UNIVERSITY OF NAIROBI

IMPACT OF SEASONAL CHANGES ON MALARIA AND RIFT VALLEY FEVER VECTOR ECOLOGY AND INFECTION STATUS IN BARINGO COUNTY, KENYA

BY

ONDIBA, ISABELLA MORAA (BEd.Sc. MSc.)

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DECLARATION

I declare that this thesis is my original work and has not been submitted elsewhere for examination or award of a degree.Where other people's work or not my own work has been used, this has been properly acknowledged and referenced.

Signature……Date……...

Ondiba Isabella Moraa

School of Biological Sciences

This thesis has been submitted for examination with our approval as research supervisors

Signature……Date ……

Prof. Florence A. Oyieke

School of Biological Sciences,

University of Nairobi

Signature.....Date……

Dr. George O. Ong'amo

School of Biological Sciences,

University of Nairobi

DEDICATION

To my late mother in law, Milka Ondari, who rejoiced with me on getting the news of my scholarship to undertake this programme. She wanted to dance and *koiririata* as I shed tears of joy but unfortunately she was bedridden.

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ABBREVIATIONS AND ACRONYMS

Ae	: *Aedes*
An	: *Anopheles*
CDC	: Centers for Disease Control and Prevention
Cx	: *Culex*
DNA	: Deoxyribonucleic acid
DVBDs	: Division of Vector Borne Diseases
ELISA	: Enzyme linked immunosorbent assay
IRS	: Indoor residual spray
ITN	: Insecticide treated nets
LLINs	: Long lasting insecticide treated nets
MAb	: Monoclonal Antibodies
NTDs	: Neglected Tropical Diseases
PBS	: Phosphate Buffered Saline
PCR	: Polymerase Chain Reaction
PSC	: Pyrethrum Spray Collection
PVC	: Polyvinyl Chloride
RVF	: Rift Valley Fever
UNITID	: University of Nairobi Institute of Tropical and Infectious Diseases
VBDs	: Vector Borne Diseases

ABSTRACT

The seriousness of vector borne diseases is expected to be altered with ongoing climate change. This is because the vectors responsible for transmission of vector borne diseases (VBDs) are climate sensitive. The current study sought to find out the impact of seasonal variations on rift valley fever (RVF) and malaria vector bionomics in Baringo County. Immature and adult mosquitoes were collected once every month from 24 sites distributed in four ecological zones based on elevation. Larvae were collected from breeding habitats using a dipper and a pipette in 2014 to 2016. Indoor adult mosquitoes were collected by pyrethrum spray collection (PSC) while outdoor adult mosquitoes were collected using Centers for Disease Control and Prevention (CDC) light traps. Both larvae and adults were morphologically identified using taxonomic keys. *Anopheles gambiae* complex mosquitoes were further genetically identified to sub species level by polymerase chain reaction (PCR). Known vectors of malaria were screened for *Plasmodium falciparum* parasites by enzyme linked immunosorbent assay (ELISA). Larval species diversity was significantly different ($p<0.000$) with highest diversity in the cold dry season ($H=1.99$) and lowest in the long rain season ($H=1.59$). Out of the nine types of habitats sampled, ditches had the highest mean of anopheline larvae (16.6 per 20 dips) while concrete tanks had the highest mean of culicine larvae (333.7 per 20 dips). Swampy habitats were preferred by both anopheline (12.4 per 20 dips) and culicine (23.5 per 20 dips) larvae. Malaria vectors were more abundant indoors (80.8%) than outdoors (19.2%) while Rift Valley fever (RVF) vectors (culicines) were more abundant outdoors than indoors. Vector abundance was highest in mud wall houses and in houses with open eaves. Most vectors were collected in the lowland (82.5 %) followed by the riverine zone (14.6 %) and these were the only two zones where *Anopheles funestus* was found. Larger numbers of mosquitoes (3,629) were collected in the drier seasons than wet seasons (2,141). Out of the 635 *An. gambiae* mosquitoes that were identified to sub species level, 626 (98.6%) were *An. arabiensis* while 9 (1.4%) were *An. gambiae* s. s. *Plasmodium falciparum* infections were detected in mosquitoes collected from the lowland and riverine zones only during dry season. Sporozoite rates were higher in *An. funestus* (10.0%) than in *An. arabiensis* (0.35%) though the latter species was most abundant and constituted 93.4% of tested mosquitoes. The dry season presented greater risk for malaria transmission when mosquito species diversity, abundance and sporozoite rates were high. *Anopheles arabiensis* was the dominant species, but results implicate *An. funestus* as a key malaria vector in Baringo County. Abundance of outdoor secondary malaria vectors and RVF vectors calls for multiple control strategies that target outdoor mosquitoes. Thus Baringo County Government should implement integrated mosquito control strategies to target both indoor and outdoor mosquitoes in the lowland and riverine zones; where vector abundance and infection rates were highest. Intervention measures should not be limited to the wet seasons only. Simple house modifications such as screening of eaves to hinder mosquito entry are recommended as part of integrated approaaches to vector control.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background information

Seasonal changes in climatic factors influence development and survival of mosquitoes, which are the known vectors of diseases (Reiter, 2001). Generally, the factors responsible for sustainability of vector life cycles are basically ecological with climate being the most important in limiting transmission and distribution of disease on a large scale (Craig *et al*., 1999; Egbendewe-Mondzozo *et al*., 2011). Therefore, vector-borne diseases (VBDs) are among the illnesses that have been linked to climate change. This is because the life cycle duration of mosquitoes and the pathogens they transmit are climate-sensitive (Paaijmans *et al*., 2009). Climate plays a role in emergence and resurgence of many infectious diseases and the most sensitive diseases are those transmitted indirectly by requiring an intermediate host or vector (Githeko *et al.,* 2000; Patz *et al.*, 2008). Effects of climate change may be felt through vectors (Mills *et al*., 2010) so understanding vector ecology is crucial in the control of VBDs (Gage *et al*., 2008). These diseases occur as epidemics probably triggered by variability in climatic conditions which may favor higher transmission rates (Egbendewe-Mondzozo *et al*., 2011). Most significant among vector-borne diseases that are climate sensitive are rift valley fever (RVF) and malaria (Anyamba *et al*., 2010; Caminade *et al*., 2014).

Rift valley fever is a zoonotic disease transmitted through the bite of female *Aedes* and *Culex* mosquitoes (CDC, 2013). It is also transmitted through contact with body fluids and tissues of infected animals (Nguku *et al*., 2010). Occasional serious outbreaks of RVF are experienced in livestock and humans. The conditions which favor transmission of RVF virus are above average rainfall associated with flooding and temperature associated with warm weather. Topography, soil type and density of competent vectors also make an area prone to RVF outbreaks (Breiman *et al*, 2010). Many species of mosquito vectors are capable of transmitting RVF virus in different countries (Chevalier *et al.,* 2004; Pepin *et al*., 2010; Seufi and Galal, 2010) but the principal vectors are the flood water *Aedes* species such as *Aedes mcinthoshi* and *Aedes ochraceus* usually referred to as primary vectors (Sang *et al*., 2017). Other mosquito species are in the *Culex* and

Mansonia genera usually referred to as secondary vectors and a few potential species in the genus *Anopheles* also play a great role in the spread of RVF during outbreaks (Sang *et al*., 2017). In Baringo County of Kenya, RVF was reported for the first time during the 2006-2007 outbreaks (Sang *et al*., 2010).

Another vector-borne disease that is associated with variability in climatic conditions is malaria which causes death and economic problems in the world (Murray *et al*., 2012). It is, probably, the most climate-sensitive VBD that causes serious health problems in Kenya and sub-Saharan Africa as a whole (Craig *et al.,* 1999). Globally malaria cases are estimated to be 219 million, 435,000 deaths, with the greatest burden on the African continent (WHO, 2018). The groups of people mostly affected are children aged below 5 years and pregnant women (WHO, 2000; WHO, 2018).

Ecological studies have revealed that anopheline mosquito species found in Baringo County of Kenya include *Anopheles gambiae* complex, the most common sub species in the complex being *An. arabiensis*. Other species identified in Baringo include *An. funestus, An. pharoensis* and *An. coustani* (Mala *et al*., 2011a). The most abundant species in the area is *An. pharoensis* which, together with *An. coustani,* mainly act as nuisance insects since their vectorial capacity has not been established. However, Aniedu in 1992 reported *An. pharoensis* as a vector of malaria in Baringo (Aniedu, 1992).

According to Githeko and Ndegwa (2001), *Anopheles* mosquitoes transmit malaria parasites when environmental and climatic conditions are suitable. *Anopheles gambiae* larvae distribution and abundance are affected by rainfall and other environmental factors (Amadi *et al*., 2018b). Therefore, understanding how disease transmission varies in the community due to these conditions is important for early warning and detection of the disease which would lead to quick implementation of targeted control programs. This study sought to determine the effects of seasonal variability in temperature and precipitation on RVF and malaria vector abundance and distribution. The study also investigated the status of malaria vector infections with *Plasmodium falciparum* parasites in relation to seasonal changes across four ecological zones namely; lowland, midland, highland and Riverine in Baringo County.

1.2 Problem Statement

Vector-borne diseases such as rift valley fever (RVF) and malaria pose a threat to livestock and human populations in Baringo County. The vectors responsible for transmission of these diseases are sensitive to changes in climatic factors such as rainfall and temperature. Baringo County of Kenya occasionally experiences unpredictably higher than usual amounts of rainfall leading to flooding. For example Lake Baringo had extended inland by about 2km in 3 years time since 2011 to 2014 (personal communication from Baringo). This implies that vector breeding sites would increase; a situation that may lead to changes in the dynamics of disease transmission intensity. Baringo is semi arid and transmission of vector borne diseases fluctuates because vector abundance varies with changes in climatic conditions. Rift valley fever which is a sporadic, zoonotic disease was experienced in Baringo for the first during the 2006/2007 RVF outbreak in Kenya following El Nino rains. On the other hand, malaria in Baringo is hypoendemic therefore epidemics do occur in regions, where the population is immunologically vulnerable, away from the Lakes region in the lowland.

1.3 Justification of the study

Mosquito vector populations fluctuate annually on a seasonal basis. Knowledge on seasonal abundance of vector species at the local level would improve vector control programs and contribute to prevention of VBDs such as RVF and malaria. Despite this, there is dearth of information on vector species diversity, breeding habitat preference, distribution and seasonal population fluctuations in Baringo County.

Previous studies conducted in Baringo County focused on RVF and malaria vectors around the lakes region (Aniedu, 1992; Sang *et a*l., 2010; Arum *et al*., 2010; Mala *et a*l., 2011; Lutomiah *et al*., 2013; Omondi *et al*., 2015). The current study covered a wider area, which included highlands and Kerio valley (riverine zone) on the western part of the county, to establish species diversity, breeding habitat preference, distribution and abundance in relation to variations in rainfall and temperature.

Rift Valley fever (RVF) is a zoonotic disease that occurs sporadically in form of outbreaks and is transmitted by diverse mosquito species in different geographic regions. Therefore, information on diversity, distribution and abundance of RVF vectors can be useful in risk assessment of RVF outbreaks. Malaria on the other hand is the most prevalent vector borne disease which is a major

cause of morbidity and mortality in Baringo County. Information on malaria vector species is only available for the lowland Lakes region in Baringo County (Sang *et a*l., 2010; Lutomiah *et al*., 2013; Omondi *et al*., 2015).

Thus, it is important to determine the effects of seasonal variability in climatic factors on RVF and malaria vector abundance to determine seasons when disease transmission is highest. Furthermore, it is necessary to screen the vectors for infections with malaria parasites to identify the areas where residents are more vulnerable to malaria transmission. The results from this study can be used to model the relationship between variability in climatic factors, vector abundance and infection status. This will also allow interventions to be adapted to specific locations and seasons of the year when risk is high hence reduce human vulnerability to VBDs.

