coldspot	1			
Hotspot	0.95	0.841	-0.533	0.434
Age in years				
0 – 5	1			
6 – 15	2.66	0.000	0.439	1.518
16– 30	1.02	0.941	-0.677	0.730
31 – 50	0.35	0.062	-2.106	0.050
>50	1.05	0.073	-4.000	0.179
Visit Number				
1	1			
2	0.61	0.153	-1.149	0.180
3	0.31	0.007	-2.008	-0.311
4	0.76	0.456	-0.958	0.430
5	0.78	0.388	-0.792	0.308

Table 11: Multi-Variate GEE Logistic Regression model for Factors Associated with Asymptomatic Parasitemia (Adjusted model)

Variable	**Adjusted Odds Ratio**	**P value**	**Adjusted Odds Ratio (95% CI)**	
Village				
Kinesamo	1			
Lukhuna	0.74	0.495	-1.165	0.563
Maruti	2.14	0.040	0.034	1.488
Nangili	0.93	0.896	-1.100	0.962
Sitabicha	1.80	0.090	-0.092	1.269
Wakulinda	0.67	0.345	-1.205	0.421
Age in years				
0 – 5	1			
6 – 15	2.67	0.000	0.434	1.533
16 - 30	1.10	0.779	-0.611	0.816
31 – 50	0.43	0.126	-1.888	0.233
>50	0.14	0.077	-4.050	0.209
Visit Number				
1	1			
2	0.60	0.191	-1.251	0.249
3	0.26	0.005	-2.289	-0.400
4	0.63	0.248	-1.239	0.320
5	0.82	0.542	-0.804	0.423

In the adjusted multi-variable model (Table11 above), the village: (A.O.R: 2.14, C.I: 0.03 - 1.488), age: (A.O.R: 2.67, C.I. 0.434 -1.533) and visit number: (A.O.R: 0.26, C.I: -2.289 - 0.400) are the main determinants for asymptomatic parasitemia in the community.

4.7 Objective Four: Risk factors for Incidence of malaria in fever hotspots and coldspots

To model the risk factors for malaria incidence in the fever hotspots and coldspots, we fitted a multi-level mixed effect logistic regression model controlling for clustering at three levels; clustering at the village, clustering at the household level and clustering of repeated values within the same individual.

Risk factors for malaria infection were identified in a univariate model and then combined in a multilevel mixed-effects logistic regression model, which simultaneously adjusts for variations between individual repeated measures, the individuals in the household and the clustering of households at the village level. The goodness of fit was measured by calculating the Pearson residual and by visually assessing the agreement between these parameters using a Bland-Altman plot.

4.7.1 Risk factors for Malaria - Descriptive

Incidence distribution was calculated for region, age and village. There were variations of incidence of malaria by age and village (table. 12 and 13).

Table 12: Incidence of malaria infections by age

Age Category	Incidence Rate per 1000 person months
0-1 year	37
2-5 years	61
6-10 years	54
11-14 years	54
15-20 years	47
21-30 years	30
31-40 years	19
41-50 years	25
≥50 years	6

Table 13: Incidence of malaria infections by Village

Village	Incidence Rate per 1000 person months
Kinesamo	60
Lukhuna	26
Maruti	47
Nangili	27
Sitabicha	52
Wakulinda	51

4.7.2 Risk factors for Malaria - Modelling

The univariate model identified the main risk factors for malaria as; Age ; older children are almost 50% less likely to get malaria infections compared to their younger counterparts (O.R: 0.51, C.I: 0.338 - 0.779), ITN use ; those not sleeping under a net are two times likely to develop malaria infections (OR: 2.04,C.I: 1.179 - 3.544), Open eaves; Individuals living in houses with open eaves are more than one and half times likely to be infected by malaria (O.R: 1.58, C.I: 1.143 - 2.196), the village one lives; people living in Lukhuna are 62% less likely to get malaria (OR: 0.38 C.I: 0.301 - 1.197) and the number of larval sites; those living in areas with more than five larval sites were more than three times likely to get malaria infections than their counterparts living in areas with no larval sites (O.R: 3.2 C.I: 1.572 - 6.694). There is a slightly increased risk of malaria infection among those living in fever hotspots compared to those in the fever coldspots (O.R: 1.25 C.I: 0.629 - 2.497) (table 14).

Table 14: Multilevel Mixed Effects Regression model for Malaria Risk factors (Unadjusted)

Variable	Unadjusted Odds Ratio	P value	Unadjusted Odds Ratio (95% CI)	
Village				
Kinesamo	1			
Lukhuna	0.38	0.008	0.187	0.779
Maruti	1.01	0.967	0.521	1.973
Nangili	0.49	0.069	0.232	1.055
Sitabicha	1.18	0.603	0.620	2.278
Wakulinda	0.60	0.147	0.301	1.197
Region				
Coldspot	1			
Hotspot	1.25	0.519	0.629	2.497
Age in years				
0 – 5	1			
6 -15	0.88	0.478	0.623	1.248
16-30	0.51	0.002	0.338	0.779
31-50	0.28	0.001	0.167	0.465
>50	0.29	0.001	0.145	0.588
Net use				
Always	1			
None	2.04	0.011	1.179	3.544
Sometimes	1.38	0.066	0.979	1.971
Type of wall				
Mud Earth	1			
Cement	0.79	0.523	0.393	1.605
Mabati	1.49	0.492	0.474	4.704
Presence of Malaria Symptoms				
None	1			
Yes	4.41	0.000	2.573	7.567

Open containers				
No	1			
Yes	1.16	0.534	0.720	1.882
Open Eaves				
No	1			
Yes	1.58	0.006	1.143	2.196
Number of Larval sites				
None	1			
1-2	1.6	0.057	0.985	2.588
3-4	1.4	0.259	0.783	2.478
>5	3.2	0.001	1.572	6.694

Table 15: Multilevel Mixed Effects Regression model for Malaria Risk factors (Adjusted)

Variable	**Adjusted O.R**	**P.Value**	**95% C.I for Adjusted Odds Ratios**	
Region				
Coldspot	1			
Hotspot	1.3	0.278	0.826	1.940
Age in years				
0 – 5	1			
6 – 15	0.8	0.385	0.607	1.212
16– 30	0.5	0.003	0.351	0.811
31– 50	0.2	0.000	0.136	0.404
>50	0.3	0.001	0.154	0.624
Net use				
Always	1			
None	2.2	0.004	1.301	3.887
Sometimes	1.5	0.012	1.097	2.153
Open Eaves				
No	1			
Yes	1.5	0.007	1.121	2.096
Type of wall				

Mud earth	1			
Cement	0.9	0.895	0.336	2.591
Mabati	1.1	0.855	0.570	1.966
Larval Sites				
None	1			
1-2	1.5	0.085	0.944	2.415
3-4	1.4	0.272	0.785	2.361
>5	3.3	0.001	1.647	6.452

After adjusting for all the other factors in the multi-level mixed effects logistic regression, the incidence of malaria was still slightly higher in the fever hotspots than the fever coldspots but did not reach statistical significance (Table 15 above). People living in fever hotspots are 1.3 times more likely to have malaria than those living in fever coldpots (A.O.R: 1.3; P; 0.27, C.I; 0.826 – 1.940).

Risk factors for malaria infections include age; younger children below five years are at greater risk of malaria compared to the older children, (A.O.R: 0.5 ; C.I: 0.351 - 0.811), ITN use; those not sleeping under are more than two times likely to develop malaria infections than those consistently using ITNs (A.O.R; 2.2 C.I. 1.301 - 3.887), open eaves (A.O.R; 1.5 C.I: 1.121 - 2.096) and the presence and number of larval sites; the more the number of larval sites,the higher the chances of getting malaria (A.O.R: 3.3 C.I 1.647 - 6.452) (Table 15).

Most of these factors were not different in both the fever coldspots and hotspots except that those in the fever hotspots were slightly more likely to get infected than their counterparts in the fever coldpsots. However, there were significant differences in larval sites between fever hotspots and fever coldspots (this is discussed under entomological factors in 4.6 below).

4.8. Objective Five: Incidence of malaria and Mosquito Characteristics

4.8.1: Mosquito Counts

A total of 870 malaria vectors were collected for the whole period of one year.

Of the mosquitoes that were captured, no=640 (73.6%) were identified as members of *Anopheles gambiae* complex.

The member species of An. *gambiae* group were morphologically identified using a microscope and the morphological key. The sibling species were further identified by polymerase chain reaction using the specific species primers as follows; no=117 (24.5%) were An. *arabiensis* while An. *gambiae s.s* consisted of no=483 (75.5%) of the total collection.

4.8.2 Abdominal Status of the Mosquitoes

To determine fed status of the mosquitoes, the blood fed, gravid and half gravid were all lumped together as fed and the rest as unfed.

In total, about 79.5% of the anopheles mosquitoes that were captured during the study were fed and only 20.5% were not fed.

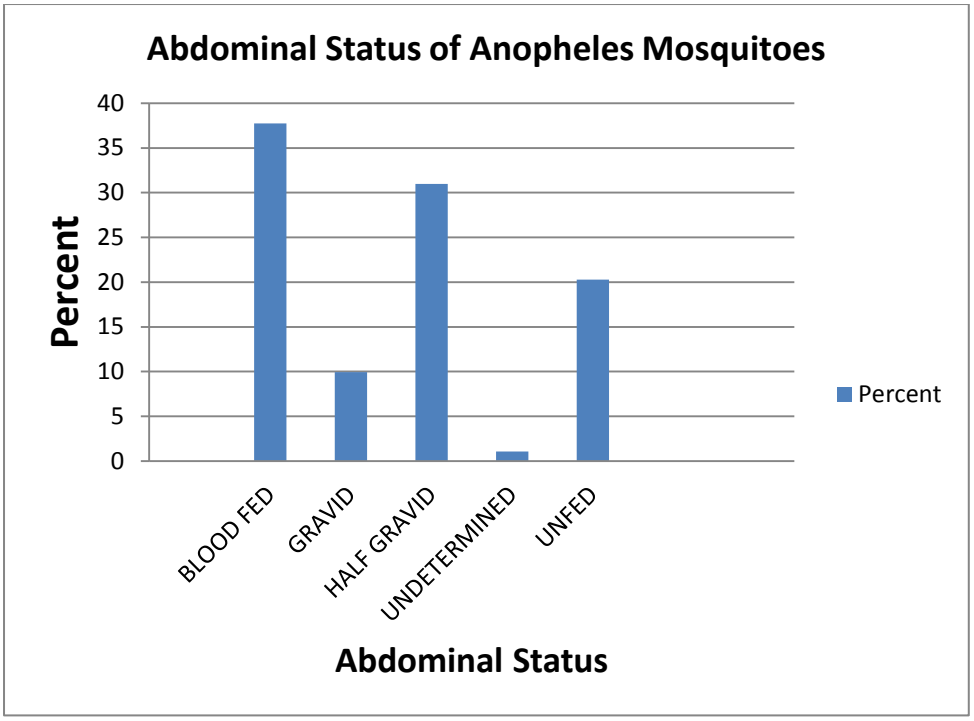

Fig 11: Abdominal Status of Anopheles Mosquitoes

4.8.3 Methods used for Monitoring Mosquitoes

The most effective means of monitoring mosquitoes was Pyrethrum Spray Catches (PSC) compared to the Window exit trap. More than 95% of all the mosquitoes were collected by PSC (Figure 12).

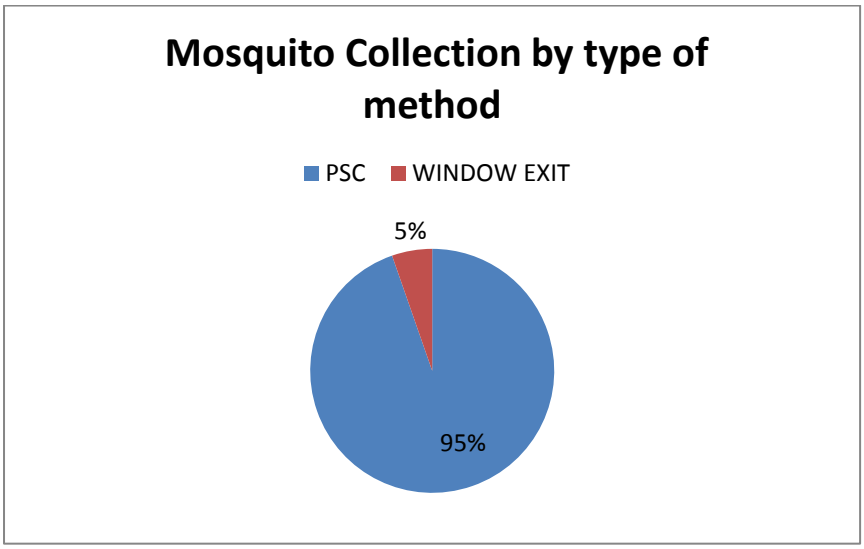

Fig 12: Mosquito Collection by Type of Collection Method

4.8.4 Mosquito counts by Region and Village

The highest number of mosquitoes was collected in Kinesamo (hotspot village) village and the lowest was in Nangili village (Hotspot village). However in general, one coldspot village (Lukhuna village) had the lowest mosquito counts as well as the lowest prevalence (already alluded before). The second coldspot village (Maruti village) had a moderately high number of mosquitoes and also recorded a high prevalence of malaria infections throughout the year (figure 13). Overall, the fever hotspots contributed the highest proportion (68%) of mosquitoes compared to the fever coldspots (32%) (Figure 14).

The highest proportions of mosquitoes were recorded in the months of May, June and July (figure 15). These months coincide with the main/long rainy season in this region.

Rainfall was monitored throughout the year using a rainfall gauge in both the fever hotspots and coldspots.

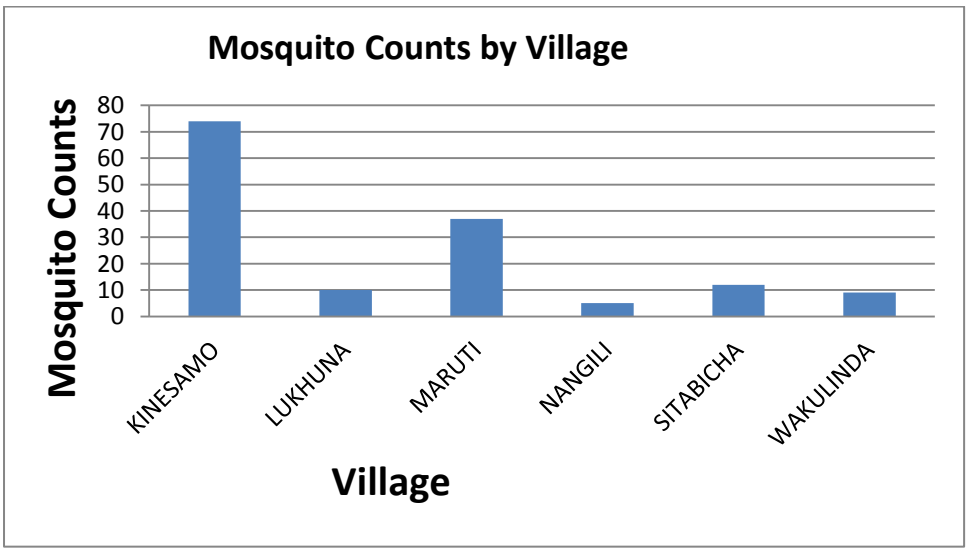

Figure 13: Mosquito Counts by Village

b) Mosquito Counts by Region

The highest proportion of mosquitoes were recorded in fever hotspots (68%) compared to the fever coldspots (32%) (Figure 14).

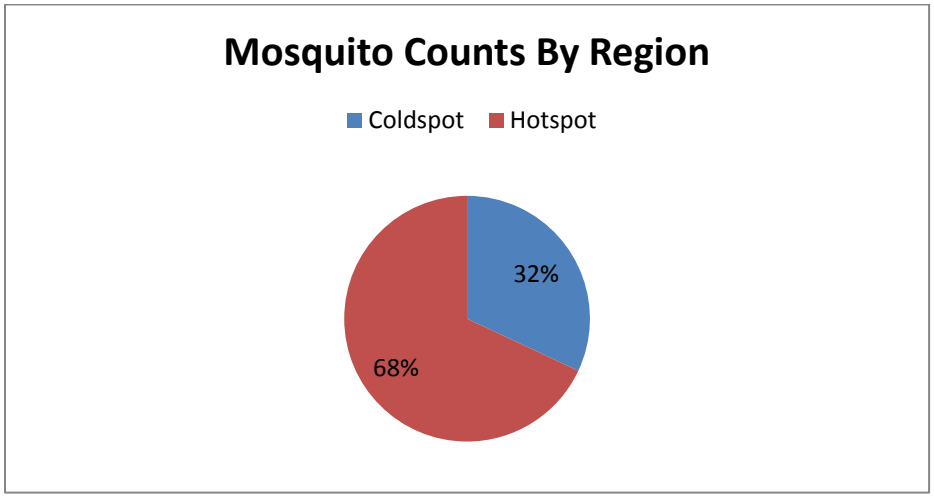

Fig 14: Mosquito Counts by Region (Fever Hotspots and Coldspots)

c) Mosquito Counts by Month

Mosquitoes were collected every month for a period of one year. The highest mosquito counts were recorded in the months of May, June and July and these correspond to the main rainy season in this area (Figure 15).

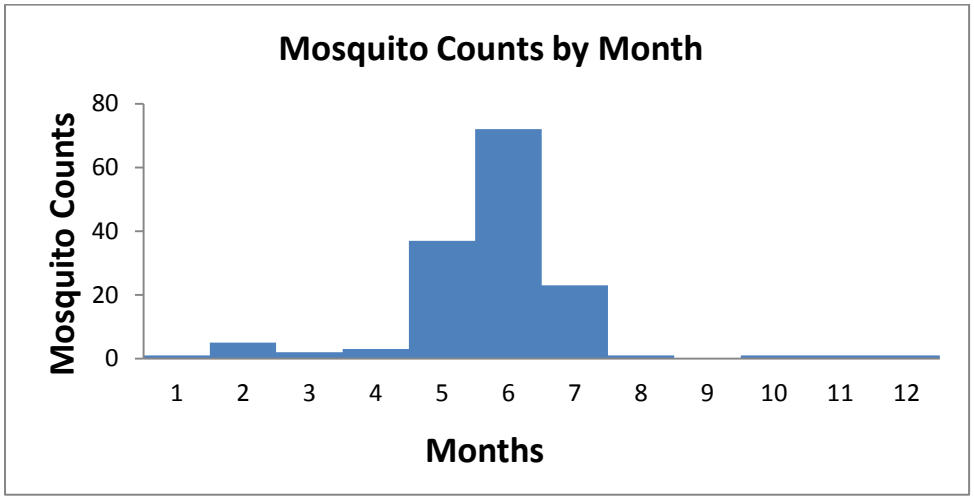

Fig 15: Mosquito Counts by Month

4.8.5 Sporozoite rates

Sporozoite rates were very low (1%). Only mosquitoes caught in Maruti village (Coldspot) tested positive for sporozoites. The rest of the mosquitoes collected in the other villages did not test positive for sporozoites. All sporozoite positive mosquitoes were *An gambiae s*,s.

4.8.6 Correlation of Mosquito Counts and Malaria Prevalence

This study sought to find out if there was a direct association between mosquito densities and the malaria prevalence in the two regions. There was a linear relationship between the total mosquito counts and the prevalence of malaria.

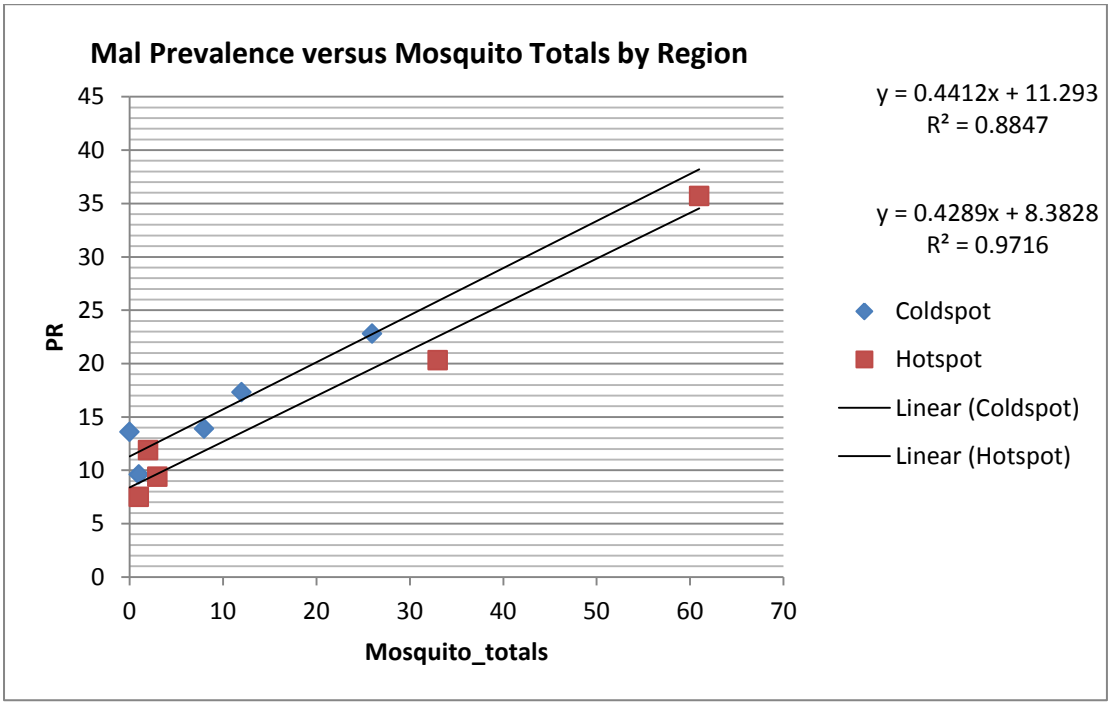

Fig 16: Malaria infection Prevalence versus Mosquito Totals by Region

4.8.7 Correlation of Incidence of Malaria and Mosquito Counts by Village

There was a clear correlation between the incidence of malaria infection and the mosquito counts by village. The incidence of malaria was highest in Kinesamo village which also correlates with the highest mosquito collections in the same village. Higher mosquito collections in a village correlated with a higher incidence of malaria infections. This is depicted in figure 17.

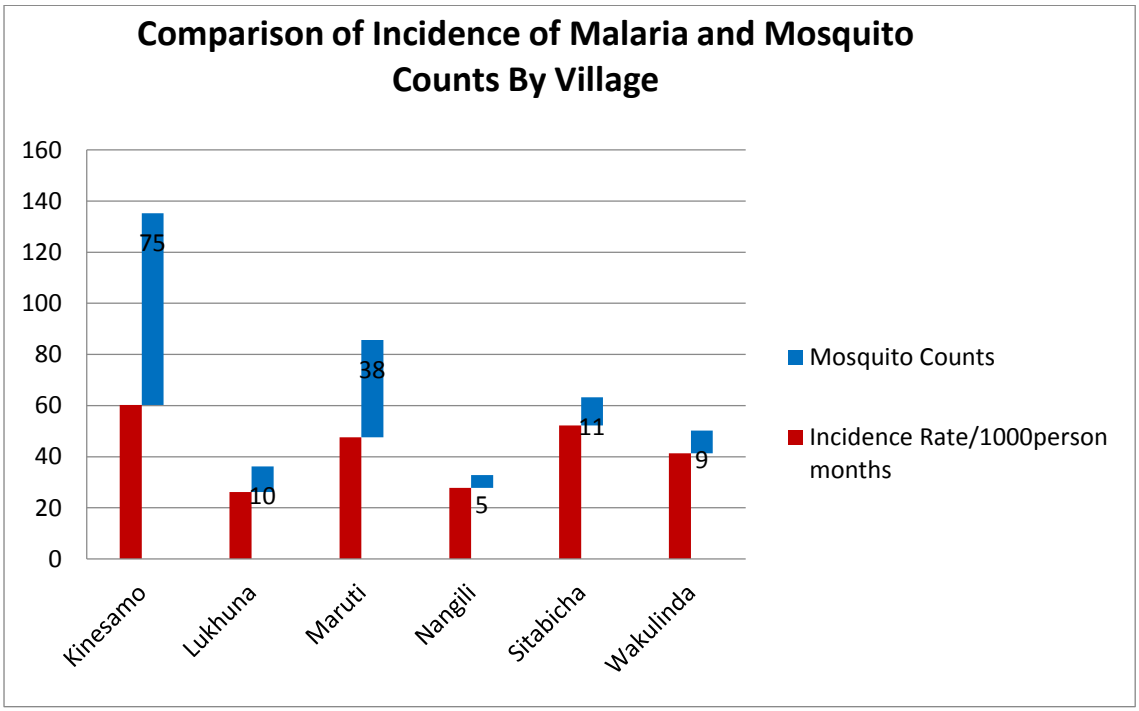

Figure 17: Graph showing correlation of Incidence of Malaria and Mosquito Counts by Village

4.7.8 Association of Larval sites with Region

The association of larval sites with region was done at household level to avoid duplication and repetitiveness since each household had many individuals.

There was a significant association between the number of larval sites and region during the first visit in July ($p = 0.035$). In the following quarterly rounds, there was no significant association between the number of larval sites and the region. However, the fever hotspots recorded the highest proportion of larval sites throughout the year. There were more larval sites recorded during the first and final rounds of parasite surveys which were conducted in July. This period coincides with the main rainy season in this region. Generally, there were more larval sites (more than 5) in the fever hotspots compared to either none or between (1-2) in the fever coldspots throughout the year (Table 19).

There was a consistent association between village and the number of larval sites through out the five surveys conducted in the year ($p = 0.001$). Households in Kinesamo village had consistently the

highest proportion of larval sites throughout the year while Lukhuna village had the lowest proportion of larval sites through out the year.

Table 16: Larval Sites in fever Coldpots and Hotspots by Visit Number/season

Number of Larval Sites	Fever Cold spot	Fever Hotspot	Statistical Test
Visit One			
None	10 (41.7%)	9 (18.8%)	
1- 2 larval sites	10 (41.7%)	16 (33.3%)	
3 - 4 larval sites	4 (16.7%)	16 (33.3%)	χ^2=8.6,P=0.03
Over 5 larval sites	0 (0.0%)	7 (14.6%)	
Visit Two			
None	11(47.8%)	14 (31.1%)	
1- 2 larval sites	11(47.8%)	19 (42.2%)	
3 - 4 larval sites	1 (4.4%)	8 (17.8%)	χ^2=5.4,P=0.15
Over 5 larval sites	0 (0.0%)	4 (8.9%)	
Visit Three			
None	12 (52.2%)	16 (34.0%)	
1- 2 larval sites	10 (43.5%)	23 (48.9%)	
3 - 4 larval sites	1 (4.4%)	7 (14.9%)	χ^2=3.4,P= 0.34
Over 5 larval sites	0 (0.0%)	1 (2.1%)	
Visit Four			
None	12 (50.0%)	14 (30.4%)	
1- 2 larval sites	11 (45.8 %)	20 (43.5%)	
3 - 4 larval sites	1 (4.2%)	10 (21.7%)	χ^2=5.8,P= 0.12
Over 5 larval sites	0 (0.0%)	2 (4.4%)	
Visit Five			
None	10 (41.7%)	9 (18.7%)	
1- 2 larval sites	8 (33.3%)	17 (35.4%)	
3 - 4 larval sites	4 (16.7%)	15 (31.3%)	χ^2=4.9,P= 0.17
Over 5 larval sites	2 (8.33%)	7 (14.6%)	

5.0 CHAPTER FIVE: DISCUSSION

5.1 Introduction

The study was conducted in at the Webuye HDSS in Bungoma East Sub-County, Bungoma County. Malaria transmission in Bungoma County is perennial with the main peaks during the rainy seasons. Although there has generally been a decline in malaria infections across most parts of the country, the disease continues to ravage this part of Western Kenya (Hamel et al., 2001; HDSS, 2009). The prevalence of malaria in Bungoma remains very high at 60% (Ministry of Health Kenya & Health Policy Project, 2015) despite most areas in the country registering a decline. Children bear the brunt of the scourge with malaria accounting for the highest morbidity and mortality in this group (Division of Malaria Control [Ministry of Public Health and Sanitation]Kenya National Bureau of Statistics, 2016).

The Webuye HDSS (located in Bungoma East Sub-County) was started in 2007, since then several cycles of half yearly cross-sectional surveys have been done within this community. Data on malaria is mainly inferred from self reported fever symptoms as well as facility confirmed cases. Currently there is no organized system in the HDSS to link malaria patients at the main referral health facility at Webuye with the community (the households they come within the HDSS). However, information on malaria at the facility can still be extrapolated to the community because the facility serves this community as part of the catchment area.

Trends in self reported fever distribution in the HDSS and a spatial analysis showed some form of clustering of fevers in particular villages. This indicates that there is possible heterogeneity of malaria infections among the villages based on the self reported fever symptom. This study was therefore informed by the need to answer the main research question on whether there was actual heterogeneity in malaria transmission within the HDSS by measuring the malaria transmission

indices. Whether there is an actual difference in malaria transmission in fever hotspots and coldspots may also be explained by other factors apart from measuring malaria transmission indices. Therefore in designing this study behavioural characteristics were included to explore their possible relationship with increased malaria infections in this area.