1.4 Research Questions

1. Does change in temperature and rainfall affect species diversity and larval habitat preference by rift valley fever and malaria vectors?
2. Does change in temperature and rainfall affect abundance of rift valley fever and malaria adult vectors?
3. Does change in temperature and rainfall affect infection status of malaria vectors?

1.5 Research Hypotheses

1. Seasonal change in temperature and rainfall does not affect species diversity and larval habitat preference by rift valley fever and malaria vectors
2. Seasonal change in temperature and rainfall does not affect abundance of rift valley fever and malaria adult vectors
3. Seasonal change in temperature and rainfall does not affect infection status of malaria vectors

1.6 Objectives

1.6.1 General objective

To assess the impact of seasonal changes in temperature and rainfall on rift valley fever and malaria vector species diversity, abundance and distribution and infection status in malaria vectors in Baringo, Kenya.

1.6.2 Specific objectives

1. To investigate effects of changes in temperature and rainfall on species diversity and larval habitat preference by vectors of rift valley fever and malaria in Baringo County
2. To assess the effects of seasonal changes in temperature and rainfall on the distribution and abundance of vectors of rift valley fever and malaria in Baringo County
3. To determine the impact of seasonal changes in temperature and rainfall on infection status of malaria vectors in Baringo County.

CHAPTER TWO

2.0 GENERAL LITERATURE REVIEW

2.1 Climate and Vector-Borne Diseases

Climatic factors influence the incidence of vector-borne diseases such as RVF and malaria. Factors such as rainfall and temperature modify the abundance of vector populations and emergence of epidemics in areas of low endemicity (Krefis *et al*., 2011a). For instance, variability in temperature, precipitation, wind, and extreme weather events have been linked to transmission of mosquito-borne diseases in some regions of China (Bai *et al.*, 2013). Similarly, inter-annual climate variability is an important determinant of epidemics in parts of Africa where climate influences both mosquito vector dynamics and parasite development rates (Egbendewe-Mondzozo *et al*., 2011). As such, climate variability has been used to predict probability of malaria incidence by observation of precipitation patterns in Botswana (Thomson *et al*., 2005). In West Africa, studies by Yamana and Eltahir (2013) have reported that rainfall may be important in shaping the impact of climate change on malaria transmission in future. Climate data has also been suggested as a possible tool for decision makers to predict malaria epidemics in the Kenyan highlands (Githeko and Ndegwa, 2001). Since the control of epidemic malaria is a priority for the international health community, specific tools for the early detection and effective control of the epidemics are needed (WHO, 2004).

Rift valley fever, which is another vector borne disease, occurs in form of sudden epizootics with prolonged inter-epidemic periods. In Kenya, 11 epizootics have occurred between 1951 and 2007 with an average of inter-epizootic period of 3.6 years (Murithi *et al*., 2011). According to the study of Anyamba *et al* (2010), cases RVF in human and livestock occurred two to four months as predicted using satellite measurements of regional elevated climatic and environmental factors in Afrrica. In terms of climatic conditions, frequent and persistent rainfall leading to flooding has been associated with RVF epizootics (Davies *et al*., 1985; Hassan *et al.*, 2011). However, in West Africa RVF outbreak has been associated with high population density of the major vectors (Soti *et al.*, 2011) which would be indirectly influenced by the amount of rainfall. In Kenya, rift valley fever has been predicted to occur in areas with suitable conditions for the vectors such as rift valley region of Kenya (Mweya *et al*., 2013).

2.2 Global burden of Rift Valley Fever and Malaria

Globally, vector-borne diseases are responsible for almost 20% of the infectious diseases affecting humans. More than 80% of the world's population is at risk of one or more vector borne diseases (WHO, 2017). Developing countries in Africa suffer most from the vector-borne disease burden and its socioeconomic consequences (Hotez and Kamath, 2009). Rift valley fever which is one of the arboviral infections has negative impacts on human and animal health and the economy (Hassan *et al*., 2011). The disease leads to significant losses due to human illnesses, livestock abortions and death (Nanyingi *et al*., 2015). The direct economic impacts on affected agricultural producers are due to loss of animals, banning of trans-boundary trade and decrease in livestock prices (Domenech *et al*., 2006; El Mamy *et al*., 2011). Global annual clinical cases of RVF have been estimated to range between 350 and 2750; though arboviral infections occur in epidemic waves based on weather phenomena (LaBeaud *et al.*, 2011). When the 2006–2007 outbreaks subsided in East Africa, more than 1,000 people had been diagnosed with RVF, and more than 300 people were confirmed to have died of the disease (Breiman *et al*., 2008). Estimated total number of people affected by RVF during the 2007 outbreak in Kenya, Somalia, Sudan, Tanzania, Madagascar and South Africa was 230,000 while reported human cases were 2,242 (Anyamba *et al*., 2010). Apart from loss of human life, outbreaks of RVF can result in devastating economic losses at household and national levels due to the fact that pastoralists usually incur great losses, including reduction in milk production, deterioration of animal health and death (Muga *et al*., 2015). In Tanzania, the latest RVF outbreak in 2007 led to an estimated loss of about US$ 4,243,250 and US$ 2,202,467 due to deaths of cattle and sheep/goats respectively (Sindato *et al*., 2011).

Malaria epidemics generally attack immunologically vulnerable populations, and their explosiveness can strain the capacity of health facilities. This leads to increase in case fatality rates by five-fold or more during outbreaks (Kiszewski and Teklehaimanot, 2004). Such cases of explosive malaria were witnessed in 2017 in Baringo East, an area that is rarely affected by malaria (Koech and Muchui, 2017). Globally, malaria alone contributed about 243 million cases and about 863,000 deaths in 2008. An estimated 219 million cases of malaria occurred worldwide and 660,000 people died mostly children in the African region in the year 2010 (WHO, 2012). Latest global estimates of malaria cases and mortality were 219 million and 435,000 respectively in 2017 with the largest proportion of deaths occurring in the African region (WHO, 2018).

Countries experiencing endemic malaria also happen to be among the poorest countries in the world with low rates of economic growth (Sachs and Malaney, 2002). Total costs per malaria episode based on disease severity in three countries representing sub Saharan Africa as estimated in 2009 ranged between US$ 5.2 to 287.81 (Sicuri *et al*., 2013). The cost burdens of malaria are the product of complex relationships between social, economic and epidemiological factors (Chuma *et al*., 2010a). Malaria mortality in the world increased between 1980 and 2004 (995,000 to 1,817,000 respectively) then decreased to 1,238,000 in 2010 (Murray *et al*., 2012). In spite of the perceived decrease, mortality burden in adults is larger than estimated previously. The observed decrease in malaria mortality has been attributed to widespread use of insecticide treated nets (ITNs) and long lasting insecticide treated nets (LLINs) (WHO, 2011). The difference between the two types of nets is that ITNs involve periodic retreatment with chemicals while LLINs do not require re-treatment.

2.3 Geographical distribution of Rift Valley Fever and Malaria

Arboviral infections belong to the group of neglected tropical diseases (NTDs) which commonly affect the rural poor in sub Saharan Africa (Hotez and Kamath, 2009). Rift valley fever is endemic in the African continent but has also spread to the Arabian Peninsula (Abdo-Salem *et al*., 2006). Though RVF was initially confined to the African continent, an unexplained hemorrhagic fever in humans and associated animal deaths/abortions from Saudi Arabia and Yemen were confirmed to be caused by RVF virus in 2000 (Shoemaker *et al*., 2002). The countries where outbreaks have occurred include; Chad, Egypt, Gambia, Kenya, Madagascar, Namibia, Mauritania, Mayotte, Saudi Arabia, Senegal, Somalia, South Africa, Sudan, Swaziland, Tanzania, Yemen, Zambia, Zimbabwe (CDC, 2013). However, highest cumulative outbreak days have been experienced in South Africa followed by Mauritania, Kenya and Tanzania (Nanyingi *et al*., 2015). Outbreaks of RVF were reported in the Republic of South Africa in 2010 mainly affecting animals and this occurred after heavy rainfall accompanied with warm temperatures (Metras *et al*., 2013). In Kenya, Somalia and Tanzania, RVF outbreaks which occurred between December 1997 and January 1998 were associated with heavy rainfall (CDC, 1998). Outside the African continent, outbreaks of RVF were reported in Saudi Arabia in 2000-2001, 2004 and in Yemen in 2000-2001(Abdo-Salem *et al*., 2006). Though RVF has not been reported in the western countries, there is fear of it being introduced and established in the United States (US) and the European Union (EU) through several pathways (Rolin *et al*., 2013). Therefore, arboviral

control should be conducted with a lot of seriousness because of disease burden and threat of emergence of infections in larger groups of susceptible populations (LaBeaud *et al*., 2011).

On the other hand, malaria as a vector borne disease has a global distribution and significant health burden (Caminade *et al.,* 2014). The spatial limits of its distribution and seasonal activity are sensitive to climatic factors, as well as the local capacity to control the disease. The epidemics occur throughout the world, and their causes are as diverse as the climate, topography, and vector ecology of the endemic regions in which they occur.

2.4 Rift Valley Fever in Kenya

Rift valley fever epizootics have been occurring in Kenya periodically since 1934 (Davies, 2010). It was first reported in 1912 in Rift Valley province and remained confined there until 1960's when it spread to other regions of Kenya (Murithi *et al*., 2011). Eleven national RVF epizootics have been reported since 1950 up to 2007 at intervals ranging between 1-7 years (Murithi *et al*., 2011). Twenty seven thousand five hundred cases of RVF are estimated to have occurred in Garissa during the RVF outbreak of 1997 (Woods *et al*., 2002). The 2006-2007 outbreaks had a devastatingly high case-fatality rate for hospitalized patients and up to 180,000 infected mildly ill or asymptomatic people within highly affected areas (Nguku *et al*., 2010). The 2007 RVF outbreak in Kenya is estimated to have caused a loss of Ksh 2.1 billion due to loss of livestock and other related sectors (Rich and Wanyoike, 2010). Investigations during and after the outbreak showed that individuals who handled aborted animal fetuses got severe disease while those who consumed products from sick animals died (Anyangu *et al.,* 2010). Mapping of ecological conditions in arid areas has indicated a close association between inter-annual climate variability and RVF outbreaks in Kenya (Anyamba *et al*., 2010). In December 2006, RVF cases were reported from several parts of Kenya which included districts from North Eastern, Coast, Rift Valley, Central provinces and one case in Nairobi (CDC, 2007).

2.5 Vulnerability to Rift Valley Fever

In humans, RVF is an acute, feverish zoonotic disease caused by a *phlebovirus* belonging to the family Bunyaviridae. Humans acquire RVF virus through exposure to the body fluids or tissues of infected animals (Nguku *et al*., 2010) and through bites from infected mosquitoes. Direct exposure to infected animals can occur during handling and slaughter. Studies have shown that spending time in rural areas and sleeping outdoors at night in regions where outbreaks occur

could be a risk factor for exposure to mosquitoes and other insect vectors (Chinwe *et al*., 2014). Animal herdsmen, abattoir workers, veterinarians and other individuals who work with potentially-infected animals in RVF-endemic areas have an increased risk for getting infections (CDC, 2013). Prevention and control strategies are complicated and expensive since this zoonotic disease is hypothesized to have mosquito-egg reservoir (Linthicum, 1985). Therefore, control should involve community education focused on reducing risk exposures which include slaughtering or handling sick animals and drinking raw milk (LaBeaud *et al*., 2011). Community education on reduction of risk should best be applied before an outbreak occurs, based on accurate predictions of RVF outbreaks.

2.6 Rift Valley Fever Mosquito Vector Species

The ability of RVF to move outside traditionally endemic countries, even out of the African continent, lies in the fact that many species of mosquito vectors are capable of transmitting the virus (CDC, 2013; Sang *et al*., 2017). The dominant mosquito species vary by region, which in turn, impacts the transmission cycles of RVF virus (CDC, 2013). A number of mosquito genera namely; *Aedes, Culex, Mansonia, Anopheles* are implicated as vectors of RVF virus, the most important being *Aedes* and *Culex* species. These two are responsible for both maintenance and amplification of RVF virus. The species which have been implicated in various parts of the world include; *Culex poicilipes* in Senegal (Lambin *et al*., 2010), *Aedes vexans, Ae. mcintoshi, Ae. ochraceus, Ae. dalzieli*, and Phlebotominae spp (Fontenille *et al*., 1998) in West Africa. In Kenya, vectors of RVF virus include: *Aedes tricholabis, Ae. mcintoshi, Ae. ochraceus, Culex pipiens, Mansonia uniformis* and *Ma. africana* (Tchouassi *et al*., 2012). *Mansonia uniformis and Ma. africana* are the most common species implicated for transmission of arboviruses in semi-arid Baringo (Lutomiah *et al.,* 2013). Other species which have tested positive for RVF virus in Kenya include *Culex quinquefasciatus, Cx. univittatus, Cx. poicilipes, Cx. bitaeniorhynchus, An. squamosus* and *Aedes pembaensis* (Sang *et al.,* 2010).

2.7 Rift Valley Fever virus transmission cycle

Mosquitoes and animals maintain the virus during the inter-epidemic periods. Rostal *et al.* have shown that the presence of RVF virus antibodies in livestock and *Aedes* mosquitoes, which are competent vectors of RVF virus in the environment, is an indication that the viral activity exists during inter-epizootic periods (Rostal *et al*., 2010). Outbreaks occur after unusually heavy and

persistent rainfall leading to flooding. Adult *Aedes* species hypothesized to be trans-ovarially infected emerge in immense numbers in floodwaters. Rift valley fever virus is then transmitted to nearby domestic animals and humans (Figure 2.1) but higher viraemia occur in animals than humans. Flooded sites become colonized by *Culex, Anopheles, Mansonia* and other species which act as secondary vectors to spread the virus to additional animals and humans (Sang *et al*., 2017). The transmission steps can be summarized as follows: 1.) Rift valley fever virus can be transmitted from female mosquitoes to offspring via the egg (vertical transmission). 2.) In the egg the virus remains viable (infectious) for several years during dry conditions. 3.) Excessive rainfall enables more mosquito eggs, commonly of the genus *Aedes, to* hatch and release trans-ovarially infected adults. 4.) As mosquito populations increase, the potential for virus to spread to the animals and humans increases. 5.) In epizootic events, there is increased handling of infected animals which also increases risk of exposure for humans (CDC, 2013).

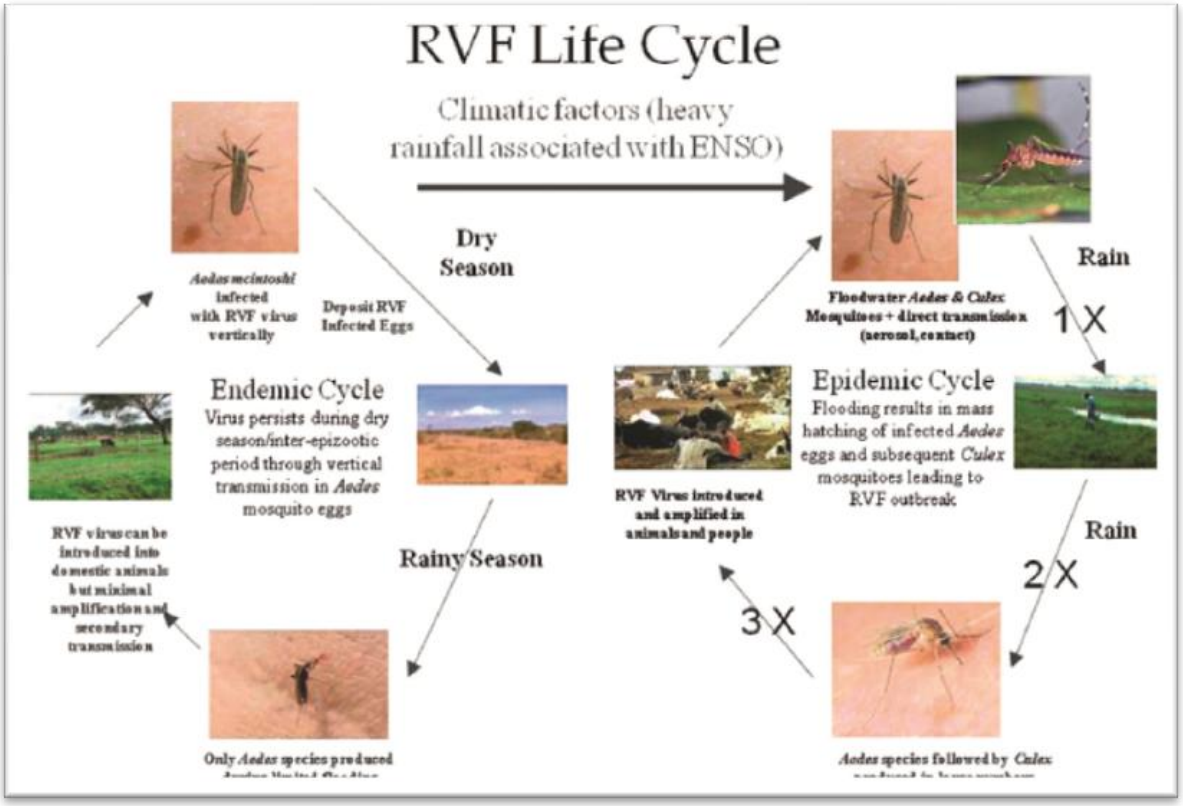

Figure 2.1 Rift Valley Fever Life Cycle (from Anyamba *et al*., 2010, conceptualized by Linthicum, K.J.)

The three Xs depicted in epidemic cycle represent critical pathways, which can be interrupted by targeted and specific mosquito control activities.

2.8 Malaria as a vector-borne disease

Malaria is an infectious vector-borne disease caused by a single-celled parasitic protozoan of the genus *Plasmodium***.** The common species of *Plasmodium* known to cause malaria are *P. falciparum, P. malariae, P. ovale* and *P. vivax. Plasmodium falciparum* is the most serious of the four species of malaria parasites common in Africa and causes more than 95% of all malaria infections in sub-Saharan Africa (WHO/UNICEF, 2005; WHO, 2017). A fifth species of *Plamodium* known as *P. knowlesi* causes human malaria in Malaysia where the natural host is the rhesus monkey (Cox-Sing *et al*., 2008). Almost all remaining malaria infections are caused by *P. malariae* (Bloland *et al.,* 1999) while infections due to *P. ovale* are rare and the fourth species, *P. vivax,* is almost absent in Africa. Anopheline mosquito vectors that transmit malaria parasites flourish well in tropical Africa where the climate and ecology provide favorable conditions (WHO/UNICEF, 2005). Therefore, the combination of the most deadly parasite, *P. falciparum,* and the most efficient *Anopheles* mosquito vectors in Africa increase malaria incidence and thus human vulnerability to the disease.

2.9 Malaria vector mosquito species

There are about 380 species of *Anopheles* mosquitoes around the world and 60 species are vectors of malaria (WHO, 1997). The dominant *Anopheles* vectors of human malaria in Africa are *An. gambiae* complex which include *An. gambiae* s. s., *An. arabiensis, An. merus*, *An. melas, An. bwambae, An. quadrinnulatus/An. amaharicus* and the recently named *An. coluzzii* (Coetzee *et al.,* 2013). Description of *An. gambiae* species complex is based on mating experiments supported by chromosomal, distributional and biological differences. Other species which are highly anthropophilic are *An. funestus, An. moucheti* and *An. nili* (Sinka *et al.,* 2010). *Anopheles funestus* complex consist of nine species that are distributed throughout Africa; these are *An. parensis* Gillies, *An. aruni* Sobti, *An. confusus* Evans and Leeson, *An. funestus* s.s., *An. vaneedeni* Gillies and Coetzee, *An. rivulorum* Leeson, *An. fuscivenosus* Leeson, *An. leesoni* Evans, and *An. brucei* Service (Gillies and Coetzee, 1987). Apart from the morphological similarities among these sibling species, their biology and vectorial competency is different. All sibling species are zoophilic except *An. funestus* s. s. which is closely associated with human dwellings (Gillies and Coetzee, 1987). Four out of the nine sibling species of *An. funestus* complex have been identified

in western Kenya. These are *An. funestus* s. s., *An. leesoni*, *An. rivulorum* and *An. vaneedeni* (Kweka *et al*., 2013). A prevoius study conducted at 10 sites in Kenya also identified four species of *An. funestus* complex and these included *An. funestus* s. s., *An. parensis*, *An. leesoni*, and *An. rivulorum* (Kamau *et al*., 2003). In agreement with the studies of Gillies and Coetzee, Kamau *et al.* (2003) also found *An. funestus* s. s. exclusively indoors suggesting its greater role in malaria transmission. Similar results have been obtained in Tanzania where the *An. funestus* s. s. was found to be the most common member of *An. funestus* complex species (Temu *et al*., 2007; Lwetoijera *et al*., 2014).

2.9.1 Sampling techniques of malaria vectors and determination of infection status

The mosquito sampling techniques depend on the objectives of the study and these range from hand collection using sucking tube (aspirator), spray sheet collection, trap collection using animal or human bait, light traps, dipping and pipetting for aquatic forms (WHO, 1975b). Some of the techniques used to determine infection status of the vectors include dissection of the salivary glands (WHO, 1975b; Fontenille *et al*., 2001) and circumsporozoite enzyme linked immunosorbent assay (ELISA) (Wirtz *et al*., 1987; Fontenille *et al*., 2001).

2.9.2 Malaria vector ecology

Anopheles gambiae favors small collection of water such as ground pools of rain water, wet foot prints, freshly flooded ditches and burrow pits for breeding. On the other hand, *An. funestus* tends to breed at the margins of Permanent water bodies such as swamps, dykes, rivers and where some emergent grass sedges or other foliage provide shelter for the larvae (Gillies and Coetzee, 1987; Minakawa *et al*., 2005). Field and similar places with shallow standing water provide an ideal situation for such mosquitoes. Studies have shown that abundance, distribution and malaria transmission by different malaria vectors are driven by different environmental conditions (Kelly-Hope *et al*., 2009). For example in coastal region of Kenya, *Anopheles gambiae* s. s. and *An. arabiensis* were found to be positively correlated with precipitation, and negatively correlated with temperature and humidity measures. This contrasts *An. funestus*, which was significantly negatively correlated with precipitation, but positively correlated with temperature, humidity and normalized difference vegetation index (NDVI). *Anopheles arabiensis* which is one of the sub species of *An. gambiae* complex prefers drier semi arid areas with high temperatures (Gillies and Coetzee 1987; Coetzee *et al*., 2000).

2.10 Life cycle of the malaria parasite

Malaria parasite has a complex life cycle which involves insect vector mosquitoes of the genus *Anopheles* and the human host (WHO, 1997; 2003). The life cycle of the malaria parasite is divided into two different phases: (i) **sporogonic cycle** in the mosquito (ii) the **erythrocytic** and **exo-erythrocytic cycles** in the human host. Mosquitoes acquire gametocyte-stage parasites from blood by feeding on an infected host. The parasites carry out fertilization in the mid gut of the vector to produce sporozoites (infective stage) which invade the salivary glands. When an infected mosquito feeds, it injects sporozoites together with its saliva into the human blood (Beier, 1998). Sporozoites are carried by the circulatory system to the liver and invade liver cells (hepatocytes). Once inside the liver cells, the intracellular parasite undergoes asexual replication known as exoerythrocytic schizogony. This culminates in the production of merozoites which are released into the blood stream where they invade the red blood cells. Further development and multiplication occurs in the red blood cells which will later burst and release the parasites into the general circulation from which they will be sucked by a feeding female mosquito (Figure 2.2). The cycle is completed in the mid-gut and salivary glands of the mosquito (Aly *et al.*, 2009). The time necessary for the development of the sporozoites varies with temperature, species of the malaria parasite and with humidity, but generally it is about 8-15 days (WHO, 1997; 2003).