A total of 400 subjects from both the fever hotspots and fever coldspots were followed up for a period of one year. The loss to follow up was quite low (7.9%) and it was missing completely at random (MCRA). This is within the acceptable levels of not more than 20% (Vicki et al., 2004) which therefore does not bias the study results. Loss to follow up has been reported before as a serious source of bias to the study results especially if it is missing not at random (Mary et al., 2013; Vicki et al., 2004). The most likely reason for this low loss to follow up is mainly because the area is predominantly a rural setup where people have settled and do not easily migrate in and out of the area. This serves as a major strength for longitudinal studies conducted within the HDSS because it reduces loss to follow up, thereby essentially lowering the non-response rate. The inclusion criteria were tightened by selecting people who had stayed in the area for atleast one year and were not planning to move out any time soon. This also enhanced the accuracy and power of the study.

5.2 Knowledge and Behavioural Characteristics Regarding Malaria in Fever Hotspots and Coldspots

The study investigated whether there were differences in malaria related knowledge and behavior between the fever hotspots and fever coldspots. These were seen as possible alternative explanations for observed differences in the incidence of malaria between the fever hotspots and the coldspots. Knowledge was mainly on whether they knew the causative agent, mode of spread, medications used for malaria, breeding sites, common biting time and preventive measures for malaria. Human behavior included risky behavioural practices such as staying out late in the night, sleeping very late, travelling outside Bungoma County and not using ITNs while sleeping at night. Previous studies

have shown that the level of knowledge on preventive measures or treatment may impact directly on the use of these preventive measures as well as uptake of treatment (Erhun et al., 2005; Wakgari et al., 2003). On the contrary, some reports indicate that despite high levels of knowledge on malaria and preventive measures, this is not always accompanied by correct practice (Oguonu et al., 2005). However, most studies are in agreement that there is a clear interdependence between human behavior and development of malaria infections (Jane et al., 2011; Obol et al., 2013).

The common malaria vector in the Western region is a member of the An. *gambie complex* which mainly bites indoors and mostly late night when people have gone to sleep. Behaviour such as staying up until very late in the night would therefore increase chances of being bitten by mosquitoes and therefore getting infected (the assumption is that those indoors are sleeping under an ITN). In addition, as the ITN coverage increases, there is increased possibility of mosquitoes adapting new behavior where they bite during non-peak hours or bite outside the house (Killeen et al., 2006). Current reports have documented the change in mosquito behavior whereby the mosquito vectors are now biting outside the house and during earlier hours/time than previously documented (Elliott, 1972; Russell et al., 2011; Sougoufara et al., 2014; Wamae et al., 2015). This therefore means those who like to stay outside in the night till very late or those who work mainly in the night are likely more predisposed to an increased number of bites which can lead to a disproportionately larger number of infections among those who practice these habits.

There were no significant differences in malaria related knowledge on the causative agent, mode of transmission, biting times and protection against malaria among those living in the fever hotspots and fever coldspots. Most studies on knowledge relating to malaria document a high level of knowledge regarding the causative organism though the respondents may not necessarily know the details of how transmission occurs (Hlongwana et al., 2009; Klein et al., 1995; Wakgari et al., 2003). Although the study does not show any major differences in the knowledge variables regarding

malaria, there was a significant difference in the knowledge relating to medications used for malaria between the fever hotspots and fever coldspots. This finding is supported by a study from Columbia by Forero et al. (2014) that found significant differences in knowledge regarding malaria medications as well as practice. Mwenesi et al. (1995) also found that mothers of children at Kilifi withdrew antimalarial treatment from children who developed complications such as convuslions because of lack of appropriate knowledge. To this end, given the fact that this study was conducted in a rural setting, this finding may not be surprising. Revisions to the the recommended first line treatment for malaria in Kenya have been made several times. It is however not clear how this information gets to the people at the grassroots and whether they are able to comprehend it in the manner in which it is communicated given that their literacy levels remain low. This may explain why there is a general confusion on which medications are used for malaria as first line treatment especially among the rural folk. This lack of clarity on which is the first line treatment for malaria means they can buy any of the other drugs such as choroquine or fansidar as long as they are available over the counter. It is important for the community to have the right information because knowledge on malaria prevention measures has been previously documented to influence the practice or uptake of malaria control measures (Erhun et al., 2005; Klein et al., 1995; Legesse et al., 2007; Vundule & Mharakurwa, 1996).

The study also found that there were no major differences in behavior such as time to go to sleep, where dinner is eaten and where people rest after dinner. Most of the people in both the fever hotspots and coldspots sleep between 9pm and 12pm which is the peak biting time for mosquitoes. Majority of the people also had their dinner early and mainly inside the house. This is probably because there are no actual physical boundaries between the fever hotspots and fever coldspots. The boundaries are not physical and people also share common cultural practices therefore making behavioural characteristics unlikely to be very different. There were no significant differences on

travel history between the fever hotspots and coldspots throughout the five rounds of data collection. This is again related to the fact that this is a predominantly rural setup and therefore there is less travel and generally movement compared to an urban setting. Travelling was therefore not associated with whether someone was in the fever hotspots or fever coldspots in any of the data collection rounds. It was also not associated with one having malaria or not. On the contrary, travel has previously been documented as a key risk factor for malaria especially in low transmission settings (Shanks et al., 2005). Travel especially to endemic regions (for those from non-endemic regions) may predispose individuals to malaria infections and hence those infected may travel back home with the disease incubating but later manifest with symptoms of malaria while in their own home area. This is basically imported malaria and may sometimes be mistaken for an original transmission episode in the area if history is not well taken. However, travel does not necessarily play any major role in increasing the number of malaria cases in endemic regions given that transmission is intense and always present in this population throughout the year unless in cases where travel is associated with sleeping outside the bednet like in the cases where they attend funerals and may have to spend the nights outside.

ITN use is also a behavioural practice which is mainly informed by appropriate knowledge and attitudes. Generally, a higher proportion of people living in the fever coldspots had slept under an ITN the previous night compared to those in the fever hotspots. The study found a significant association between the ITN use and region during the second (October) and fourth (April) data collection rounds. This might be associated with the use of ITNs related to mosquito abundance that become a nuisance especially during the rainy seasons (Shililu et al., 1998). After the rainy season, the mosquito numbers reduce and the nuisance also reduces, this may therefore lead to some people not sleeping under the bednets (Baume et al., 2009). The second visit was conducted in October, at this point there are likely to be fewer mosquitoes. This means that those people who normally get

prompted to use ITNs by the buzzing of mosquitoes may stop using the ITNs when they don't get this nuisance. The same scenario is reflected in the fourth visit which was carried out in April. The heavy rains begin around this time and therefore mosquito larvae are washed off by the running water. As a result of this, there will be fewer mosquitoes which also mean less buzzing and less nuisance until later in the month or the following months. Those who mainly protect themselves because of the nuisance produced by the noise from mosquitoes may therefore not use the ITNs on some of the days when they don't hear this noise. Appropriate and consitent use of ITNs has been extensively studied and documented by previous studies to protect individuals and families against mosquito bites and therefore malaria infections (Agusto et al., 2013; Alonso et al., 1993; D'Alessandro et al., 1995; Habluetzel et al., 1997). This finding is also supported by evidence from previous studies that have also shown seasonal variations in the use of ITNs recording a higher use of ITNs during the rainy season which also coincides with high mosquito abundance (Atieli et al., 2011; Baume et al., 2009; Frey et al., 2006). The finding may partly explain the differences in the incidence of malaria infections between the fever hotspots and coldspots although the possible difference in use of ITNs is only noted and recorded at two survey points. Malaria prevention behavior such as appropriate use of ITNs has been shown to influence prevalence of malaria in an area (Ingrid et al., 2009; Killeen et al., 2006; Sunday et al., 2014; Yvonne et al., 2007). Although there is 100% ownership of ITNs in this area, sleeping under a net or not remains largely an individual's decision which is affected by many factors. Differences in ITN use may therefore result in variations in malaria prevalence even on a small scale. When there is a group of households using ITNs well and another group of households not using them correctly, there is bound to be differences in malaria prevalence in the two groups. This may provide some answers to some of the differences noted between fever hotspots and coldspots within the HDSS. However, this interperetation is done with a lot of caution since there is no sufficient information beyond just an association and this is only present in the second and fourth rounds of data collection.

5.3 Malaria Burden (Incidence and Prevalence) in Fever Hotspots and Coldspots in Bungoma East County

Previous studies have reported that malaria transmission continues to become heterogeneous as malaria infections decline. Heterogeneity becomes even more pronounced in areas with low transmission (Cattani et al., 1986). However, there is scant information on heterogeneity of malaria in areas of high transmission. This research sought to determine whether there was a difference in the incidence of malaria between the fever hotspots and fever coldspots in a region with high transmission.

The study has shown that the burden of malaria in this region is still relatively high. The highest prevalence recorded in the fever hotspots during the five rounds was 35.7%. However, among the individual villages, the prevalence varied significantly and the highest prevalence recorded was in Sitabicha village at 45.7%. This prevalence is still much lower than the prevalence of 60% recently recorded in this area (Ministry of Health Kenya & Health Policy Project, 2015) or a much earlier previously recorded prevalence of upto 90% (Hamel et al., 2001). The highest prevalence of malaria in both the fever hotspots and coldspots was recorded in the month of July. This coincides with the main rainy season within this area. During this time, the main rains have just subsided and there is a lot of stagnant water that become the main mosquito breeding sites. This corroborates other reports that have shown that although malaria transmission in endemic areas may always be present throughout the year, it generally demonstrates seasonal variations with peaks mainly during the main rainy seasons (Baird et al., 2002; Fontenille et al., 1997; Hamad et al., 2002). This variation has mainly been attributed to the seasonal abundance of mosquitoes related to an increased number of breeding sites (Galardo et al., 2009; Shililu et al., 1998).

Although there was no statistically significant difference in the incidence of malaria between the fever hotspots and fever coldspots, there was a slightly higher risk of malaria infections among those living in fever hotspots compared to their counter parts in fever coldspots. There were significant micro-epidemiological differences in the incidence of malaria within villages. Generally, the entire larger HDSS area is referred to as a "hotspot" for malaria transmission and therefore the other hotspots are within the larger "hotspot". Our findings are supported by earlier reports that documented existence of differences in malaria transmission at very small scale such as households resulting in formation of clusters of higher than average transmission of malaria reffered to as "hotspots."(Greenwood, 1989; Ye et al., 2007). Therefore in this study, villages were the smallest hotspot units within the larger "hotspot". This concept has been described before by Bejon et al. (2014) who did a micro-epidemiological analysis of febrile malaria at the Kenyan Coast and found hotspots at the level of villages forming hotspots within hotspots.

The study showed a slightly increased risk for malaria infections among people living in the fever hotspots compared to those living in the fever coldspots. However, this did not reach statistically significant levels. This is probably brought about by the fact that one of the villages in the coldspot had consistently high prevalence of malaria throughout the year therefore contributing to actual increased risk of malaria in the fever coldspots as well. Studies have also shown that it is also possible to have a high prevalence of malaria in a coldspot because prolonged lack of exposure to the parasite removes the acquired immunity and therefore leads to frequent and severe infections (Doolan et al., 2009; Schofield & Mueller, 2006). However, this is a very unlikely explanation in this case given that the fever coldspots and hotspots are within the same homogeneous geographical region. A plausible explanation would be the fact that not all the fevers are caused by malaria (O'Meara et al., 2015; Rooth & Björkman, 1992), there are other infectious agents that could be responsible for the fevers (Dicko et al., 2005). The clustering of villages into fever hotspots and

coldspots was based on a spatial analysis of self-reported fevers from longitudinal follow ups of participants in the HDSS (O'Meara et al., 2014). Another possible and plausible explanation is that symptomatic/febrile hotspots keep changing and are not as stable as the asymptomatic hotspots. This is because when people have symptoms, they are likely to seek treatment and therefore this clears the parasite from the blood. This is in agreement with previous studies that have shown symptomatic hotspots as temporally unstable and may keep varying over time (Bejon et al., 2010; Bejon et al., 2014; Bousema et al., 2012).

A total of five quarterly visits for the parasitology surveys were conducted throughout the year. There was some form of consistency in the incidence and prevalence of malaria among some of the villages throughout the whole year. Two villages; Maruti in the fever coldspot and Kinesamo in the fever hotspot accounted for the highest incidence of malaria consistently through the visits (seasons) therefore becoming the actual true hotspots of malaria transmission within the HDSS while Lukhuna village had consistently the lowest incidence of malaria throughout the year making it the true coldspot within the HDSS. Among the fever hotspot villages, Kinseamo, Sitabicha and Wakulinda villages consistently had high prevalence of malaria throughout the year. In the fever coldspot villages, Lukhuna village had the lowest prevalence of malaria throughout the year. However, Nangili Village had the lowest prevalence of malaria among the hotspots while Maruti village in the fever coldspot had very high prevalence of malaria.

The differences in the incidence of malaria observed at village level form a basis for targeting of interventions on clusters to increase efficacy of the interventions (Bejon et al., 2014).

5.4: Asymptomatic Malaria Infections in Fever Hotspots and Coldspots

This study also sought to determine if there were differences in the proportion of those with malaria infection who do not present with any symptoms (asymptomatic) and the risk factors for asymptomatic status between the fever hotspots and the fever coldspots. This is important since

asymptomatic individuals remain infectious for a long time yet they do not present with any symptoms and therefore are not likely to get treatment. They become a major silent reservoir for malaria parasites. A high proportion of asymptomatic cases in an area could mean that this group of people does not get treatment and stays with the infection for long periods hence causing persistence of malaria transmission and hence infections in that area. This in turn will continue to hamper the elimination efforts using the current malaria control tools.

Among those who were tested for malaria infections and turned out positive, almost half of these were asymptomatic cases. This means that if they had not been tested actively through the study, these particular infections would not have been picked up. The number of asymptomatic infections could be much higher than what our study found because we used RDT for testing. Although the RDT sensivitity has been shown to be very high (Katharine et al., 2011), its not comparable to PCR (Johnston et al., 2006) especially for the subpatent infections. PCR has a sensitivity of 100% and has been documented as the most effective method for detecting low density parasitemia as well as mixed infections (Laban et al., 2015). Therefore, there is likelihood that the very low density parasitemia might not have been detected by the RDT test hence giving a possibility of higher undetected asymptomatic cases in this area.

Overall, the highest proportion of asymptomatic infections were found in the fever hotspots compared to the fever coldspots although there was no statistically significant difference between these two regions.

From this study, we can therefore deduce that the prevalence of asymptomatic infections remains very high. However, this is still lower than what was previously documented in this area of upto 93% of malaria infections presenting asymptomatically (Hamel et al., 2001). Given that there is no active surveillance within the HDSS in Bungoma East Sub-County, many people in this area are

infected but remain untreated for long periods of time hence maintaining the reservoir within this population. Although hospital records can be used to infer the prevalence rate of malaria in this community, the group of infected asymptomatic individuals cannot be captured by the hospital records since they do not present to the health facilities for diagnosis and treatment. The presence of high asymptomatic cases in endemic regions is a common observation documented by other studies (Bottius et al., 1996; Chen et al., 2016). Previous reports have attributed this to the fact that those living in endemic regions get frequent mosquito bites and therefore this frequent exposure to malaria parasites leads to development of partial immunity (Doolan et al., 2009). This partial immunity suppresses the severity of infections thus individuals do not present with symptoms (Lindblade et al., 2013). Since asymptomatic individuals do not manifest with any symptoms, they do not seek treatment and therefore remain as actual parasite reservoirs who continue to transmit the infection to the general population (Lindblade et al., 2013). Asymptomatic individuals remain a big threat to malaria control and malaria elimination efforts in this area and Kenya in particular. Studies have shown that asymptomatic individuals still carry gametocytes and therefore can transmit gametes to the mosquitoes (Alves et al., 2005; Bousema et al., 2004; Karl et al., 2011). Besides gametocyte carriage, the asymptomatic individual may suffer other complications related to malaria such as anemia as the parasite continues to destroy red blood cells (Maina et al., 2010; Newton et al., 1997). Indeed, some studies even advocate for testing of malaria in any individual with a derangement in hematological indicators since they could also be a pointer to chronic infection with malaria (Bottius et al., 1996; Maina et al., 2010).

Besides the mosquito conducive environmental factors, asymptomatic infections may explain why malaria transmission remains persistent in this region and why there are differences in the general incidence of malaria between the fever hotspots and coldspots. This evidence is supported by previous reports which attributed persistence of malaria in some of the regions to the existence of the

low density parasitemia asymptomatic cases which do not get treated because the infected individuals do not show any symptoms (Bottius et al., 1996; Das et al., 2015). This is because asymptomatic infections act as the "silent" reservoirs and continue to fuel transmission of malaria infections without being cleared. It still remains unclear how long the asymptomatic parasitemia lasts before clearing from the blood without treatment, although some studies average this to about 70-90 days in children above 10 years and adults but the mean is much higher (179 days) among younger children (Bretscher et al., 2015). Given that they do not present with any symptoms and do not feel sick, it may not be possible for such individuals to seek treatment and therefore remain the reservoirs of the malaria parasite for a long time. This implies that asymptomatic individuals may remain infectious to the mosquitoes for very long periods (Agusto et al., 2013; Bottius et al., 1996; Bretscher et al., 2015) therefore prolonging the infections in the community which is a major threat to the malaria control measures.

This study also explored factors associated with asymptomatic parasitemia in this population. Similar to what was found for malaria infections; generally there were micro-epidemiological differences in asymptomatic parasitemia at village level. The village where one lives is a significant predictor of asymptomatic status when an individual gets infected with malaria. People living in Lukhuna village (which is one of the villages in the coldspot) are the least likely to be asymptomatic when they get malaria parasitemia. These small scale differences in malaria transmission have been reported before (Bejon et al., 2014; Greenwood, 1989). Most malaria infections in this coldspot village manifested with symptoms. Individuals who manifest with symptoms are more likely to seek treatment for the infection and therefore clear the parasites and cease to be the parasite reservoir. This could partly explain why this village is a coldspot for malaria within the HDSS. On the contrary, in Maruti village which was initially classified as a fever coldspot, individuals are more than twice likely to be asymptomatic when they get malaria infection. This may have come about by

the fact that the initial classification of villages into cold and hotspots was based on self reported fevers. As alluded to earlier in the discussion, fevers may signify other illnesses and not necessarily malaria (O'Meara et al., 2015). Some authors have even argued that the use of fevers in estimation of clinical malaria may result in an over-estimation of clinical cases (Rooth & Björkman, 1992). Another plausible and supported explanation by previous reports is that symptomatic hotspots are quite unstable and may keep shifting depending on various factors (Bejon et al., 2010; Bejon et al., 2014; Bousema et al., 2010; Ernst et al., 2006). Arguments against the need to target interventions to hotspots have mostly cited the changing/shifting nature of hotspots especially the febrile/symptomatic hotspots.

Generally, most of those who had asymptomatic parasitemia were more likely to be from villages classified as fever hotspots. Studies have shown that people living in the hotspots for malaria transmission have a much higher tendency to be asymptomatic reservoirs of the parasite (Bottius et al., 1996; Bretscher et al., 2015). This can be explained by the fact that those in areas of high transmission tend to develop some partial immunity due to repeated exposures to the malaria parasite and this shields them from developing clinical symptoms when they get infected. This has been demonstrated by previous studies (Doolan et al., 2009). Nevertheless, there is evidence that the natural immunity acquired from the repeated exposures does not necessarily shorten the length of these asymptomatic infections (Bretscher et al., 2015) and therefore the infections may become chronic (Bottius et al., 1996). These persistent infections are detrimental to the affected individual posing serious health challenges through chronic low-grade hemolysis which leads to anemia as well as cognitive impairment in school going children (Chen et al., 2016; Newton et al., 1997). The implications on malaria control programs are enormous. The presence of high prevalence of asymptomatic infections threatens to reverse the gains already made and documented. Effective control programmes therefore need to reach everyone at risk inorder to reduce the prevalence.

Notwithstanding, people with no symptoms will definitely not seek treatment even with the best treatment guidelines and subsidized effective drugs yet they remain infectious. The greater challenge is tracking down all infected people and offering treatment.

The study also found that the age of an individual is a significant predictor of asymptomatic parasitemia. The younger children (below 5 years) have a much higher tendency to develop symptoms while those between 6 to 10 years are more likely to remain asymptomatic when they get malaria infection. Similar evidence has been reported elsewhere (Riley et al., 2001). Infants (0-6months) are mostly asymptomatic when they get infected by malaria parasites because of the acquired maternal antibodies (Snow et al., 1998). However, this passive immunity from the mother begins to wane after six months and the infant exposed to the parasite may begin to get very severe malaria infections (Sehgal et al., 1989). Older infants and young children in holoendemic regions become more prone to manifestation of clinical disease after a parasite infection because they don't have immunity against the parasite (Baird, 1995; Sehgal et al., 1989). However, these repeated exposures to the malaria parasite enables them to acquire immunity specific to the parasite (Bruce et al., 2000; Doolan et al., 2009; Eli et al., 2001; Ladeia-Andrade et al., 2009) making the children semi-immune to the parasite and therefore less prone to the severe attacks. The semi-immune status makes them less prone to development of clinical symptoms whenever they get infected with the malaria parasite. Unfortunately, the acquired immuntiy against malaria is partial and not life long (Doolan et al., 2009). When semi immune individuals move out of an endemic area, the exposure reduces and therefore they begin to lose their immunity (Doolan et al., 2009) and if taken back to an endemic region, they are likely to develop severe malaria. Contrary to previous studies that have indicated that older people living in malaria endemic regions are less likely to develop malaria (Ladeia-Andrade et al., 2009) and when they do, they are not likely to present with symptoms, our results present an opposite picture. Similar to the younger children below five years, older adults

infected with malaria in this cohort were more likely to present with symptoms. This is an interesting finding that may form a basis for further research on what changes take place in the partial immunity for malaria as one grows older. Some reports have shown greater vulnerability to malaria by older adults, however these studies have mainly been conducted among the non-immune populations especially the tourists (Stich et al., 2003). Age is therefore a major predictor for asymptomatic disease. Our findings are corroborated by those of other studies that have shown age as one main predictor of whether one develops symptoms or not (Eli et al., 2001; Frederick et al., 2009; Ladeia-Andrade et al., 2009).

These findings have implications on malaria control in a high transmission region. The younger children present with symptoms when infected and therefore are taken for treatment while older children and adults likely remain asymptomatic carriers of the parasite and therefore keep re-infecting the children in their households. Since the older children don't present with symptoms when infected, they may suffer debilitating effects of chronic infection such as chronic anemia, increased co-bacterial infections and impaired cognition that may lead to low performance in school among others (Chen et al., 2016).

This study has shown that asymptomatic status is associated with the season. We recorded the least proportion of asymptomatic infections during the dry season in January and the highest during the wet season. This is probably because generally there is a reduction in the number of malaria infections during the dry season. Some of the previous reports conducted in similar endemic settings have reported similar findings (Dhiman et al., 2015). In another study conducted in Cameroon by Kimbi et al. (2005), they attributed the lack of seasonal differences in asymptomatic cases on the fact that the area had perennial transmission. On the contrary, these observations contradict what is generally documented by most studies. Asymptomatic infections are usually highest during the dry season mainly because of the reduced incidence and density of the parasites at this time (Geiger et al., 2013). In many endemic regions, malaria transmission is seasonal peaking during the rainy

season. This is probably because mosquito breeding is at its highest immediately after the long rains due to availabilty of many breeding sites (Binka et al., 1994; Briet et al., 2008; Galardo et al., 2009; Hamad et al., 2002; Singh & Sharma, 2002). The asymptomatic cases form the epicenter of transmission during this high transmission season. Mosquitoes bite the infected asymptomatic individuals and flare up the infection by passing it on to others (Das et al., 2015). Given that malaria infections during the rainy season are more likely to have high density parasitemia as compared to the dry season, this can possibly explain why majority of those infected during the rainy season manifest with symptoms (Odongo-Aginya et al., 2005; Ouedraogo et al., 2013).

During the rainy season, there was a higher proportion of symptomatic disease in the fever hotspots than the fever coldspots while during the dry seasons; there were more symptoms in the fever coldspots than the fever hotspots. This is in agreement with a study conducted in Mali that showed an increase in fevers during the rainy seasons (Dicko et al., 2005). This is probably because the asymptomatic individuals in the hotspots act as reservoirs of the parasite and fuel new infections during the rainy seasons. This finding is supported by evidence from a study conducted by Bousema et al. (2010). It is therefore important to identify hotspots of asymptomatic infections because they form foci of transmission where individuals with asymptomatic disease act as the main reservoirs of the parasite during the dry season and fuel new infections during the rainy seasons which spread to the neighbouring areas (Bousema et al., 2012; Bousema et al., 2014; Das et al., 2015).

5.5 Risk Factors for Malaria Infections in Fever Hotspots and Fever Coldspots

The study also sought to identify risk factors for heterogeneity of malaria in a geographically homogeneous area within Western Kenya. Although the study has shown a slightly increased risk of malaria infection among people living in fever hotspots compared to those in fever coldspots, this difference does not reach statistical significance. Therefore region clusters based on fever (fever coldspot or fever hotspot) is not a significant risk factor for malaria in this region. This is probably brought about by the fact that the regions were clustered around self reported fevers which is

presumed to be the main symptom for malaria and therefore a sign of clinical disease. A study carried out in western Kenya showed that there was misdiagnosis and therefore wrongful treatment for malaria based on the presumption that fevers represented malaria (O'Meara et al., 2015). For a long time, diagnosis of clinical malaria has been based on presence of fever/febrile illness and based on this recommendations for presumptive treatment made. Current reports indicate that diagnosis of clinical malaria based on fevers may generally lead to overdiagnosis of malaria and therefore unnecessary administration of malaria treatment to those who don't need it hence increasing chances of resisitance (O'Meara et al., 2015; Rooth & Björkman, 1992; Roucher et al., 2012). They argue that diagnosis of malaria should be based on actual testing of malaria parasites using the various available testing tools even in the low transmission regions to eliminate the over exegeration of malaria cases based on fevers. It is also possible that since the villages are porous, people interact on a day to day basis and therefore those in the hotspots keep re-introducing the infection to their counterparts in the coldspots. Another possible and plausible explanation for the lack of statistically significant differences in the incidence of malaria between the fever hotspot and fever coldspot is the fact that symptomatic hotspots are not stable, they keep changing with time. This is supported by other studies that have shown that symptomatic hotspots are actually not stable over time (Bejon et al., 2010; Ernst et al., 2006). In this study, some of the fever hotspots such as Kinesamo, Sitabicha and Wakulinda villages were stable in terms of consistent transmission of malaria all year round while one coldspot village remained consistently a cold spot all year through.

Age is a significant risk factor for malaria infection in both the fever hotspots and fever coldspots. The younger children (below) five years are more likely to be infected by the parasite than the older children and adults. The main explanation for this lies with the fact that there is acquisition of natural immunity after repeated exposures. Young children have not yet developed immunity against the paraite and therefore get infected with every exposure to an infectious mosquito bite (Baird,

1995; Bruce et al., 2000; Carneiro et al., 2010). Children under five years are the most vulnerable group and they tend to get particularly very severe disease that may progress to death (Eli et al., 2001; Okiro et al., 2009). This forms the basis for the current WHO recommendations that children under five years should be well protected from mosquito bites by sleeping under long lasting insecticidal nets (LLINs) as well as intermittent preventive therapy for infants (IPTi) (World Health Organization, 2015). Although this kind of argument is true, the risk is that children get re-infected by malaria parasites repeatedly because the reservoir is mainly the asymptomatic adults who remain untreated because they do not present with overt symptoms to propel them to seek treatment.

Consistent use of the ITNs by household members is protective against the parasite and the opposite is also true. In this study, members who lived in a household where they never used ITNs at all were more than twice likely to get infected compared to those who used a bed net always. Although we have had few studies indicating a shift in the biting behavior of mosquitoes, generally most of the feeding happens at night (Elliott, 1972; Ferguson et al., 2010; Shililu et al., 1998; Wamae et al., 2015) and therefore the effective use of the current control methods reduces the chances of infection among household members. This finding is supported by an overwhelming body of research evidence that has shown great reduction in malaria infections where ITNs have been used appropriately (Agusto et al., 2013; Alonso et al., 1993; Atieli et al., 2011; Habluetzel et al., 1997; Okiro et al., 2007) as well as a reduction in malaria vectors (Bayoh et al., 2010). Individuals who go to bed without a bed net remain at greater risk of getting a mosquito bite that may result in transmission of the parasite if the mosquito is already infected. Infact, the higher the number of people using ITNs within the household, the more likely that people in the household are protected and this extends even to the neighbours conferring herd immunity when a larger group of households are actually using these ITNs (Alonso et al., 1993; Hawley et al., 2003) .

Although not reflected by the results from this study, the current change in mosquito biting times that has been documented in some of the studies may present a major challenge to this kind of malaria control since malaria mosquitoes have began biting during the day and even bite earlier than the usual times when people are sleeping for example at dusk (Reddy et al., 2011; Russell et al., 2011; Sougoufara et al., 2014).

The study found that people living in houses with open eaves are more likely to get infected with malaria compared to their counterparts in houses that do not have open eaves. Open eaves are open spaces between the wall and the roof. These open spaces allow free access of mosquitoes into the houses even during the night hence increasing the chances of man and malaria vector contact (Lindsay & Snow, 1988; Ye et al., 2006). The increased contact with the mosquitoes results in an increase in the number of bites and hence increased chances of getting malaria infection. Housing design has been previously documented as a major risk factor for malaria infection (Gamage-Mendis et al., 1991; Lindsay & Snow, 1988). Unfortunately, for most rural areas within Western province, most people live below poverty line and are not able to afford expensive construction materials. Therefore most houses are made of mud walls and are grass thatched. As part of designing these houses, a space is left between the wall and the roof because the grass thatch cannot appose to the wall. For houses roofed with iron sheets, some have this space as well. This is therefore likely the main construction design in this area. This kind of spaces allow for mosquitoes to enter the house any time and especially at night when they are looking for a blood meal therefore allowing for the man vector contact. Although this study does not show roofing as a significant risk factor for malaria, other studies have shown the use of iron sheets to have a protective effect against malaria (Ye et al., 2006).