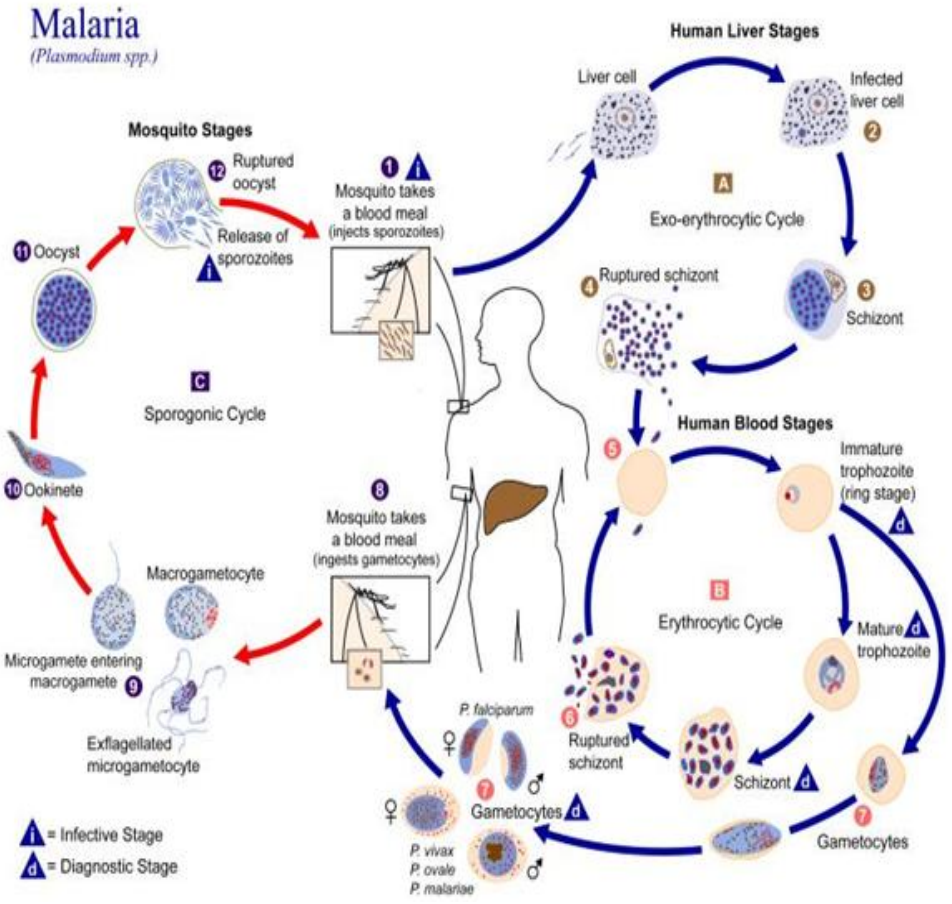

Figure 2.2 Life Cycle of Plasmodium spp. in man and the mosquito (WHO/CDS/CPE/SMT/2002.18 Rev.1 Part I)

2.11 Factors favoring RVF and malaria vector proliferation and disease transmission

Climatic factors like temperature and rainfall and environmental factors like land cover influence occurrence of vectors of RVF and malaria.

2.11.1 Effect of climatic factors on vector bionomics

Meteorological factors such as temperature, humidity and rainfall influence ecology and physiology of most insect vectors (WHO, 1975b). Mosquitoes vary in their vector potential because of climatic factors that affect their abundance, blood feeding behavior, survival and ability to support parasites (Gage *et al*., 2008). A study done in western Kenya showed that *An. gambiae* s. s. was more adapted to moist conditions and its density increased during wet season contrary to trends observed in *An. funestus* (Minakawa *et al*., 2002). These findings indicate that

future changes in climatic factors may alter species distribution. Furthermore, temporal variation of *P. falciparum* malaria prevalence in the highlands of western Kenya has been shown to be affected by meteorological variables such as rainfall, temperature and humidity (Wanjala *et al*., 2011). Increase in global temperature has led to the emergence of threat of diseases in human and animals particularly those of vector borne diseases (Dhama *et al*., 2013). As temperature increases, it disturbs the seasonality of the vector in causing disease by reducing or enhancing the survivability. Life span of vectors varies because feeding pattern, population growth rate and incubation period get affected. This has direct impact on the vector's susceptibility to pathogens and capacity to transmit them. A study done in Vanuatu showed that malaria was correlated with temperature and rainfall at the seasonal scale (Chaves *et al*., 2008).

(i) Effect of temperature on vector distribution and development

Temperature is one of the climatic factors that limit the geographical distribution of mosquito species (WHO, 1982) and affects development of both vector and parasite. In vectors, it affects rate of development of egg, larva, pupa and adult (Munga *et al*., 2007). High temperatures shorten time required for mosquito development from egg to adult and vice versa. Long development cycle due to low temperature reduce mosquito generations, puts larvae at high risk of predators and eventually reduces adult population size. By prolonging aquatic stage, temperature also determines the timing and abundance of mosquito vectors following adequate rainfall (Thomson *et al*., 2005). High temperature increases blood digestion and feeding frequency hence more host contact leading to increase in population of infective mosquitoes (Afrane *et al*., 2005). This increases fecundity and improved reproductive fitness (Afrane *et al*., 2006). Increase in temperature also shortens malaria parasite development time thus increasing proportion of infective vectors and vectorial capacity (Githeko *et al*., 2000; Afrane *et al*., 2008).

(ii) Effect of humidity on vector life span

Humidity affects rate of evaporation of water in breeding sites. Low humidity also subjects adult mosquitoes to desiccation because of their tracheal system of respiration so they only become activated when moisture increases (WHO, 1975a). High humidity favors metabolic processes in the vector, prolongs survival and allows the vector to complete its life cycle so that it can transmit the parasite to the host. Optimal humidity also enables mosquitoes to survive for longer periods allowing them to disperse further and transmit the disease (Yamana and Eltahir, 2013a). A survey

on distribution and abundance of malaria vectors in Kenya revealed that relative abundance of *An. gambiae* was positively associated with moisture index, suggesting that this species is more adapted to moist conditions (Minakawa *et al*., 2002). On the contrary, proportion of *An. funestus* was found to be higher in the dry season than the wet season.

(iii) Effect of Rainfall on vector abundance

Changing precipitation patterns increase number of good quality habitats and density of vegetation which in turn offer suitable resting sites for mosquitoes (Githeko and Ndegwa, 2001). Rainfall benefits mosquito breeding when it is moderate but may also destroy habitats and flush out larvae when it is excessive (WHO, 1982; Paaijmans *et al*., 2007). The development rate of mosquito larvae depends on hydrological types and biotic factors in water bodies (Kroeger *et al*., 2014). Water insolation and abundance of algal food determine rate of mosquito larval development (Lydzanicz and Lonc, 2003) in addition to prevailing climatic conditions. Rainfall in Kenya is variable especially in arid and semi arid lands (ASALs) and the interannual variation is higher in short rains than long rains (Koskei, 2016). In Baringo County of Kenya, rainfall is eratic, highly variable and unreliable suggesting climate variability (Muriithi *et al*., 2017; Koskei, 2016). In terms of ecology, amount of precipitation is a good predictor of abundance of vectors, their survival and possible disease transmission. In northern Mauritania, severe outbreaks of unexpected malaria and RVF were reported in several oases after a few weeks of heavy rainfall followed by flooding (El Mamy *et al*., 2011). Likewise, studies conducted in four districts of Kenya where RVF is prevalent revealed that heavy rainfall and moisture preceded RVF outbreak peaks by one month (Nguku *et al*., 2010). In Baringo, occurrence of heavy rainfall was more than one month before the onset of RVF outbreak in 2007. This suggests that these vector-borne diseases are accelerated by the amount of precipitation and that a time lag exists between onset of rainfall and disease outbreak.

Generally, it should be noted that mosquito vectors transmit disease pathogens when environmental and climatic conditions such as availability of water, temperature and humidity permit.

2.11.2 Effect of land cover on vector distribution

Malaria and RVF vector distributions are influenced by environmental factors. Studies done in India have revealed that early warning systems based on climatic variability alone cannot

correctly predict resurgence of malaria in desert and semi-desert areas (Baeza *et al.,* 2011). This is because changes in land use patterns that arise from irrigation and agriculture influence malaria incidence hence should be included in modeling (Baeza *et al.,* 2011). For example, distinct cultivation in the proximity of homesteads has been associated with childhood malaria in rural Ghana (Krefis *et al.,* 2011b). This is because landscape cover influences the distribution of vector species whereby *An. gambiae* prefers to breed in open sun-lit shallow pools of water. *Anopheles funestus* on the other hand prefers more permanent bodies of water with emergent vegetation while *An. arabiensis* prefers to breed in low and hot areas (Gillies and Coetzee, 1987). The presence of *An. arabiensis* in high altitude areas could be as a result of human activities such as deforestation or swamp reclamation, which modify the micro-climatic conditions and create environmental conditions that favor mosquito breeding and invasion of new species (Afrane *et al.,* 2011). The unmitigated environmental changes lead to rise in temperature and thus enhancing the spread and survival of malaria vectors and development of malaria parasites (Himeidan and Kweka, 2012).

On the other hand, high vegetation density index around ponds has been shown to correlate positively with RVF transmission (Soti *et al*., 2013) suggesting suitable environment for the vectors. Soil type may also determine the prevalence of RVF as it influences moisture retention, vegetation type, drainage and flooding (Nguku *et al*., 2010). Furthermore, the *Aedes* eggs infected with RVF virus remain infectious in the soil until heavy flooding when mass breeding resulting in epizootics and epidemics. The soil types which have been associated with RVF are solonetz, solonchaks and planosols; a classification based on Food and Agriculture Organization (FAO, 2006).

2.11.3 Effects of breeding sites and animal shed on adult mosquito abundance

Proximity to breeding sites and presence of animals near human habitation can affect mosquito vector abundance and distribution. A study in the Gambia found that mosquito density was lower in villages more than 3km from breeding sites than those closer to the breeding site (Clarke *et al*., 2002). Proximity to breeding sites (dams) in Ethiopia was found to have effect on mosquito abundance and infection rates of malaria vectors (Kibret *et al*., 2009). The villages nearer the water reservoirs had high abundance of mosquitoes and infection rates while control villages had no infected mosquitoes. A study in western Kenya found a strong association between distance

from breeding site and presence of cattle on indoor resting density of *An. arabiensis* (McCann *et al.*, 2017). Animals tethered close to the house lowered mosquito abundance in the house (Kirby *et al*., 2008) in the Gambian villages and a shift of biting mosquitoes from human to animals outdoors has also reduced malaria in coastal Kenya (Wanganui *et al*., 2013).

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS

3.1 Study area description

The study was conducted in Baringo County of Kenya from 2014 to 2016. The area lies between 35.602^0 - 36.277^0 E, and 0.541^0- 0.723^0 N at altitudes ranging between 870 and 2499 m above sea level. The region is inhabited by Tugen, Ilchams and Pokot communities who are mainly agro-pastoralists.

The mean annual rainfall is about 650mm with temperature ranges between 30 ^{0}C to 37 ^{0}C. There are four lakes within the study area, three to the east and one to the west. Two of the lakes on the eastern side are permanent and these are the 130 sq km L. Baringo with fresh water, located to the north and the 34 sq km L. Bogoria with salty water, located to the south. Between the two permanent lakes is Lake 94 which lies in a marshy area and is seasonal. Lake Kamnarok is an oxbow lake which is also seasonal and lies next to the Kerio River within the Kerio valley on the western side of the county. The county has several rivers most of which are seasonal except R. Perkerra, R. Molo and R. Kerio. Both R. Perkerra and R. Molo drain into L. Baringo. The numerous seasonal rivers in the area cease to flow during the dry season and are most often characterized by pockets of small pools along the riverbed, which provide suitable breeding habitats for mosquitoes. There also exist dams that form focal points where humans and livestock aggregate to access water especially during the dry seasons.