Living in a cemented wall house is protective against malaria infection compared to a mud walled house although not of statistical significance. The mud walled houses are likely to have cleaves which form good resting places for the mosquitoes. On the other hand, cemented houses smoothen out the walls and there are no spaces to allow the mosquitoes to rest. These findings are corroborated by other studies that have shown that risk of malaria increases with the design of housing and housing materials used for construction (Gamage-Mendis et al., 1991; Kirby et al., 2008). Previous reports have also indicated that modification of housing designs especially in the rural areas would reduce malaria infections quite significantly (Kirby et al., 2008; Lindsay et al., 2002). Previous studies have associated housing design with increased chances of getting malaria infection (Brooker et al., 2004; Kirby et al., 2008; Lindsay & Snow, 1988; Ye et al., 2006). Nonetheless, it remains unclear why current malaria interventions have not been targeted towards housing modifications in the rural areas despite the available evidence. Although most of the people living in rural areas are generally poor and may not be able to afford decent housing because of cost, there are cheap and simple ways of modifying a mud house to reduce the cleaves which act as hiding places for mosquitoes (Brooker et al., 2004). There is need to communicate with the community on simple ways of housing modification that will make their houses safer from these mosquitoes.

5.6 Mosquito Characteristics and Malaria infections in Fever Hotspots and Coldspots.

The study found an increased proportion of larval breeding sites in most of the villages within the fever hotspots compared to the fever coldspots. Presence of these larval sites/mosquito breeding sites remains a significant risk factor for malaria in this area. Living close to breeding sites has previously been shown to be a significant risk factor for malaria (Subramanian et al., 1991). Individuals living in villages such as Kinesamo village (fever hotspot) which had the highest number of breeding sites were more likely to have malaria infection compared to those in villages such as Lukhuna which had lower number of breeding sites. The higher the number of breeding sites, the more the number of mosquitoes and therefore increased contact/bites and exposure which leads to

infection. This is also evidenced in our study by the fact that the highest collections of mosquitoes were done in Kinesamo village (fever hotspot) and the lowest number in Lukhuna village (fever coldspot). The study also showed the highest incidence of malaria infections in Kinesamo village which correlates with the high number of mosquito counts as well as the lowest incidence of malaria in Lukhuna village which also correlates with lower densities/counts of mosquitoes.

The clustering of infections around mosquito breeding sites explains the formation of 'hotspots' of intense transmission. Households closest to the breeding sites are more likely to have high densities of mosquitoes. These findings concur with other studies that have shown environmental conditions being the main risk factors for variation of malaria risk as well as spatial heterogeneity in malaria transmission. Clusters of higher than average transmission of malaria form closest to the breeding sites (Jane et al., 2011; Staedke et al., 2003; Subramanian et al., 1991). Studies have also shown that significant differences in transmission of malaria occur even when breeding sites exist within very short distances (Clarke et al., 2002). This is reflected in this study since the highest incidence of malaria is recorded in the same villages with the highest counts/densities of mosquitoes. On the other hand, some authors argue that although the general and well accepted view is that higher densities of mosquitoes correlate with higher prevalence of malaria, this may not necessarily be the case. There are areas with high densities of mosquitoes which do not ncecessarily correlate with higher levels of malaria infections (Clarke et al., 2002; Meyrowitsch et al., 2011). Instead some areas with lower densities of mosquitoes have documented greater risk of malaria infection (Clarke et al., 2002). Both arguments notwithstanding, there is an overwhelming body of evidence in support of environmental factors playing a key role in malaria transmission (Castro et al., 2009; Ferguson et al., 2010; Jane et al., 2011; Obala et al., 2015; Staedke et al., 2003). Based on this, some studies have suggested that implementing simple interventions measures targeting the environment such as larviciding may reduce malaria infections significantly (Castro et al., 2009).

Almost 95% of all the mosquitoes collected in this study were done using the Pyrethrum Spray Catches method (PSC). The Window Exit Traps (WET) collected very few mosquitoes. This finding is supported by current studies on mosquito surveillance that have shown that the number/density of mosquitoes collected is largely influenced by the method of mosquito collection (Ndiath et al., 2011). Mosquito collection studies have also shown that the Window Exit Trap is the least sensitive mosquito collection method compared to most of the other mosquitoe collection methods (Govella et al., 2011; Ndiath et al., 2011). Although this study did not set out to investigate the best method for monitoring mosquistoes, the PSC was able to collect almost all the mosquitoes during the entire period of time. Additionally, it was noted that mosquitoes collected by WET were more likely to be gravid compared to those captured by PSC. This is mainly because mosquitoes captured by WET are likely those that are endophagic and exophilic- leave the houses where they fed to seek resting places outdoors. These will be captured when bloodfed. Another possible explanation could be that these mosquitoes are endophillic and rested indoors to digest blood meal and develop eggs. These will exit the houses as fully gravid; seeking ovisposition sites outside hence will be captured by WET as gravid mosquitoes.

The greatest number of anopheline mosquitoes collected during our study was from the An. *gambiae* complex. This has been documented as the main species in sub-saharan Africa (Coetzee et al., 2000; White, 1974). The main sibling species was An. *gambiae ss* while An. *arabiensis* comprised slighltly less than a quarter of the total.

An. *gambie ss* is mainly an obligate endophillic, endophagic and anthropophagic vector biting its victims mainly late night until morning which coincides mainly with the time when individuals are already sleeping. Most of the current malaria control measures such as ITNs target this property of the vector (Lindsay et al., 1991; White, 1974). This vector has been documented as the most common vector responsible for malaria transmission in most parts of Africa (Antonio-Nkondjio et al., 2012; Pinto et al., 2000; White, 1974). In Kenya, studies conducted in Western Kenya showed

An. *gambie ss* as the primary vector responsible for malaria transmission in this region (Shanks et al., 2005; Shililu et al., 1998). A study conducted in Kenya by Wamae et al. (2015) reports changing vector biting behavior somewhat to early hours of the night and early morning, although most biting still occurred in the late night when people are in bed. However, studies conducted elsewhere depict a different picture documenting high levels of outdoor biting by anopheles senso stricto attributed to the likely evasion of the current indoor based anti-vector measures such as increased use of ITNs (Reddy et al., 2011; Russell et al., 2011). On the contrary, some of the recent studies indicate that mosquito behavior for An. *gambie s.s* in Western Kenya and generally most parts of Africa has largely remained the same, mostly biting late at night when people have gone to bed (Bayoh et al., 2014; Kabbale et al., 2013; Shililu et al., 1998). This observation affirms that the use of current malaria interventions to target this vector as still largely viable.

On the other hand, An. *arabiensis* which is part of the An. *gambie complex*, in this study comprised less than one quarter of the Anopheles gambie complex collected. The vector is mainly exophyillic, exophagic and is mostly zoophagic but in the absence of livestock, it may feed on human blood. Recent studies indicate that this vector is becoming more prevalent (Lwetoijera et al., 2014; Onyabe & Conn, 2001; Oyewole et al., 2007) and has also shown resistance to the common pyrethroids used in controlling malaria (Jones et al., 2012). This poses a threat to the malaria control measures since this vector bites and rests in outdoor habitats which make it difficult to use the current malaria control measures. However, this study showed that the prevalence of An. *arabiensis* although low but is slightly higher than what is documented in a previous entomology study conducted in Western Kenya by Shililu et al. (1998).

These findings provide evidence that the predominant vector for malaria is the one targeted by the current malaria control measures. Current malaria control measures are therefore essentially still viable for this region for the control of malaria, although numerous challenges still abound. Recent

reports have indicated that the An. *gambie ss* mosquitoes are changing their biting behavior to early dusk (before people go to bed) or very early morning (when people wake up) (Wamae et al., 2015). Several other studies are currently reporting mosquitoes feeding outdoors (Russell et al., 2011; Sougoufara et al., 2014). In addition, there are reports of mosquitoes resistance to the chemicals used to impregnate ITNs (Badolo et al., 2012; Nanaan et al., 2006; Obala et al., 2015; Ranson et al., 2011; Trape et al., 2011).

This study found a very low sporozoite rate (1%). Some of the likely reasons could be the fact that fewer mosquitoes were analysed and also possibly poor preservation of the samples before transportation to the laboratories for processing. However, it is also possible that there were no problems and this was the actual sporozoite rates of the area. Some of the explanations for this observation could be that most of the mosquitoes collected were new and young mosquitoes and therefore are unlikely to have sporozoites. Mostly the older mosquitoes have sporozoites. Recent studies have shown similar findings where there is a high prevalence of malaria but low sporozoite rates (Manyi et al., 2014). Previously most studies correlated a high prevalence of malaria with a high sporozoite rate (Shililu et al., 1998; Thomson et al., 1994) and low sporozoite rates corresponding to low prevalence of malaria infections (Kouassi et al., 2016). A recent study indicated that An. *fenestus* is re-emerging in the western Kenya and therefore could be the current drivers of the infection in Western Kenya (McCann et al., 2014).

The highest collection of mosquitoes was immediately after the long rains. This can be explained by the fact that during the main rainy season, the heavy rains wash away larvae but immediately after the long rains, there is an increase in stagnant water which forms the larvae breeding habitats. Similar findings have been posted by previous reports (Bogh et al., 2003; Shililu et al., 1998).

5.7 Study Limitations

- The hotspots and coldspots were based on spatial clusters of self reported fevers hence the geographical dermacations are basically villages. The villages remain quite porous in terms of social relations.
- In this study, mosquitoes were collected indoors, with the assumption that most bites occur indoors. However, some studies have clearly shown that mosquitoes are increasingly biting outdoors (Russell et al., 2011).
- Treatment of all malaria positive individuals is likely to interfere with the number of malaria episodes an individual is likely to get because transmission is lowered (Lucy et al., 2008; Shah et al., 2013). The other option would be to test them and only provide the results at the end of the study. This option though likely to give the true picture of the incidence but would have been unethical.
- Lastly, many studies conducted on prevalence and incidence of malaria mainly focus on children who are usually more susceptible to malaria than adults (Jensen et al., 2009; Mwangangi et al., 2013; Nahum et al., 2010). However, in this study we tested all age groups.

6.0 CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1: Conclusions

6.1.1 Knowledge and Behaviour Related to malaria

- The general level of knowledge regarding malaria was quite high in both fever hotspots and coldspots. However, there are some misconceptions especially regarding transmission of malaria with some people attributing the disease to dirty water.
- Knowledge regarding the first line of treatment for malaria was low and is associated with whether one is in the fever hotspot or coldspot.
- The practice of using ITNs for protection against malaria was also associated with whether someone is in the fever hotspot or coldspot during some seasons of the year especially the dry season.
- Travel does not contribute to the number of malaria infections in this region.

6.1.2 Burden of Malaria in Bungoma East Sub-County

- Generally the burden (prevalence and incidence) of malaria in this region is still very high. Highest prevalence of malaria in the fever hotspots was 35.7%.
- Children below five years and older adults (above 50 years) were more likely to be affected by malaria than other age groups.
- People living in fever hotspots have almost one and half times increased risk of developing malaria infection compared to their counterparts in the fever cold spots.
- There was marked and statistically significant heterogeneity in malaria risk across the villages within the HDSS in Bungoma East County. Living in Kinesamo village (fever hotspot village) increases the risk for one to get malaria infection while living in Lukhuna village (fever coldspot village) decreases the risk of getting infected.

6.1.3 Asymptomatic Infections

- Almost half of all the people infected with malaria do not have any symptoms which is a great danger given that they do not seek treatment since they do not feel sick. However, they remain infectious to the mosquitoes and therefore act as “silent” reservoirs.
- There was a higher proportion of asymptomatic infections in the fever hotspots compared to the fever coldspots. However, there was no statistically significant difference in asymptomatic infections among the villages.
- The school going children (6-14) were more likely to have malaria infection and yet not present with any symptom which makes them likely vulnerable to the negative effects of chronic malaria such as cognitive impairment, anemia, repeated bacterial infections among others.
- A higher proportion of the total malaria infections during the dry season are mainly symptomatic infections.

6.1.4. Risk Factors for Malaria in fever Hotspots and Coldspots

- Risk factors for malaria in both the fever hotspots and coldspots include; age, presence of open eaves, wall material and ITN use. These were not significantly different between the fever hotspots and coldspots except for ITN use which only differs in the second and fourth round of data collection but overally, there are no differences.
- There is a significantly high proportion of breeding sites/larval sites in fever hotspots compared to the fever coldspots.
- People living in the fever hotspots have atleast a one and half times increased risk for developing malaria compared to those in the fever coldspots.
- There is a significant difference in the incidence of malaria infections by village correlating to entomological indices.

6.1.5 Contribution to New Knowledge

- The study has shown that malaria infections in Bungoma East Sub-County are significantly spatially heterogeneous at a very small scale (village level) and this correlates with entomological risk factors such as the high proportion of breeding sites and high density of mosquitoes. These could likely explain the persistence of infections and malaria transmission in this area hence the need for targeted control based on the presenting local situation. Previous studies on heterogeneity of malaria infections have mainly concentrated in the low transmission regions. This study therefore adds to the body of knowledge regarding heterogeneity of malaria risk and transmission in high transmission regions.

- Although previous studies have recorded a relationship between malaria infections and malaria behavior including ITN use, this has been done on a large scale. Micro-epidemilogical studies have not found the same. This study therefore brings out the fact that micro-epidemiological differences in malaria infections could actually result from local differences in behavior related to the use of ITNs as well. This is indeed a significant contribution to knowledge in the area of micro-epidemiology of malaria.

- In Kenya, statistics for prevalence of malaria infections are largely drawn from facility data. However, this study has shown that there is a high likelihood that the figures provided on malaria prevalence through the facility data may not be necessarily correct. In this study, nearly half of all those infected by malaria were asymptomatic. This implies that these asymptomatic carriers remain in the community and maybe a continuous source of parasite transmission to the mosquitoes and then man. This could partly explain the persistence of malaria transmission and infections within this area. In addition, we found the highest proportion of asymptomatically infected individuals among the 6 -14 years age group. This

is a unique finding given that most of the previous studies have not recorded such findings. It may form a basis for further research in trying to understand why this group is asymptomatic. Finally, the study has also shown that asymptomatic infections display spatial heterogeneity even on a small scale. This is also not a commonly reported finding elsewhere.

6.2 Recommendations to the Stakeholders

6.2.1 The Webuye HDSS/Bungoma East County

- There is need to hold health education campaigns through and by the public health department. The campigns should focus on providing simple knowledge on malaria including the treatment options as well as proper and consistent use of ITNs throughout the year irrespective of the season. Developing culture appropriate IEC materials on the first line treatment for malaria may be useful and create easier understanding.
- The presence or existence of community units in this area provides an opportunity for training of community health workers on malaria prevention and treatment in order to enable them pass the correct information to the local people. This will ensure self sustainability.
- Health information that focuses on basic environmental larval site management to reduce larval breeding sites such as habitat modification, habitat manipulation, larviciding and biological control should be taught to communities by the Community Health Extension Workers and the community health workers.
- Almost 50% of all the ITNs are currently torn; there is need for replacement campaigns for the torn and worn out ITNs to be done on or before the end of every five years.
- Although the community is generally poor and they may not necessarily afford very good housing, some modifications in their housing structures may reduce the entry of mosquitoes through eaves. The community can be taught to explore methods of sealing eaves as well as smoothening of mud walls inorder to remove crevices where mosquitoes hide. Open eaves

provide a free access for mosquitoes to get into the house while the crevices in the mud wall provide the best resting places. Simple housing modifications using the available and cheapest raw materials that are easily accessible by the community can be embraced such as sealing the eaves with fine wiremesh that will still allow ventilation but no mosquitoes.

6.2.2 The National Malaria Control Programme (NMCP)

- Put up active surveillance programs in areas of high endemicity to allow for detection of asymptomatic cases and put them on treatment. This will considerably reduce the reservoir and therefore reduce the burden of malaria in these areas.
- Once the active sureveillance programs have been put in place, they are likely to pick up areas with higher than average transmission "hotspots" and therefore provide an opportunity for targeting those areas.
- Although microscopy remains the gold standard for testing of malaria infections, as we head towards elimination, there is need to delve into newer testing technologies such as PCR to enable detection of the asymptomatic and sub-patent infections that are very common in endemic regions.
- In areas where active surveillance of malaria is ongoing because of the HDSSes, there is need for support in case of the need for additional interventions. For example in the Webuye HDSS, villages within the hotspots may require extra interventions.
- Target the hotspots of malaria transmission with additional tools for malaria control such as larviciding to eliminate the extra breeding sites.
- Develop IEC materials on malaria control and medications that are appropriate for the less literate group and mainly rural population for them to easily understand the first line of treatment.

6.2.3 The Ministry of Health

- Although health in Kenya has been devolved, the ministry of Health still has a major role to play inorder to ensure there is active malaria surveillance in endemic malaria regions. This can be done through various methods at their disposal including lobbing the County Governments in the affected areas to allocate more funding for active surveillance and strengthening the community strategy.

6.2.4 Areas for Further Research

This study provides an opportunity to build on the current research findings and conduct further research on the following areas;

- Explore further the malaria related behavioral practices and how these impact on malaria infections within the area. This will likely be a mixed methods approach with both quantitative and qualitative data collection. The qualitative component will allow for a complete community enagagement process as well as augment and explain away the quantitative results. Thereafter, there is need to set up an intervention on behavior modification and assess its impact in further reduction of malaria cases in this area.

- Further research on asymptomatic malaria cases. In this study, the highest proportion of asymptomatically infected individuals was 6 -14 years. There is need to follow up this age group to understand why they don’t present with symptoms.

- Use of blood spots and mosquito collections to understand better the population dynamics of malaria in this region & identify the reservoir of infection. There is need to understand who in the population gets more affected by the malaria parasites and from whom these parasites came from,whether the parasites from members of the same household are genetically identical and if they were bitten by the same or different mosquitoes. This can only be done through genetic analysis.

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APPENDIX 1A): CONSENT STATEMENT (ENGLISH)

MOI TEACHING AND REFERRAL HOSPITAL/MOI UNIVERSITY

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

UNIVERSITY OF NAIROBI/KENYATTA HOSPITAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

INFORMED CONSENT STATEMENT FOR

Parasitological Surveillance of Malaria in Bungoma East District

INTRODUCTION:

You are being asked to provide consent for your household to participate in this research study that is designed to find out the incidence of malaria in Bungoma East district. I'm conducting this study as a sub-study within the larger study that we have been conducting on malaria infections and bednet use. This consent form gives you information about the study. The research staff will talk with you about this information. You are free to ask questions about this study at any time. If you agree for your household to take part in this study, you will be asked to sign this consent form. You will get a copy to keep. Before you decide if your family should be a part of this study, we want you to know all about it.

STUDY PURPOSE:

The purpose of this study is to find out how many times individuals in this area get infected with malaria within one year and whether this has something to do with the current change of mosquito characteristics. We will try to find out if mosquitoes have been changing and which specific mosquitoes are infecting people in this area.

Please note that:

It is entirely your choice whether or not you want your household to participate in this study.

You may stop taking part in the study at any time.

NUMBER OF PEOPLE TAKING PART IN THE STUDY:

If you agree to participate, your household will be one of about 80 households that will take part in this study, all from Bungomaa East district. All the household members in the selected households will be requested to take part in the study unless they refuse.

PROCEDURE FOR THE STUDY:

You have been asked to participate in this study because your household is already participating in the mosquito surveillance study or you are a very close neighbour to the household where mosquitoes are collected every month. After you have read and signed this consent form, we will ask you some general questions on malaria prevention, bednet use and also take a drop of your blood to test for malaria.

Each member of your household will be offered a malaria test to see if they also have malaria parasites. If any person in your household has malaria, a small amount of blood will be taken and they will also be given drugs to treat the malaria.

All information collected during this research study will not be released to anyone in a way that could identify you, your child, or your family. Information about mosquito nets and malaria infection in your household may be shared with other researchers. However, your identity will remain confidential and will not be available under any circumstances to other researchers.

Research records are maintained for at least six years or until after the study is completed, whichever is longer. No end date has been set for analyzing results from the study. At such time as the study is considered completed, research information will be destroyed.

RISKS OF TAKING PART IN THE STUDY:

Risks of Blood Drawing

Risks associated with drawing blood from a finger stick for a malaria test include momentary discomfort. Risks associated with drawing blood from your arm include momentary discomfort and/or bruising. Infection, excess bleeding, clotting, or fainting are also possible, although very unlikely.

Risks of Breach of Confidentiality

Although the investigators will take care to maintain confidential records, taking part in this study may risk study information becoming known to other people.

BENEFITS OF TAKING PART IN THE STUDY:

If your family participates in this study, any one with malaria infection will be detected and treated. Even if they don't feel sick on that day, they could still have malaria in their blood. This could prevent illness later.

ALTERNATIVES TO TAKING PART IN THE STUDY:

You have the choice of not participating in this study. If you choose not to participate, no action will be taken against you

CONFIDENTIALITY:

The results of this study will be shared in different research forums. However, it will not be possible to identify any individuals from this information. Any publication of this study will not use your name or any member of your household. Efforts will be made to keep your personal information confidential.

COSTS: There are no costs to you or your family for participating in the study.

PAYMENTS/COMPENSATION: There is no compensation for participating in this study.

CONTACTS FOR QUESTIONS OR PROBLEMS:

For questions about the study, contact the principal Investigator; Judith Nekesa Mangeni, P.O Box 512-30100, Eldoret. Tel. No.0738692058. For questions about your rights as a research participant or complaints about a research study, contact Moi/MTRH IREC (Institutional Review and Ethics Committee) office at 0532033471/2/3/4, extension 3008 or University of Nairobi/Kenyatta hospital on (254-020)2726300 Ext 44355 .

VOLUNTARY NATURE OF STUDY:

Taking part in this study is completely up to you. You may withdraw from this study at any time.

PARTICIPANT'S CONSENT:

In consideration of all of the above, I give my consent for my household to participate in this research study. I have read this consent form (or had it read and explained to me), all my questions have been answered, and I agree for my household to take part in this study. I acknowledge receipt of a copy of this informed consent statement.

PARTICIPANT'S SIGNATURE OR MARK:_____Date:_____

(Must be dated by the parent/guardian if literate)

SIGNATURE OF WITNESS: _____Date:_____

(if parent/guardian is illiterate) (Must be dated by witness)

SIGNATURE OF PERSON OBTAINING CONSENT:___Date:_____

APPENDIX 1B): CONSENT STATEMENT (KISWAHILI)

HOSIPITALI YA RUFAA YA CHUO KIKUU CHA MOI

KAMATI YA TAASISI YA UCHUNGUZI NA MAADILI

CHUO KIKUU CHA NAIROBI/ HOSPITALI YA RUFAA YA KENYATTA

KAMATI YA TAASISI YA UCHUNGUZI NA MAADILI

Uchunguzi wa Maabukizi ya malaria katika Wilaya ya Bungoma Mashariki

KIBALI KILICHOFAHAMISHWA

UTANGULIZI:

Unaombwa kutoa kibali kwa niaba ya familia yako kushiriki katika uchunguzi unaolenga kubaini sababu ya kuongezeka kwa maambukizi ya malaria Bungoma Mashariki. Fomu hii ya kibali ina kupa habari kuhusu utafiti. Maafisa wa utafiti watazungumza nanyi kuhusu habari hii. Uko huru kuuliza maswali juu ya utafiti wakati wowote ule. Iwapo unakubali nyumba yako kushiriki katika utafiti huu, unatauliza kutia sahihi kwenye fomu ya kibali. Aidha, utapewa nakala ya kuweka. Tungependa ufahamishwe kwanza kuhusu malengo ya utafiti kabla hujaamua iwapo nyumba yako itashiriki au la.

LENGO LA UTAFITI:

Lengo la utafiti huu ni kubaini sababu zinazowafanya watu wa sehemu hii kuendelea kuambukizwa malaria. Tungali tunajaribu kuelewa ni mara ngapi watu wa familia yako watapata malaria kwa muda wa mwaka mmoja na pia kama mbu wamekuwa wakibadilika.

Kumbuka :

Ni uamuzi wako kuchagua iwapo utashiriki na familia yako katika utafiti au la.

Una uhuru wa kukoma kushiriki katika utafiti huu wakati wowote.

IDADI YA WATU WANAO SHIRIKI KATIKA UTAFITI:

Iwapo utakubali kushiriki, nyumba yako itakuwa miongoni mwa nyumba takriban 80 watakaoshiriki katika utafiti huu. Washiriki wote ni wakazi wa Wilaya ya Bungoma Mashariki. Wahiriki wote wa familia yako wata ombwa kushriki katika huu utafiti. Hivyo, tutaelewa ni mara ngapi washiriki wa familia yako hupata malaria na wale mbu wanaoambukiza viini vya malaria.

HATUA ZA UTAFATI:

Unaombwa kushiriki katika utafiti kwa sababu nyumba yako tayari inashiriki katika utafiti wetu wa kuchunguza mbu au wewe ni jirani wa karibu mno na yule amabye utafiti wa mbu unafanywa kwake. Baada ya kusoma na kutia sahihi fomu hii ya kibali, tutakuuliza maswali machache kuhusu kuzuia ugonjwa wa malaria na matumizi ya neti, kisha tutachukua kiasi kidogo cha damu yako ili kupima malaria.

Kila mmoja wa familia yako atapimwa ili kubaini iwapo wao pia wana vimelea vya malaria. Mmoja wenu akipatikana na vimelea vya malaria, atapewa dawa za kutibu malaria.

Timu ya utafiti watakuomba ruhusa ya kukutembelea nyumbani mwako mara moja kwa kila miezi mitatu hadi baada ya mwaka moja.

Habari yoyote itakayokusanywa katika utafiti huu ni ya siri: haitatolewa kwa njia itakayokukutambulisha kwako au kwa mtu yeyote katika familia yako. Habari kuhusu neti za mbu na uambukizaji wa malaria inaweza kupeanwa kwa watafiti wengine. Hata hivyo, utambulisho wako utabakia wa siri na hauwezi kutolewa kwa mtafiti yeyote katika kila hali. Watafiti wanohusika na utafiti huu moja kwa moja wataangalia uwezekano wa thamani ya utafiti uliopendekezwa na wachunguzi wengine kabla

Rekodi za utafiti zitahifadhiwa kwa miaka sita au hadi pale ambapo utafiti utakamilika. Hakuna wakati ambapo umewekwa wa ukamlishaji wa uchanganuzi wa data ya utafiti. Baada ya utafiti kukamilika, habari zote za utafiti zitaharibiwa.

HATARI ZA KUSHIRIKI KATIKA UTAFITI HUU:

Hatari za kutoa damu

Kuna hatari zinazohusiana na utoaji wa damu kutoka kwa kidole na mkono. Hatari hizi ni kama vile: Uchungu wa muda mfupi sana na majeraha madogo. Aidha, kuna hatari ya kuambukizwa, kutokwa na damu nyingi, kuganda kwa damu na ama kuzimia ambayo ni kwa nadra sana.

Hatari ya uvunjaji wa siri

Wachunguzi watahifadhi rekodi zote kwa njia ya usiri. Kushiriki kwako kunaweza kuhatarisha habari ya utafiti kujulikana kwa watu wengine. Hata hivyo, hakuna habari itakayotolewa ambayo itatambulisha jina lako na la familia yako.

MANUFAA YA KUSHIRIKI KATIKA UTAFITI:

Familia yako ikishiriki katika utafiti huu, kila aliye na maambukizi ya Malaria atachunguzwa na kupata tiba. Tiba hizi zitasaidia kwa sababu unaweza kuwa hauhisi maumivu yoyote wakati huo lakini malaria iko katika damu yako.Tiba hii itazuia ugonjwa wa malaria wa baadaye .

UCHAGUZI WA KUSHIRIKI KATIKA UTAFITI.

Uteuzi wa kushiriki katika utafiti huu. Ukichagua kushiriki au kutoshiriki katika utafiti, watoto wako wataendelea kupata tiba za kawaida katika hosipitali. Hakuna matokeo hasi yanayohusishwa na kutoshiriki kwako.

USIRI:

Hakuna uwezekano wa kutambulisha mtu yeyote anayeshiriki katika utafiti huu. Timu ya utafiti itampa kila moja wenu numbari ya kujitambulisha. Nambari ya utambulisho (ambao sio jina la

mtoto wako au habari yoyote itakayomtambulisha) inatumika kutambulisha habari. Jarida lolote litakalotolewa kutokana na utafiti huu halitatumia jina lako au mshirika yeyote wa familia yako. Juhudi zote zitafanywa ili habari zote kukuhusi ziwe za siri.

GHARAMA: Hakuna gharama yoyote utakayogharimia kwa kushiriki katika utafiti.

MALIPO/FIDIA: Hakuna fidia utakazopata kwa kushiriki kwako kwenye utafiti. Hata hivyo, iwapo mmoja wenu atapatikana na vimelea vya malaria, atapewa dawa.

MAWASILIANO:

Ukiwa na shida au swali lolote, unaweza kuwasiliana na mtafiti mkuu; Judith Nekesa Mangeni,P.O Box 512-30100,Eldoret,Nambari ya simu ni o738692058. Kuhusu masuali na haki zako kama mshiriki unaweza kuwasiliana na Moi/MTRH IREC (Taasisi ya Kamati ya Kuangalia Maadili) kupitia numbari 0532033471/2/3/4, enezo 3008 au UON/KNH ERC (Taasisi ya Kamati ya Kuangalia Maadili) kupitia namabari (254-020)2726300 Ext 44355.