Baringo County experiences four seasons described as follows: cold dry season in the months of June, July and August; short rains in September, October and November; Hot dry season in December, January and February; long rains in March, April and May. However, recent analysis of rainfall trends in Baringo showed extreme seasonal variation in monthly rainfall. The County suffers from intensive floods with severe droughts leading to alterations of seasons (Koskei, 2016).

The study area was divided into four ecological zones lying adjacent to each other in a west-east direction (Figure 3.1). From east to west, the zones were: the lowland (≤1000m above sea level), the midland area (1000-1500m above sea level), the highlands (1500-2300m above sea level),

and the riverine zone (1000-1300m above sea level). There was an over wrap in altitude between riverine zone and midland zone but riverine zone was different from midland by having different vegetation cover and a gentle slope compared to midland whose landscape was interspersed with low hills. Riverine zone was parallel to the Kerio valley on the western side of the highland zone while midland was on the eastern side of the highlands and next to lowland zone.

(i) Lowland zone

The lowland zone receives an annual rainfall of about 600mm and the main vegetation cover is *Prosopis juliflora* ("Mathenge" plant). There is mixed farming in this zone and main crops are onions, tomatoes, water melons and maize in the Perkera irrigation scheme. Bee keeping for commercial production of honey is also practiced. This zone has a slope of less than 4° making it prone to seasonal flooding during the rainy seasons. The occasional flooding is particularly worse around Lake Baringo where homes, farms and some schools are sometimes submerged under water leading to displacement of inhabitants. Permanent rivers; R. Perkera and R. Molo also flood during the rainy seasons. Marigat town, the second largest urban centre in Baringo is found in the lowland zone. The region experiences extremely high temperatures throughout the year.

(ii) Midland zone

The midland zone is interspersed with seasonal rivers that flow only after the heavy rains in the highlands. The seasonal river beds are dry most of the time with small stagnant pools of water which are sunlit therefore suitable for mosquito breeding particularly anopheline species. The midland slope is between 20-30° and the main vegetation cover is Acacia/Commiphora bushes which appear dry and dead but become green when there is rainfall. Except for the acacia bushes, there is no ground vegetation as the ground surface is covered with small stones and no fertile soil. Therefore, there is no crop farming in the midland due lack of fertile soil. Water pans and dams are the main sources of water for both humans and there livestock.

(iii) Highland zone

The highland area comprises of the Tugen Hills with indigenous forests as well as planted exotic forests. The area has very steep terrain of between 30-40° and rainfall ranges between 1000-1500mm per annum (KIRA, 2014). The zone has cooler temperature than lowland and midland.

Permanent water springs are common and provide areas suitable for mosquito breeding. The highland region has well drained soils hence farming of crops like maize and vegetables is practised besides keeping animals. The county head quarter in Kabarnet town is located in the highland zone.

(iv) Riverine zone

The riverine zone has a slope of less than 6° making it also prone to flooding. The region is characterized by dry riverbeds with small pools of stagnant water similar to midland zone. Altitudes above 1200m asl are mainly rock with expansive ground areas covered with continous rock. There is mixed vegetation of acacia and other tree species. The main water sources are Lake Kamnarok and Kerio River. Swamps, dam and lake edges provide suitable breeding sites for mosquitoes throughout the year. The lower part of riverine zone along the Kerio River has fertile soil hence farming is practiced.

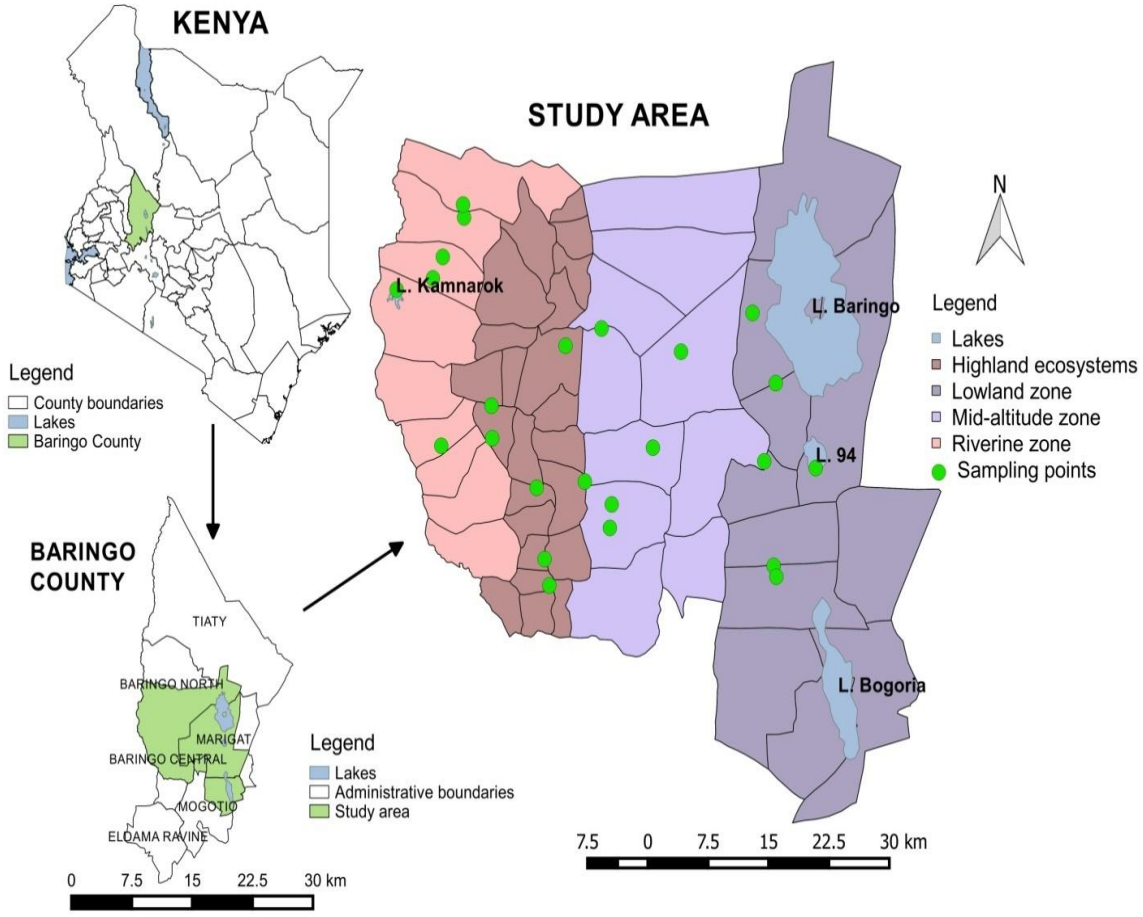

Figure 3.1 Map of study area showing sampling sites in Baringo County

3.2 Study design

The study design was longitudinal in which the mapped mosquito breeding habitats and houses were sampled for mosquitoes repeatedly. The area was stratified into four ecological zones based on elevation. Randomized and purposive sampling of mosquitoes was done. Sampling points were determined by generating 100 random points per stratum using the random points tool in Quantum GIS. The random points were converted to a KML file (Keyhole Markup Language for storing geographic data**)** and exported into Google Earth where only points close to water bodies and accessible by road were chosen (Ochieng *et al*., 2016).

The breeding sites were mapped in each of the four zones making a total of 24 sites in the entire study area. The sample of six per zone was used to include all possible breeding habitats and to sample equal number of sites in all zones so as to determine mosquito species diversity and

distribution. Purposive sampling was done in areas known to be hotspots of rift valley fever like Salabani and Sirata in the lowland zone of the study area

Houses nearby breeding sites were selected for monthly indoor adult sampling. The houses were categorized based on materials used for roofing and making the wall.

3.3 Sampling and identification of mosquitoes

Larvae and pupae were sampled from different types of breeding habitats spread across all the study zones. They were collected once a month using a standard 350ml dipper (Walton, 2005) and a pipette from the 24 mapped sites.

Adult mosquitoes were sampled from both indoor and outdoor places once every month in the 24 mapped sites. Indoor resting mosquitoes were collected from different types of houses by pyrethrum spray catch method (WHO, 2003) while outdoor resting mosquitoes were collected by CDC light traps (Lutomiah *et al.,* 2013). Pyrethrum spray catch method was used because it is well suited for collecting indoor resting mosquitoes which are assumed to have fed on humans while CDC light trap attracts outdoor mosquitoes at night.

Both adult and larval mosquitoes were identified morphologically under the dissecting microscope but pupae were left to emerge then identified at adult stage. Some of the features used to identify larvae included siphon, mesopleural hairs and abdominal tergal plates. *Anopheles gambiae* and *Anopheles funestus* larvae were distinguished from each based pleural hairs and tergal plates (Gillies and Coetzee, 1987). The features used to identify adult mosquitoes included palps, scales, wing and leg markings. *Anopheles gambiae* complex adult mosquitoes were further identified to sub species level by polymerase chain reaction (PCR) technique. Briefly the method is based on species-specific nucleotide sequence in the ribosomal DNA intergenic spacer (Scott *et al*., 1993).

3.4 Determination of mosquito infection status with *Plasmodium falciparum*

Anopheline mosquitoes (*An. gambiae* and *An. funestus*) were screened for *Plasmodium falciparum* sporozoites (malaria parasites) by enzyme-linked immunosorbent assay (ELISA) technique (Wirtz *et al*., 1987). Briefly the sandwich ELISA was used to identify *Plasmodium falciparum* antigen. The wells of microtiter plate were coated with monoclonal (MAb) antibodies. Non-specific binding sites were blocked using bovine serum albumin. Mosquito triturate sample

to be tested was then added to the coated wells after washing. Enzyme linked MAb antibody was added to bind antigens in the test sample to display a visible colour.

3.5 Meteorological factors

Monthly maximum and minimum air temperatures were sourced from online website of the International Research Institutes (IRI) of climate and Society's database (Ceccato *et al.,* 2010; Funk *et al.,* 2014) during the study period. The monthly average rainfall data used was obtained from University of California Santa Barbara (UCSB) Climate Hazards Group Infra Red Precipitation with Station Data (CHIRPS) v2p0 (Funk *et al.*, 2014).

3.8 Ethical considerations and clearance

The study involved intrusion of privacy during collection of indoor resting mosquitoes, therefore prior to sampling, signed consent was sought from the household heads. The investigators involved in mosquito sampling were provided with protective gear including overalls, face mask and gum boots. The study was approved by the Kenyatta National Hospital and University of Nairobi Ethics and Research Committees (KNH-ERC/R/75) (Appendix 1).

3.9 Community involvement

The study was introduced to the administrators in the County headquarters in the Minisry of Health, Ministry of Agriculture and Veterinary Services and Ministry of Education. The information about the intended research was then communicated to the Sub County heads and down to the area chief and assistant chiefs who were sensitized on the benefits of the investigation to the community. The field assistants who were involved in data collection were from the nearest villages where they are known. At the end of the study, a series of meetings to disseminate research findings were held starting from Sub County levels to the villages where public education was done and publicity materials printed with control and protection messages were distributed (Appendix 2). This was possible because the current study was part of a larger project.