UHIARI KATIKA UTAFITI HUU:

Kushiriki kwako katika utafiti ni kwa hiari. Unaweza kujitoa katika utafiti huu wakati wowote ule.

KIBALI CHA MSHIRIKI:

Baada ya kupitia yote yaliyotajwa hapo juu, nakubali nyumba yangu ishiriki katika utafiti. Nimesoma/nimesomewa, nimeelewa na maswali yangu yamejibiwa kulingana na yaliyoelezwa kwenye fomu ya kibali. Nimedhibitisha yaliyomo kwenye fomu ya kibali na nimepata nakala yangu.

SAHIHI AU ALAMA YA MSHIRIKI:____Tarehe:_____

(Ni lazima tarehe iwekwe na mzazi/mlezi iwapo ana kisomo)

SAHIHI YA MSHAHIDI: __Tarehe:_____

(Iwapo ana kisoma) (Lazima iwekwe na mshahidi)

SAHIHI YA ANAYEOMBA KIBALI:____Tarehe:_____

APPENDIX 2A): HOUSEHOLD CONSENT FORM

MOI TEACHING AND REFERRAL HOSPITAL/MOI UNIVERSITY

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

UNIVERSITY OF NAIROBI/KENYATTA HOSPITAL

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

HOUSEHOLD CONSENT FOR

Parasitological surveillance in Bungoma East District

Household number: _____ Date: _____

Hello, my name is Judith Nekesa Mangeni, a PhD student at the University of Nairobi ,School of public health and also a CARTA fellow. I am working with Moi University, College of Health Sciences. We are conducting a survey about malaria infection and bed net use in Bungoma East. Additionally, as a PhD student, I would also like to determine the incidence of malaria and correlate this with changes in mosquito characteristics over time. We will try to understand how often individuals are infected with malaria and whether these changes are related to changes in the mosquito behavior. I would like to speak with the head of the household. If the head of household is not available, we would like to speak to the wife or their children if they are over 18 years of age. We have general questions about your household and health issues. We may need to ask some of the questions to all members of your household who are over 18 years.

We will be visiting your household every three months over the course of one year. The information obtained from this study will allow us to draw conclusions about which mosquitoes carry malaria and which ones infect humans.

Your household was selected because you accepted to participate in the mosquito surveillance or are a close neighbor to that household. A mosquito trap was set up and or Pyrethrum spray catches are being conducted in your household/nearest neighbor's household on a monthly basis. We will ask you some questions about your household and malaria. The questions will take about 30 to 45 minutes. All of the answers you give will be confidential and will not be shared with anyone other than members of our survey team. If you are not comfortable participating in the survey, kindly let me know, but we hope you will agree to participate. Your views will be very important in this survey. If I ask you any question and you do not want to answer, just let me know and I will go on to the next question. You can also stop the interview at any time as you wish.

Kindly call us at any time and let us know if anybody in your household gets sick before the end of three months. The number to call when you have any questions or information is 0738692058.

Name of respondent …….

Relationship to head of household……..

PARTICIPANT'S SIGNATURE OR MARK…….Date:……..

SIGNATURE OF PERSON OBTAINING CONSENT:……Date:----------

APPENDIX 2B): HOUSEHOLD CONSENT FORM (Kiswahili version)

HOJAJI YA NYUMBA

Utafiti wa Maabukizi ya Malaria katika Bungoma Mashariki

Numbari ya Nyumba: _____ Tarehe: _____

Habari, Jina langu ni Judith N. Mangeni.Mimi ni mwanafunzi wa PhD katika chuo kikuu cha Nairobi. Pia, ninafanya kazi katika Chuo Kikuu cha Moi kitengo cha Sayansi ya Afya. Tunaendeleza uchunguzi kuhusu ugonjwa wa malaria na matumizi ya neti katika Bungoma East. Nami kama mwanafunzi wa PhD ningependa kujua ni mara ngapi mtu huambukizwa ugonjwa wa malaria kwa muda wa mwaka mmoja. Tungali tunajaribu kuelewa kama haya maambukizi ya malaria ya mara kwa mara yanahusika na tabia za mbu. Ningependa kuzungumza na mkuu wa nyumba na ikiwa hayupo, ningependa kuzungumza na mke wake au watoto waliopitisha umri wa miaka kumi na nane.

Habari tutakayokusanya,itatusaidia kutambua au kuelewa wale mbu wanao beba viini vya malaria na wale ambao wanaambukiza binadamu ili kuweza kutafuta mbinu mpya za kukabiliana hali hiyo. Tutawatembelea nyumbani kila baada ya miezi mitatu hadi mwisho wa mwaka mmoja.

Nyumba yako iliteuliwa kwa kuwa tayari ulikubali kuhusika katika uchunguzi wa mbu ama wewe ni jirani wa karibu na yule ambaye anafanyiwa uchunguzi wa mbu. Maswali yatachukua dakika 30 hadi 45. Majibu utakayotupa yatakuwa ya siri na hayatatolewa kwa mtu mwingine isipokuwa watafiti wa utafiti huu. Iwapo hungependa kushiriki katika utafiti huu tanakuomba utujulishe, lakini tunaamini kwamba utakubali kushiriki. Mchango wako utakuwa muhimu sana katika utafiti. Iwapo tutakuuliza swali ambalo hungependa kujibu, utueleze ili twende kwa swali linalofuata.

Unaweza kuwasiliana nasi kuhusu jambo lolote linalo husiana na utafiti huu katika nambari 0738692058.

Jina la Mhojiwa_____

Uhusiano wa mhojiwa na mwenye nyumba _____(Iwapo mhojiwa si mwenye nyumba, mke au watoto, katiza mahojiano)

SAHIHI AU ALAMA YA MSHIRIKI_____Tarehe_____

SAHIHI YA ANAYEOMBA KIBALI_____Tarehe:_____

APPENDIX 3A): RDT CONSENT FORM (ENGLISH VERSION)

Parasitological surveillance in Bungoma East District

HOUSEHOLD NUMBER _____ Date_____

Hello, my name is Judith Nekesa Mangeni, a PhD student at the University of Nairobi, School of public health and also a CARTA fellow. We are conducting a research about malaria infection and bednet use in Bungoma East. In addition, as a PhD student I would like to know how frequently individuals in your family get malaria in a period of one year. Your household has already agreed to participate in our survey. We are asking for your additional consent to test you for the presence of malaria parasites. Malaria is a parasite that is spread by mosquito bites and lives in the blood. You may have malaria parasites even if you don't feel sick. This test will tell us if you have malaria.

The test requires a finger stick to take one or two drops of blood. The blood is placed on the cassette and after 10 minutes the results can be seen. If you don't have malaria (the test is negative), there is no additional action required.

If you do have malaria (the test is positive), you need to get malaria drugs even if you don't feel sick. You will be referred to the nearest health facility where malaria drugs will be given to you. The drugs are free and you will not have any charges at the facility.

Risks of participation – There is a small risk of discomfort at the site of the finger prick. There is a very small risk of infection at the site. Our research assistants have been trained in blood safety and a new needle and test is used for every person so the test is very safe.

Benefits of participation – By participating, you will know in the next few minutes whether you have malaria. If you do, you can be treated. Treatment may prevent you from feeling sick later and may also prevent your parasites being transmitted to someone else.

If you agree to be tested for malaria please sign or make your mark next to your name in the table.

If you have any questions, you may contact the study at any time. You may also withdraw your consent at any time. Contact: 0738692058

If you are a parent or guardian consenting for a child under the age of 18, please indicate by checking the box.

Unique Identifier (Code)	**Age**	**Signature**	**Signature of parent or guardian (if required)**	**RDT Result**	**AL given and Referral Made**

APPENDIX 3B): RDT CONSENT FORM (KISWAHILI)

Uchunguzi wa Malaria Katika Bungoma Mashariki

Numbari ya Nyumba_____ Tarehe_____

Habari, Jina langu ni Judith N. Mangeni. Mimi ni mwanafunzi wa PhD katika chuo kikuu cha Nairobi. Pia, ninafanya kazi katika Chuo Kikuu cha Moi kitengo cha Sayansi ya Afya. Tunaendeleza uchunguzi kuhusu ugonjwa wa malaria na matumizi ya neti katika Bungoma East. Nami kama mwanafunzi wa PhD ningependa kujua ni mara ngapi mtu huambukizwa ugonjwa wa malaria kwa muda wa mwaka mmoja. Nyumba yako imekubali kushiriki katika utafiti huu. Tunaomba kibali zaidi kutoka kwako ya kuturuhusu kukupima ili kuchunguza iwapo una vimelea vya malaria. Malaria ni kimelea ambacho kinapitishwa kwa kuumwa na mbu na huishi katika damu. Unaweza kuwa na vimelea vya malaria hata ingawa haujihisi mgonjwa. Upimaji huu basi, utakusaidia kujua iwapo una vimelea vya malaria.

Upimaji utahitaji kifaa kinachotumiwa kutoa tone moja ya damu kutoka kwa kidole chako. Damu hii itawekwa kwenye kaseti kwa muda wa dakika kumi na kisha matokeo yatajulikana. Matone mawili au matatu zaidi yatapakwa kwenye karatasi ya chujio. Karatasi hii itawekwa na kupimwa baadaye ili kuona iwapo una vikinga mwili vya malaria.

Iwapo huna malaria hatutahitaji kufanya uchunguzi zaidi.

Iwapo utapatikana na vimelea vya malaria au matokeo ni chanya, utahitaji dawa hata kama huhisi maumivu yoyote. Tutakupa dawa za malaria au kukuelekeza katika Zahanati iliyo karibu nawe. Dawa zenyewe zinapeanwa bila malipo hivyo hutalipishwa chochote katika kila zahanati.

Hatari za kushiriki – Kuna hatari inayohusu uchungu kidogo katika sehemu ndogo ya kidole panapodungwa ili kutoa damu. Ni nadra sana kuwepo na hatari ya kupata maambukizi katika sehemu

hiyo. Wasaidizi wa utafiti wamefundishwa kutumia sidano mpya kwa kila mshiriki hivyo hatari za kuambukizwa hazipo.

Manufaa ya kushiriki katika utafiti ni kwamba, utajua baada ya dakiki chache iwapo una vimelea vya malaria au huna. Iwapo unavyo, utapewa tiba. Tiba hii itaweza kuzuia kuugua malaria baadaye na pia kuzuia vimelea vyako kupitishwa kwa mtu mwingine.

Ukiwa na maswali yoyote unaweza kuwasiliana na watafiti wakati wowote. Uko huru pia kutoa kibali chako wakati wowote.Wasiliana kupitia: 0738692058.

Kama wewe ni mzazi au mlezi unayetoa kibali kwa mtoto wako aliye chini ya miaka 18 kushiriki, tafadhali andika kufuatilia yalioandikwa kwenye jedwali.

Nambari ya Mhusika	**Mwaka**	**Sahihi**	**Sahihi ya mzazi au mlezi**	**Matokeo ya RDT**	**Amepewa dawa au la**

APPENDIX 4A): ADULT MOSQUITO COLLECTION FORM

Collection method: _____ Date: _____

District: _____ Division: _____

Location: _____ Sub location: _____

Village: _____ Household ID: _____

*1) Abdominal status of female **anopheles** mosquitoes caught*

Household ID	*Abdominal status of female anopheles mosquitoes (No)*						
	Unfed	Blood fed	Half gravid	Gravid	Undetermined	Total	Stored? (Y/N)

2) Number of male anopheles_____

3) *Abdominal status of **culicine** mosquitoes caught*

Household ID	*Abdominal status of culicine mosquitoes (No)*					
	Unfed	Blood fed	Half gravid	Gravid	Undetermined	Male

4) Number of male culex_____

NOTES:

Form checked by----------sign----------date----------

Form entered by----------sign----------date----------

APPENDIX 4B): INDIVIDUAL ANOPHELES DATA ENTRY FORM

Collection method……Date……

District…… Division……

Location…… Sub location……

Village…… Household ID……

Sample ID	Abdominal status (g/hg/fed/unfed/undetermined)	Comment

Form checked by----------sign----------date----------

Form entered by----------sign----------date----------

APPENDIX 5A): HOUSEHOLD QUESTIONNAIRE (ENGLISH VERSION)

Malaria Prevention Study

HOUSEHOLD QUESTIONNAIRE: Parasitological surveillance

Study ID No:……..

Date……..Interviewer name…….

GPS coordinates..... Latitude ..... Longitude.....Altitude……

Location…….....Sub location……Village…….

REGION .....

Fever hotspots/fever cold spots (Hotspots=1, coldspots =2)

Household number.....

Interviewer visit no (Up to a maximum of four visits including baseline)……

Number of buildings in the compound……..

Number of buildings with sleeping spaces……

Household Characteristics

1. Type of house:	Roof (check)	Floor (check)	Walls (check)
	Thatched	mud/earth	mud/earth
	Mabati	cement	cement
	Cement	wooden planks	stone
	Open	tiles	mabati
	Other	Other	Wood

b) Are there gaps between the wall and roof (eaves)?

2. Do you keep any open water containers? (Check)

a) Yes

b) No

3. Has anyone sprayed the interior walls of your dwelling against mosquitoes in the last 6 months?

4. Does your household have any mosquito nets?

(a) Yes

(b) No

(c) Don't know

5. If yes, how many mosquito nets do you have?

How many of those nets are currently being used?

6. How many people in your household slept under the net last night?

a) All

b) Some

c) None

d) Other (specify)…….

Sleeping spaces and Nets

7. a) How many sleeping spaces does the house have?

Does each of the sleeping spaces have a mosquito net?

Yes, All of them have a mosquito net

No, None of them has a mosquito net

Some sleeping spaces have nets, others don't have

c) When did you acquire each of the nets? Net 1(sleeping space 1)……Net 2 (sleeping space 2……. Net 3 (sleeping space 3…….) Net 4 (sleeping space 4)…….

d) Did you pay for the net in the sleeping space above?

a)Sleeping space 1

b) Sleeping space 2

c) Sleeping space 3

d) Sleeping space 4

d) Was the net in each of those sleeping spaces treated?

a) Sleeping space 1

b) Sleeping space 2

c) Sleeping space 3

d) Sleeping space 4

e) Have you ever treated any of the nets with chemicals since you got it?

f) If yes, when did you treat it and which chemical did you use?

8. Condition of the net

Observe each net and record the following:

Presence of holes a) Yes b) No

Number of the holes a) 5 b) 5-10 c) More than 10

Size of the holes a) Larger than a coin b) Larger than a hand

9. List of Household Members

	Unique Identifier Code	**Age**	**Date of Birth**	**Education level**	**Employment**	**Relationship to head of household**	**Did you travel outside your home in the last 3 months?**
Head of the Household							
Spouse of the head							
Member 1							
Member 2							
Member 3							
Member 4							
Member 5							
Member 6							

10. Malaria awareness among household members

	Head of the Household	Spouse of the head	Member 1	Member 2	Member 3	Member 4
Mode of spread: a)Mosquito bite b)Fly bite c)Dirtydrinkingwater d)unhygienic food e)Don't know						
Medicine against malaria a)ACT b)Chloroquine c)Fansidar d)Quinine e)Other f)Don't know						
Commonbreeding site a)Standing dirty water						

b)Garbage/Trash c)Standing clean water e)Running clean water f)Plants/vegetation g)Don't know						
Mosquito biting time a)Sunset/dusk b)Sunrise/dawn c)Night d)Noon e)Don't know						
Preventive measures a)Smoke away mosquitos b)Mosquito coils/spray c)Sleep under a net d)Drain stagnant water e)Covering water containers f)Others g)Don't know						

Behavior related to protection against malaria

Behavioral Characteristics	Head of the Household	Spouse of the head	Member 1	Member 2	Member 3	Member 4
How often do you use the mosquito net? a) Daily b) Sometimes c) Occasionally d) Never e) Other (Specify)						
What prompts you to use the mosquito net? a) The buzzing sound of mosquitos. b) Need to protect myself against malaria. c) It has become routine d) Only when I remember						

e) Other (Specify)						
Eating location for dinner a) Indoor b) Outdoor **c)** Other (specify)						
Dinner time a) Before 7 pm b) Between 7 and 8.30 pm c) After 8.30 pm **d)** Other (specify)						
Resting location after dinner a) Indoor b) Outdoor **c)** Other (specify)						
Approximately what time do you go to bed daily a) 8 am b) 10 am c) 12 midnight **d)** Other(specify)						
Do you try to protect yourself against malaria in any other way apart from using ITNs? a) Yes **b)** No						
If yes to the above question, what do you use? a) A coil b) Spray c) Repellant **d)** None						

RDT Testing and Treatment Behaviour

	a)Unique Identifier	**b)Consent for RDT results**	**C)RDT results (Indicate whether positive or negative)**	**d)If positive, do they have symptoms (atleast in the last two weeks)(list the symptoms)**	**e)Referral done/AL given (yes/no)**
Head of the Household					
Spouse of the head					
Member 1					
Member 2					
Member 3					
Member 4					
Member 5					
Member 6					

	Head of the Household	**Spouse of the head**	**Member 1**	**Member 2**	**Member 3**	**Member 4**
Have you had any symptoms relating to malaria in the last three months? a). Yes b). No						
If yes, what did you do? a) Bought drugs from Pharmacy b) Went to the nearest facility for treatment c) Didn't do anything d) Others (Specify)						
During the last three months, have you had malaria infection confirmed by the doctor? a) Yes						

b) No						
If yes to the above question, did you get AL? a)Yes b)No						
If yes, where did you get the AL? a)Health facility b)Shop c)Other (Specify)						
Did you complete your dose? a)Yes b)No c)Other (include sharing of drugs here)						
Have you taken AL in the last 3 months without any doctor's prescription? a) Yes b) No						

APPENDIX 5B: HOUSEHOLD QUESTIONNAIRE (KISWAHILI VERSION)

Utafiti wa Maambukizi ya Malaria

HOJAJI YA NYUMBA: Uchunguzi wa Malaria

Kitambulisho cha Utafiti:……

Tarehe yakuhojiwa…….Jina la mhojaji ……

Vilinganishi vya GPS..... Latitude ..... Longitude.....Altitude……

Lokesheni…….....Lokesheni ndogo……Kijiji…….

Makazi .....

Fever hotspots/fever cold spots (Hotspots=1, coldspots =2)

Kitambulisho cha nyumba ya DSS.....

Nambari ya Hojaji (Isizidi hojaji tano)……

Idadi ya majengo katika boma ……..

Idadi ya majengo yenye nafasi za kulala ……

SEHEMU YA KULALA NA NETI

1. Jengo 1:	Paa	Sakafu	Walls
	Nyasi	ya kubomwa	ya kubomwa
	Mabati	ya sementi	ya sementi
	Cement	ya mbao	ya mawe
	wazi	Kigae	ya mabati
	Lingine	Lingine	ya mbao

b) Je, kuna shimo kati ya ukuta wa juu na Paa?

2. Unayo mitungi yoyote ya maji isiyofunikwa?

a) Ndio

b) La

3. Je, nyumba yako imepuliziwa dawa ya kuwaua mbu katika miezi sita iliyopita?

4. Je,una neti kwa nyumba yako?

(a) Ndio

(b) La

(c) Sijui

5. Kama jibu lako ni ndio, una neti ngapi?

Ni ngapi katika neti ulizo nazo ambazo zinatumika kwa sasa?

6. Watu wangapi walilala ndani ya neti jana usiku?

a) Wote

b) Wengine

c) Hakuna hata mmoja aliye lala ndani ya neti

d) mengine …….

Sehemu za Kulala

7. a) Idadi ya sehemu za kulala katika jengo hili ni ngapi?

Kuna neti katika hizi sehemu za kulala?

Ndio

La

Zingine zinazo

c) Ulipata lini hizi neti? Net 1(Malazi ya kwanza)……Net 2 (Malazi ya pili)

……. Net 3 (Malazi ya tatu…….) Net 4 (Malazi ya nne)…….

d) Je,ulilipia hiyo neti?

a)Malazi ya kwanza

b) Malazi ya pili

c) Malazi ya tatu

d) Malazi ya nne

d) Je,neti ilikuwa imetibiwa wakati ulipo ipata?

a)Malazi ya kwanza

b) Malazi ya pili

c) Malazi ya tatu

d) Malazi ya nne

e) Je, umewahi tia kemikali tangu upate?

f) Kama jibu ni ndio, Ulitia kemikali ngani na ni lini?

8. Hali ya ile neti

Kagua na uandike yafuatayo:

Shimo? a) Ndio b) La

Numbari ya shimo a) 5 b) 5-10 c) More than 10

Saizi ya shimo a) Kubwa kuliko sarafu b) Kubwa kuliko mkono

9. Orodha ya watu wa nyumba

	Nambari ya mhusika	**Umri**	**Tarehe ya kuzaliwa**	**Kiwango cha elimu**	**Kuajiriwa**	**Uhusiano na mwenye nyumba**	**Umesafiri nje ya Bungoma Mashariki miezi tatu iliyopita?**
Mwenye nyumba							
Mkewe							
Mhusika 1							
Mhusika 2							
Mhusika 3							
Mhusika 4							
Mhusika 5							

10. Hamasisho juu ya Ugonjwa wa malaria

	Mwenye nyumba	**Mkewe**	**Mhusika 1**	**Mhusika 2**	**Mhusika 3**	**Mhusika 4**
Maambukizi ya malaria: a)Kuumwa na mbu b)Kuumwa na Inzi c)Kunywa Maji machafu d)Chakula kichafu						

e)Sijui						
Madawa za kutibu malaria a)ACT b)Chloroquine c)Fansidar d)Quinine e)mengine f)sijui						
Mahali wanamozaania mbu a)Maji machafu yaliyosimama b)Uchafu c)Maji masafi yanayo simama e)Maji yanayo teremka kwa kasi f)Mimea g)Sijui						
Mbu huuma saa ngapi a)Jioni b)Asubuhi c)Usiku						

d)Mchana e)Sijui						
Njia za kuzuia malaria a)Moshi b)Kupiga spray c)Kulala ndani ya neti d)Kutoa maji yanayo simama e)Kufunika mitungi iliyo wazi f)mengine g)sijui						

11. Tabia inayohusika na kujikinga na malaria

	Mwenye Nyumba	**Mkewe**	**Mhusika 1**	**Mhusika 2**	**Mhusika 3**	**Mhusika 4**
Ni mara ngapi unatumia neti? a)Kila siku b)Wakati mwingine c)Nadra d)Sijawahi kutumia e)Mengine						

Ni nini hukumbusha kutumia neti? a) Sauti ya mbu. b) Kujikinga na malaria. c) Nimezoea d) Ninapo kumbuka e)mengine						
Mahali pa kula chakula cha jioni a)Ndani ya nyumba b)Nje ya nyumba c)mengine						
Wakati wa kula jioni a)Kabla ya saa moja jioni b)Kati ya saa moja na mbili na nusu c)Baada ya saa mbili na nusu d)mengine						
Mahali unapopumzika baada ya chakula cha jioni a)Ndani						

b)Nje c)mengine						
Wewe hulala saa ngapi kila jioni a)Saa mbili usiku b)Saa nne usiku c)Saa sita ya usiku d)mengine						
Je,unajaribu kujikinga na malaria kwa njia yoyote? Ndio La						
Kama jibu lako ni ndio,unatumia nini? a)kuchoma ubani b)Anapiga spray c)Kutumia kiziuzi d)Sifanyi chochote						

12. Orodha ya watu wa nyumba kupimwa RDT

	a) Nambari ya Mhusika	**b) Kibali cha upimaji**	**c) Matokeo ya damu**	**d) Iwapo kuna dalili ya malaria**	**e) Amepewa dawa**	**f) Dose**
Mwenye nyumba						
Mkewe mwenye nyumba						
Muhusika 1						
Muhusika 2						
Muhusika 3						
Muhusika 4						
Muhusika 5						
Muhusika 6						

	Mwenye Nyumba	**Mkewe mwenye Nyumba**	**Mhusika 1**	**Mhusika 2**	**Mhusika 3**	**Mhusika 4**
Je,ulikuwa na malaria wakati uliopita? a)Ndio b)La						
Kama ulikuwa na malaria,ulimeza dawa ya malaria? a)Ndio b) La						
Ulipata wapi dawa za malaria? Kwa duka Zahanati Madaktari wa vijiji Wakufunzi wa DSS Mengine						
Je,ulmaliza dose ya dawa? a)Yes b)No						

APPENDIX 6: STANDARD OPERATING PROCEDURE (SOP)

University of Nairobi

Project title: Incidence of malaria and Vector characteristics in a High transmission region in rural western Kenya: implications for development of targeted malaria elimination strategies.

PURPOSE/ APPLICABILITY

Purpose: To provide guidelines for the procedures to be followed when performing an RDT to diagnose malaria.

Applicability: Study coordinator and all field staff

ABBREVIATIONS

QA: Quality Assurance

SOP: Standard Operating Procedures

RDT: Rapid Diagnostic Test

EQUIPMENT AND MATERIALS

RDT test packets

Capillary tubes

Alcohol swabs

Sterile disposable Lancet

Disposable examination gloves

Assay diluent

Timer

Sharps disposable box

General disposable containers

Pencil or pen

Note book

REPSPONSIBILITIES

It is the responsibility of the research assistants to ensure that they assemble all the materials before they visit the household.

It is the responsibility of the research staff to adhere to good diagnostic practice and blood safety when performing an RDT.

PROCEDURES

1. At the household level, the research assistant must introduce himself/herself and seek written consent from each of the household members before conducting an RDT.
2. Once consent has been obtained, explain in detail what the procedure is all about and that they will feel some discomfort during the finger prick.
3. The research assistant must check the expiry date on the test kit.
4. Put on new gloves for each patient.
5. Open the test packet and remove both the test and desiccant
6. Write the patient's name on the test.
7. Open the alcohol swab. Grasp the 4^{th} finger on the patient's left hand. Clean the finger with alcohol swab. Allow the finger to dry before pricking.
8. Open the lancet. Prick the patient's finger to get a drop of blood. Do not allow the tip of the lancet to touch anything before pricking the patient's finger
9. For children below six months, perform a heel stick.
10. Discard the lancet in the sharps box immediately after pricking the finger.
11. Use the capillary tube to collect the drop of blood.
12. Discard the capillary tube in the sharps box.
13. Put four (4) drops of assay diluents into the square hole.
14. Read the test results after 15 minutes of adding assay diluent.

15. How to read the results:

15.1. A line in “C” and a line in “T” means the patient does have *falciparum* malaria

15.2. A line in “C” and no line in “T” means the patient does not have falciparum malaria.

15.3. No line in “C” and a line or no line in “T” means the test is INVALID.

15.4. If no line appears in “C,” repeat the test using a NEW unopened test packet and a NEW unopened lancet.

16. Dispose of the gloves, alcohol swab, desiccant sachet and packaging in a non-sharps waste container.

17. Record the test results in your CHW register. Dispose of cassette in non-sharps waste container.

18. Each test can be used ONLY ONE TIME. Do not try to use the test more than once.

Note. Remember to keep all the information obtained in the household confidential.

APPENDIX 7: STUDY BUDGET

Item		**Quantity**	**Unit price**	**Total price**
General Supplies and materials	RDT Kits	1,800 (360 quarterly + baseline)	Ksh.2500 per pack of 25test kits	180,000
	Clean gloves	25 boxes	350	8,750
	Notebooks	10	100	1,000
	Box file	5	200	1,000
	Spring files	5	50	250
	Printing paper	10 reams	450	4,500
	Toner	2	10,000	20,000
Training of research assistants		2	20,000	20,000
Pilot study		3 days	20,000	20,000
Local travel to households by research assistants		400sh for each research assistant to & from the field	8,800 Ksh/month (data will be collected quarterly for five months	88,000
Accommodation and Local travel by PhD student + supervisors (sometimes)		The student has to be there during every round of data collection		150,000
Smart phones (Android phones for		2-One android phone for each of the research	10,000	20,000

data collection)		assistant to collect data		
Salary for research Personnel	Research assistant	2	20,000/ month (One month contracts times 5 months of data collection)	200,000
	Data manager	Data programming into the phones (one-time payment)	15,000	15,000
		Re-programming and Extraction of the information from the phones & data cleaning for each round	10,000 (per round of data collection times (four data collection points)	40,000
Anti-malaria treatment (Artemether Lumefantrine)				160,000
Consultation of Biostatistician		(one-time payment)	15,000	15,000
Subtotal				768,000
Contingency (10% of the total budget				76,800
Grand total				844,800

APPENDIX 8: STUDY TIMELINE

	Year 1				**Year 2**				**Year 3**			
	1st Qtr	**2nd Qtr**	**3rd Qtr**	**4th Qtr**	**1st Qtr**	**2nd Qtr**	**3rd Qtr**	**4th Qtr**	**1st Qtr**	**2nd Qtr**	**3rd Qtr**	**4th Qtr**
IREC amendment Approval												
Training of two research assistants												
Data collection												
Data entry												
Data analysis												
Thesis writing												
Thesis defense												
Presentation of papers at a conference												
Publication of at least 3 papers from the thesis work												

APPENDIX 9A) KNH IRB APPROVAL-TO ATTACH PHOTOCOPY OF HARD COPY

APPENDIX 9B) IREC APPROVAL-TO ATTACH PHOTOCOPY OF THE HARD COPY.

APPENDIX 10: STUDY TEAM IN THE FIELD