CHAPTER FOUR

4.0 EFFECTS OF SEASONAL VARIABILITY ON RVF AND MALARIA LARVAL VECTOR SPECIES DIVERSITY AND BREEDING HABITAT PREFERENCE IN BARINGO COUNTY

4.1 Introduction

Mosquitoes are responsible for the transmission of many diseases among human and other animal populations across the world. Out of the 3,000 known species of mosquitoes, about 100 are vectors of human diseases (WHO, 1997). Anopheline vectors that transmit malaria flourish optimally in tropical Africa where the climate and ecology provide ideal conditions (WHO/UNICEF, 2005). Mosquito borne diseases are particularly common in tropical climates of developing countries where the risks are greater especially among the poor. In most cases the vulnerable households reside close to high risk areas such as vector-breeding sites (Konradsen *et al.*, 2003).

Information on the diversity and habitat preference of endemic vector species is essential to develop vector monitoring and control strategies. However, this depends on the identity of mosquito species present in each locality for effective implementation of vector control and management strategies (Kweka *et al*., 2012; Bond *et al*., 2014; Vanlalruia *et al*., 2014). Larval control is one feasible strategy that can form part of an integrated vector management policy particularly in semi-arid areas where larval habitats are discrete and limited in time and space (WHO, 2013). Knowledge on larval species diversity and habitat preference in different ecological settings is useful in integrated vector control. Seasonal changes have been demonstrated to have different effects on mosquito vector species (Kweka *et al*., 2012). It is important, therefore, to sample the same area at different seasons due to fluctuations in climatic conditions (Mendoza *et al*., 2008). Seasonal sampling gives information on species richness and abundance of vector species. Ecological surveys on mosquitoes depend on the objective of the study and some researchers prefer to sample aquatic larval stages because it is easier to locate the potential breeding habitats (Mendoza *et al*., 2008). Baringo County is highly heterogeneous in topography, thus this study endeavored to determine larval species diversity and habitat preference of potential vectors of RVF and malaria in selected ecological systems. Previous

studies in Baringo have been limited to surveys around Lake Baringo and Lake 94 ecological systems (Aniedu, 1992; Sang *et al*., 2010, Mala *et al*., 2011a, b; Omondi *et al*., 2015). Rift valley fever and malaria vector diversity and abundance in other ecological systems in Baringo have not been documented. The effect of seasonal changes on species diversity and their breeding habitats also remain un-established. The objective of this study was to investigate the effect of seasonal changes on larval species diversity and habitat preference of RVF and malaria vectors in more diverse ecological systems covering a large area of Baringo County.

4.2 Literature review

4.2.1 Mosquito larval habitats

Larval habitats or breeding sites are places where eggs are laid, hatch into larvae and pupate then emergence of adults occur (Rejmánková *et al.*, 2013). Female mosquitoes lay eggs on water where they hatch into larvae then develop further into pupae, both of which are purely aquatic. Diversity of mosquito breeding habitats include; small rain pools, hoof-prints, drains and ditches, brackish water, stream edges, ponds, lakes, swamps and marshes where larvae usually occur in vegetation around the edges (WHO, 1997; 2003). Knowledge of larval habitats, their distribution, and productivity is important in planning and implementing larval control strategies (Shililu *et al.*, 2007). Collecting larvae from different types of breeding sites in an area makes it possible to determine the species present and ascertain the preferred breeding habitats of each vector species (WHO, 1997; 2003).

4.2.2 Larval habitat preference by mosquitoes

The water bodies where mosquitoes breed are more or less specific to each species (Bashar *et al*., 2016). *Anopheles* mosquitoes prefer to breed in water that is exposed to the sun. The anopheline mosquito larvae are supposedly not common in urban polluted water bodies but there is evidence to show that they are adapting to survive in polluted water (Mattah *et al*., 2017). This corroborates the findings of a study in Tanzania in which it was not possible to identify clear ecological characteristics of the breeding requirements of *Anopheles* species larvae (Sattler *et al*., 2005). It is, therefore, important that every stagnant open water body even the polluted ones be considered as potential malaria vector breeding habitats. Culicine mosquitoes, however, breed in a wide range of habitats with slight differences among individual species (Muturi *et al*., 2007). A

survey conducted in Kibwezi, Kenya showed that *An. gambiae* and *Culex quinquefasciatus* were found in all surveyed habitats while *Aedes aegypti* was only found in water storage tanks (Mwangangi *et al*., 2009). This shows that different species prefer some specific breeding habitats which make it easier to target them during control efforts.

4.2.4 Larval mosquito species diversity and effect of seasonal changes

Many factors can affect mosquito species diversity in an area. These factors include but not limited to altitude, season and type of breeding places (Mendoza *et al*., 2008). Representative zones should, therefore, be selected when carrying out a larval survey. Mosquito diversity parameters like species richness and abundance can be compared between areas and seasons (Mendoza *et al*., 2008). It is possible to get differences in mosquito diversity between different breeding habitats with varying levels of physicochemical factors (Emidi *et al*., 2017). Areas with more diverse breeding habitats are likely to have higher mosquito species diversity than areas with few breeding sites (Muturi *et al*., 2007).

Seasonal changes affect availability of breeding habitats and their productivity. A study in southern Ghana found significantly higher larval mean in the wet season than dry season (Mattah *et al*., 2017). However, dry season and end of short rainy seasons were found to be highly productive in western Kenya though the habitats were few (Kweka *et al*., 2012). Management of larval habitats particularly during the dry season may help reduce adult vector densities and consequently disease transmission (Mwangangi *et al*., 2009). It was also found that seasonal changes affect species differently whereby *An. gambiae* s.l. showed no seasonal fluctuations while *An. funestus* and culicine larvae were significantly different in seasons (Kweka *et al*., 2012). Monthly variation in species diversity was observed in a village in Mwea, central Kenya (Muturi *et al*., 2007) an indication that season has an effect on some species.

4.2.5 Larval control strategies

Targeting immature mosquitoes is an appropriate vector control strategy since they are confined in small aquatic habitats where they cannot escape as opposed to adults which are highly mobile (Killeen *et al*., 2002). The control of larvae, also referred to as source reduction, involves environmental management which is categorized into two forms namely environmental modification and manipulation (WHO, 1982). Environmental modification is long-term and can be achieved through alteration of the breeding sites of the vectors by filling ponds and marshes

on a permanent basis. Environmental manipulation is short-term and can be done repeatedly by removing vegetation from ponds and canals and clearing premises (WHO, 1997). Larval control through environmental management should be implemented during the dry season when habitats are fewer and manageable (Mala *et al*., 2011a; Kweka *et al*., 2012; WHO, 2013). Another method of source reduction is application of larvicides to breeding sites. Spraying breeding water surfaces with synthetic inorganic larvicides like temephos or petroleum oils is utilized to kill the immature stages of mosquito vectors. However, this may lead to environmental pollution and development of resistance by mosquitoes to the chemicals used (WHO, 1997).

Biological control is the use of natural enemies to control pests and disease vectors mostly by targeting the immature stages of the mosquito vectors without polluting the environment. The biological agents commonly used to control mosquitoes include larvivorous fish, viruses, parasites (protozoa, fungi, parasitic worms) and bacteria that infect mosquito larvae (Benelli *et al*., 2016). Other organisms used include predatory mosquitoes and spiders. Most of these organisms have been suggested and tested experimentally but have not been implemented on large scale. The ones that have been implemented for mosquito control include Mermithid nematodes, fish and bacteria. Mermithid nematodes have been used to control mosquitoes in Mexico and U.S.A. (Kerwin and Washino, 1985). The mosquito fish, *Gambusia affinis*, is widely used by public health and mosquito control agencies throughout the world to help reduce mosquito breeding. This species is preferred because of its adaptability, resistance to unfavourable conditions and the ability to produce many young ones within a short period of time. Larvae of *Toxorhynchites* mosquitoes and nymphs of dragonflies are also predatory hence feed on mosquito larvae. Cyclopoid copepods are tiny crustaceans that attack first and second instar larvae of mosquitoes have been used successfully in Vietnam (Nam *et al.,* 1998). Plant products like Neem (*Azadirachta indica*) oil extracts have larvicidal properties while Azolla, which is a free-floating fern that covers the water surface completely, may kill the mosquito larvae (WHO, 1997). Other biological control agents are parasitic nematode worms, fungi that grow on bodies of mosquito larvae and bacteria that produce toxic products. Bacterial larvicides such as *Bacillus thuringiensis-Bti* and *B. sphaericus-Bs* are among the most widely used biological agents. Biological control methods have an advantage over chemical methods because they are non-toxic to fish, mammals and most other non-target organisms in the environment.

4.3 Materials and Methods

4.3.1 Mapping of breeding habitats

Briefly, during the preliminary survey, diverse larval habitats were observed and after randomization, the habitats representing rivers, swamps, ditches, water pans, lake margins from the road and foot paths accessible were chosen for sampling in each zone. Twenty four sites (figure 3.1) with potential mosquito breeding habitats were identified and mapped with geo-positioning equipment (GPS) during preliminary survey. Breeding habitats found in the heterogeneous topography of Baringo were selected and sampled longitudinally. They were sampled for a period of 24 months to assess vector species diversity as a risk of mosquito borne diseases to humans and their livestock. The breeding habitats that were mapped and sampled regularly included L. Kamnarok covered with floating plants, water pits with hoof prints, water springs, water pans, riverbeds, ditches and dams (plate 4.1).

Plate 4.1 Breeding habitats that were sampled regularly between 2014 and 2016 in Baringo County

A- Lake Kamnarok covered with small floating plants, **B**- Water pit with hoof prints, **C**- Water Spring, **D**- Water pan without vegetation, **E**- Water pan with algae and grass, **F**- River bed pool, **G**- Edge of Lake Baringo at Salabani, **H**- Ditch

4.3.2 Sampling and identification of larvae

Larval mosquitoes were sampled on a monthly basis between June 2014 and May 2016 for a period of 24 months. Each breeding habitat was assessed by visual inspection then a dipper and pipette were used to scoop water and pick the larvae respectively. Ten to twenty dips, depending on size of habitat, were made using the standard dipper (350ml) to collect immature mosquitoes. Habitats which were more than 50 metres square received a maximum of 20 scoops. The larvae in the dipper were transferred into a collecting vial by a pipette while counting (WHO, 1975a). Larvae from highland and riverine zones were transported to Kabarnet hospital where a room was provided for further processing which involved immobilizing larvae before identification or preserving was done. Mosquitoes from lowland and midland zones were taken to Marigat laboratory for division of vector borne diseases where further processing was carried out. The two laboratories were used for convenience and efficient because the sampling sites were far away from each other. Marigat is located in the lowland (Baringo South Sub County) while Kabarnet is located in the highland zone (Baringo Central Sub County).

Larvae were examined under the dissecting microscope and identified morphologically using taxonomic keys described by Highton 1983; Gillies and Coetzee, 1987. Features that were used for morphological identification of mosquito larvae included; shape of siphon, number of combs, pectin on edge of siphon, length of siphon relative to saddle, tergal plates and abdominal hairs. Figure 4.1 shows features of a larva usually used for identification.

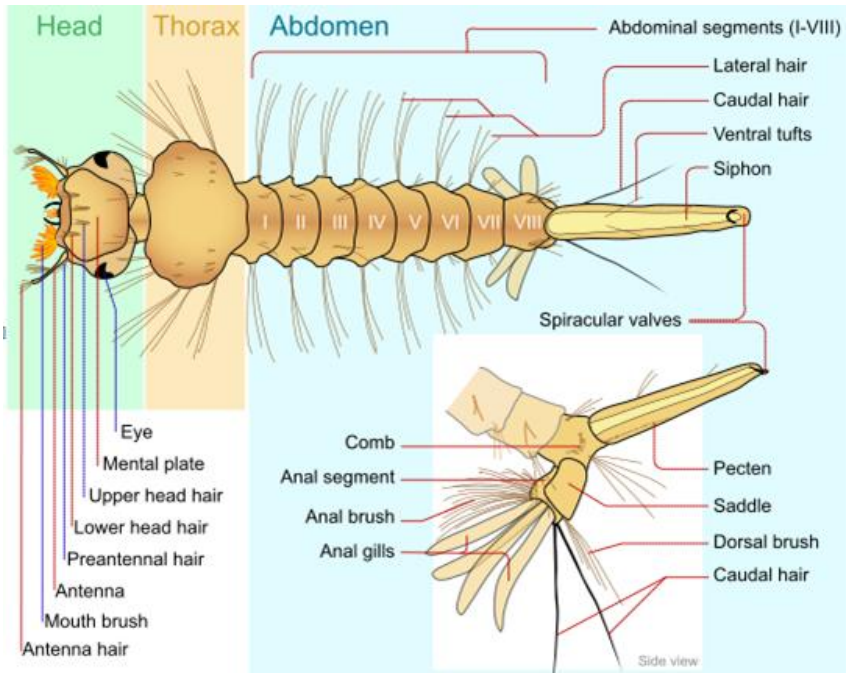

Image obtained from Wikimedia commons authored by Lady of Hats for use in the public domain

Figure 4.1 Diagram of culicine larva showing morphological features

Pupae were collected from the breeding habitats together with the larvae. They were included in the study of larvae to caution against the mistake of missing any species that might not be captured at larval stage. Since the morphological features of pupae are not clear, they were left to emerge to adults for proper identification. The container of water in which they were collected was covered by gauze or net material to prevent the newly emerged adults from escaping (WHO, 1975a). Alternatively, pupae in the open container with natural habitat water in which they were collected were left to emerge in a re-sealable improvised container where they could not escape. After emergence of adults, identification was done by observing morphological features under the dissecting microscope (Highton, 1983; Gillies and Coetzee, 1987) and recorded in the immature/larval mosquito database. All *Mansonia* species were collected at pupa stage only and left to emerge before identification was done.

4.3.3.1 Analysis of seasonal species diversity

Average monthly temperature and rainfall were used to represent the climatic conditions for each season during the sampling period. Information on mosquito species diversity in Baringo County

for each season was estimated using Shannon diversity index. This index was selected because it combines species richness and abundance and also sensitive to rare and abundant species (Morris, 2014). Berger Parker index was also considered to indicate the proportion of the most abundant species in each climatic season. Pair wise comparison of larval species diversity was made between the seasons using Shannon diversity *t*-test as proposed by Hutcheson (Hutcheson, 1970) based on the following equation:

$$t = \frac{H'_1 - H'_2}{s_{H'_1 - H'_2}} \text{ where } s_{H'_1 - H'_2} = \sqrt{s^2_{H'_1} + s^2_{H'_2}}$$

with each variance of H' estimated by

$$s^2_{H'} = \frac{\sum fi \log^2 fi - (\sum fi \log fi)^2 / n}{n^2}$$

H- Shannon diversity index for each of the two samples

S- Species richness (total number of species)

S^2- Variance of each sample (H_1 and H_2)

n- Total abundance (number of individuals)

fi- proportion each species makes towards total

4.3.3.2 Analysis of habitat preference

Larval density per habitat was determined by dividing total number of larvae by number of dips to get the mean. The mean was then standardized by multiplying by highest number of dips since different numbers of dips were taken (10 to 20) based on size of breeding habitat. A test of the data for normality using Shapiro-Wilk test showed that data was not normally distributed. Thus it was log transformed ($Log_{10}n+1$ to get rid of zeros) to reduce skewness and improve normality. After the transformation and re-testing it showed normal distribution. One way ANOVA was then used to compare mean larvae in each habitat so as to determine habitat preference by malaria and RVF vectors. When significant differences were observed in ANOVA, Tukey test was used to separate the means (Muturi *et al*., 2007).

4.4 Results

4.4.1 Larval species diversity and effect of season in Baringo County

A total of 7724 immature mosquitoes comprising of 17 species belonging to four genera (*Anopheles, Culex, Aedes* and *Mansonia*) were identified from various sites in the four ecological zones in the study area. The 17 species included five anopheline species, three *Aedes* species, eight *Culex* species and *Mansonia* species. Among the 17 species identified three *Anopheles* species are responsible for malaria transmission; *An. gambiae* (8.1%), *An. funestus* (0.1%) and *An. pharoensis* (15.4%). *Anopheles gambiae* was notably low in abundance during the long rain season (n=61) and recorded highest abundance during short rain season (n=263) while *An. pharoensis* recorded lowest abundance (n=127) during short rain season. Statistically there was no significant difference between seasons in *An. gambiae* abundance ($F_{3, 406}$ =2.115, p=0.098) but *An. pharoensis* was significantly different in abundance between seasons ($F_{3, 406}$ =4.544, P=0.004) with short rain season producing less larvae than long rain season (p=0.012). On the other hand, *Mansonia* species constituted 0.42% while *Culex* species which are secondary vectors of RVF constituted 59.97%. The genus *Culex* was represented by 8 species dominated by *Cx. quinquefasciatus.*

Highest Shannon diversity index of species was observed during the cold dry season (*H*=1.99) whereas the lowest diversity was recorded during the long rain season (*H*=1.60) (Table 4.1). *Culex quinquefasciatus* was the dominant species during three of the four seasons except long rain season when *Aedes aegypti* was the most dominant species constituting 42.9% of total larvae collected (Berger-Parker=0.429). *Culex quinquefasciatus* constituted 40%, 28.1% and 28.3% respectively during the short rain, cold dry and dry season. These variations in abundance were not statistically different ($F_{3, 406}$ =0.036, P=0.991). Species evenness was similar for three seasons (e^H/S=0.4) except for the cold dry season which had a slightly higher index (e^H/S=0.56).

Table 4.1 Effect of season on species diversity of larval mosquitoes in Baringo County

Species/Season	Cold dry Season	Dry season	Long rain	Short rain
An.gambiae s.l.	179	113	61	263
An. Pharoensis	346	375	326	127
An.coustani	77	3	0	4
An.funestus	6	0	0	0
Cx.pipiens	316	116	267	190
Cx.quinquefasciatus	533	497	568	569
Cx.annulioris	209	417	177	166
Cx.poicilipes	117	48	11	56
Cx.tigripes	62	43	29	21
Cx.dutoni	18	123	14	6
Aede taylori	5	4	0	3
Aedes aegypti	5	4	1099	1
Aedes africanus	0	4	0	11
Mansonia spp	21	4	3	4
Cx. univittatus	0	0	6	0
Cx.vansomereni	0	1	0	0
An.rufipes	0	0	2	0
Taxa_S	13	14	12	13
Individuals	1894	1752	2563	1421
Shannon_H	1.99	1.832	1.599	1.716
Evenness_e^H/S	0.563	0.446	0.412	0.428
Berger-Parker	0.281	0.284	0.429	0.400

Pair wise comparison between the four seasons by Shannon diversity t-test showed that they were all significantly different from each other in species diversity with cold dry season having the highest Shannon diversity index and long rain with lowest index. Species richness was highest during the dry season (S=14) and lowest during the long rain season (S=12). When the four seasons were merged into two groups referred to as dry and wet seasons, still there was a significant difference ($t=7.57$, $df=7570.1$, $p<0.000$) with the dry season having a higher species diversity than the wet season.

Analysis of average rainfall and temperature for each season was done to find out which component of climate affected species diversity. The cold dry season which had a moderate mean rainfall of 79.66mm, lowest minimum and maximum temperatures had the highest species diversity index (H=1.99) and highest species evenness (e^H/S=0.56). It was also noted that the

cold dry season unexpectedly had more rainfall amount than the short rain season (Appendix 3). The long rain season which had highest amount of rainfall had the lowest diversity index (H=1.59).

4.4.4 Habitat preference by culicines and anophelines

A total of 29 breeding habitats classified into 9 categories were sampled for several months when they contained water. Overall, 411 samples of larvae were taken from all habitats during the study duration and the most sampled habitat type was seasonal river bed at 5 sites totaling 81 samples (Table 4.2). This was closely followed by lake margin at 4 sites and water spring at 3 sites. The less common habitats were Dams and swamps found only in two sampling sites.

The ditch had the highest mean of anopheline larvae (16.6 larvae per sample) followed by swamp (12.4 per sample) and seasonal river bed (10.7 per sample). The ditch was the only habitat which had higher mean of anopheline larvae than culicine larvae unlike the swamp and seasonal river bed. In the swamp, culicine larvae mean was almost double that of anopheline larvae. Concrete tank was the least sampled type of habitat but had highest culicine larval mean (333.7 larvae per sample) with a low mean of anopheline larvae (2.6 larvae per sample). Water pit and water pan had the lowest mean of anopheline larvae, 1.4 and 1.8 larvae per sample respectively (Table 4.2).

Table 4.2 Average number of larval species in different habitat types

Habitat type	Number of habitats	Number of samples	Anophelines x20 dips	Mean per sample	Culicines x20 dips	Mean per sample
Concrete tank	3 (10.3%)	7	18	2.6	2336	333.7
Dam edge	2 (6.9%)	25	164	6.6	333	13.3
Ditch	3 (10.3%)	54	896	16.6	546	10.1
Lake margin	4 (13.8%)	63	477	7.5	1099	17.4
River bed	5 (17.4%)	81	867	10.7	1444	17.8
Swamp	2 (6.9%)	35	434	12.4	799	23.5
Water pan	3 (10.3%)	27	48	1.8	370	13.7
Water pit	4 (13.8%)	50	70	1.4	844	16.9
Water spring	3 (10.3%)	69	271	3.9	2682	38.9
Total	29 (100%)	411	3245		10453	

Overall, breeding habitats were significantly different in terms of larval density ($F_{8, 334}$=2.090, p=0.036). Multiple comparisons by post hoc test showed that the concrete tank with a mean of 333.7 per sample was significantly different from dam edge, lake margin, river bed; water pan and water pit (Appendix 4). However, the concrete thank was not statistically different from ditch, swamp and water spring which had relatively high means per sample (Figure 4.2). When concrete tank was excluded from the analysis because of its outstandingly high mean, there was no significant difference between all habitats ($F_{7, 328}$=0.866, p=0.534).

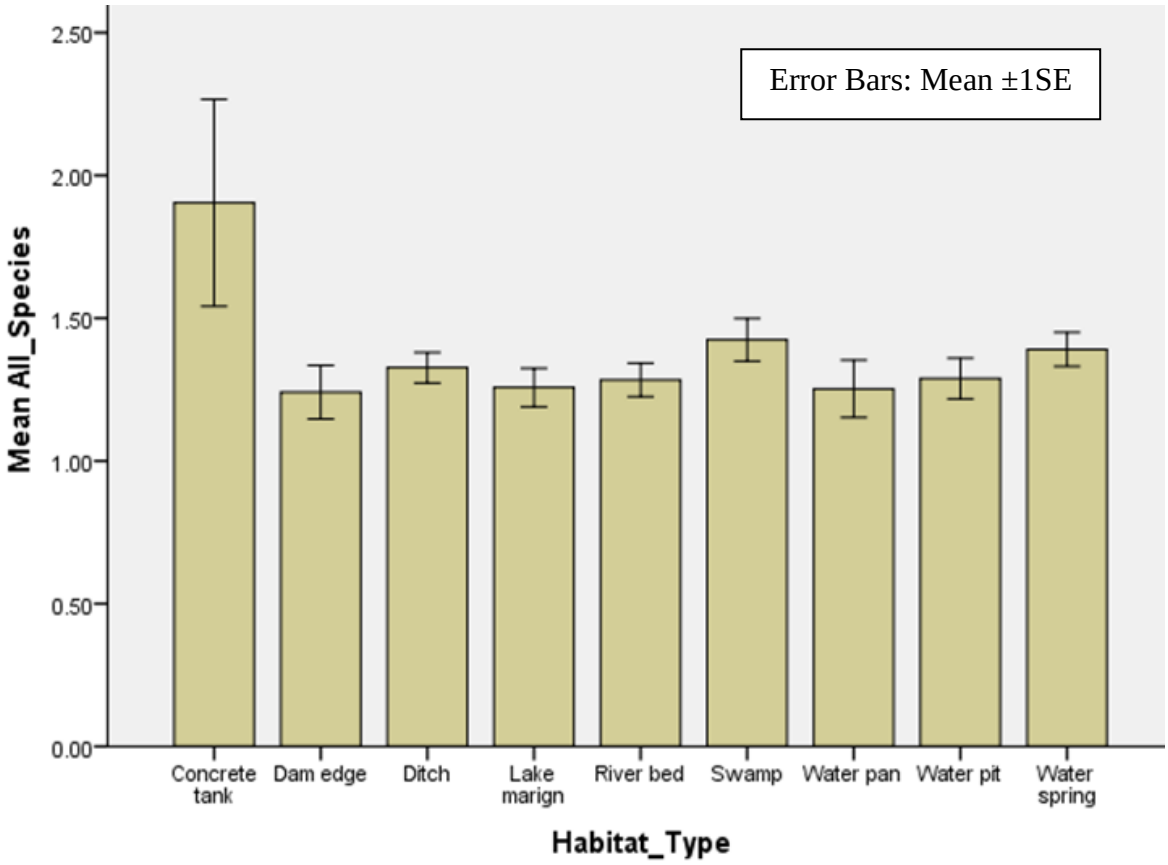

Figure 4.2 Mean of all larval species in all sampled breeding habitats in Baringo

A separate analysis involving anophelines only showed that habitats were significantly different in terms of larval mean per sample ($F_{8,401}$=9.595, p<0.000). Multiple comparisons by post hoc test showed that the ditch which had the highest mean of ano1pheline larvae was significantly different (p<0.05) from all other habitats except Swamp (p=0.233), dam edge (p=0.728) and concrete tank (p=0.162) (Appendix 5). There was no significant difference between concrete tank and all other habitats in terms of anopheline larval mean (Figure 4.3).

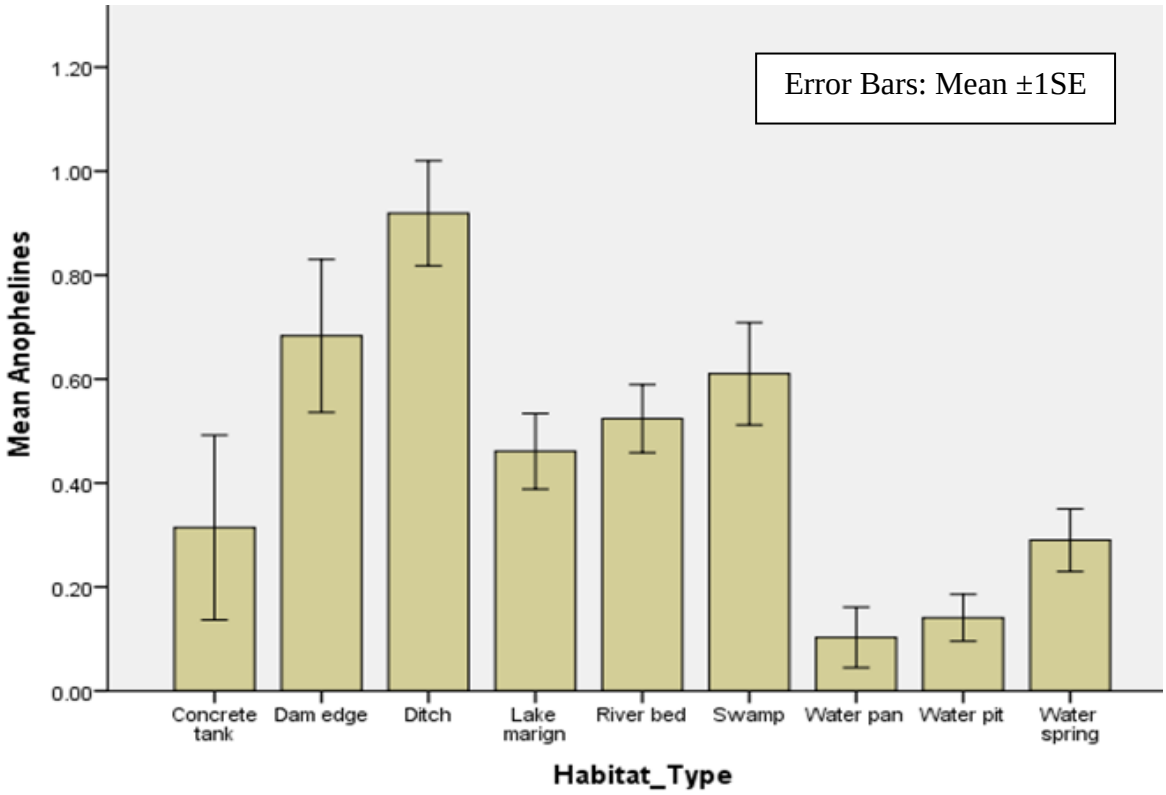

Figure 4.3 Mean of anopheline larvae in all sampled breeding habitats in Baringo

When analysis was done for culicines only, there was also a significant difference between all sampled habitats ($F_{8, 401}$=4.903, p<0.000) and when concrete tank was excluded ($F_{7,395}$=5.670, p<0.000). Concrete tank, dam edge and water pan were not significantly different from all other breeding habitats in terms of culicine larvae mean (Appendix 6). On the other hand, water spring which had second highest culicine larvae mean was significantly different from ditch, lake margin and river bed (P<0.05). Though the swamp had a larval mean ranking 3rd highest, it was only different from ditch and lake margin (Figure 4.4).

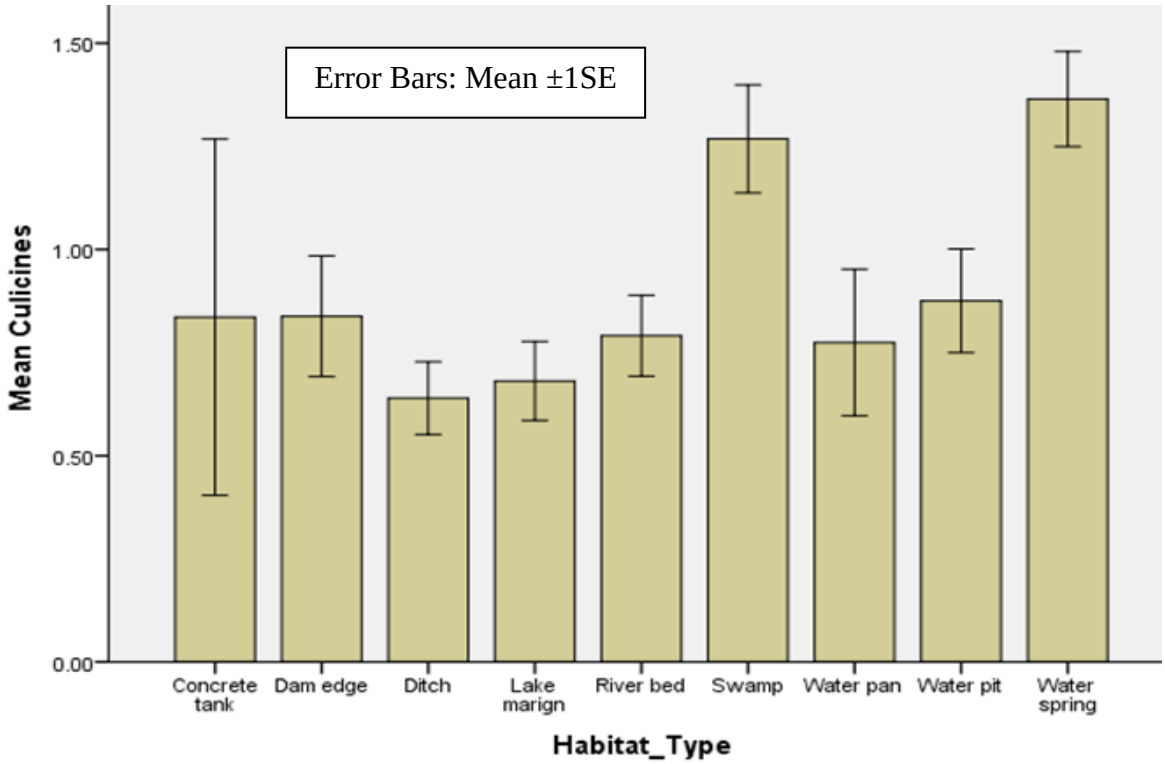

Figure 4.4 Mean of culicine larvae in all sampled breeding habitats in Baringo

4.4.4 Occurrence of malaria and RVF vectors among the surveyed larval habitats

Out of the five anopheline species identified, *An. funestus, An. gambiae* s.l. and *An. pharoensis* are the known malaria transmitting mosquitoes in Baringo. *Anopheles funestus* was only collected from the ditch in the lowland zone while *An. gambiae* and *An. pharoensis* were collected from all types of breeding habitats in all ecological zones. For *An. gambiae,* riverbed pool had the highest mean of larvae per sample (2.6) followed by ditch and swamp with a mean of 2.5 and 2.1 per sample respectively. *Anopheles pharoensis* on the other hand was highest at 6.9 larvae per sample in the ditch followed by swamp and riverbed pools with a mean of 4.0 and 3.5 per sample respectively. Ditches, river bed pools and swamp were the most three preferred breeding habitats by malaria vectors followed by lake margin (Table 4.3).

Further analysis to determine *An. gambiae* habitat preference showed a significant difference between habitats ($F_{8, 401}$=3.891, p<0.000). Post hoc test (Appendix 7) showed that the water pit which had the least mean of *An. gambiae* larvae per sample was significantly different from the ditch (p=.001) and river bed (p=0.011) which had relatively high means. The water pan and water

pit were the least preferred habitats by *An. gambiae*. Analysis to determine habitat preference by *An. pharoensis* showed an overall significant difference between habitats ($F_{8, 401}$=5.949, p=0.000). Post hoc test (Appendix 8) showed that the ditch which had the highest mean of *An. pharoensis* was significantly different from river bed and lake margin although they also had relatively high means (p<0.005). There was no significant difference between the ditch and the swamp which had the second highest mean of *An. pharoensis* larvae (p=0.674).

Table 4.3 Malaria vector mosquito larval mean distribution in different breeding habitats

Habitat type	No. of samples	Total malaria vectors	*An. gambiae* (Mean)	*An. pharoensis* (Mean)	*An. funestus* (Mean)
Concrete tank	7	8	1(0.1)	7 (1.0)	0 (0.0)
Dam edge	25	65	5 (0.2)	60 (2.4)	0 (0.0)
Ditch	54	512	135 (2.5)	371 (6.9)	6 (0.1)
Lake margin	63	257	67 (1.1)	190 (3.0)	0 (0.0)
River bed	81	491	207 (2.6)	284 (3.5)	0 (0.0)
Swamp	35	213	74 (2.1)	139 (4.0)	0 (0.0)
Water pan	27	24	13 (0.5)	11 (0.4)	0 (0.0)
Water pit	50	35	3 (0.1)	32 (0.6)	0 (0.0)
Water spring	69	144	65 (0.9)	79 (1.1)	0 (0.0)
Total		1749	570	1173	6

Mansonia species which are the only known vectors of RVF in Baringo were collected from swamps, water pit, river bed, and water spring in small numbers. *Culex quinquefasciatus* and *Cx. pipiens* which have only been implicated in RVF virus transmission were collected from all habitats. Generally, *Cx. quinquefasciatus* was the most abundant larval species constituting 51.4% of potential arboviral larval species collected from all habitats. Of the three *Aedes* species collected, only *Ae. aegypti* and *Ae. africanus* are known vectors of yellow fever virus which is also an arbovirus belonging to the same group as RVF virus. *Aedes aegypti* larvae were collected from water pit and water spring in small numbers (6, 12) but a large number (1091) was collected from concrete tanks. The concrete tank contributed 98.9% of *Ae. aegypti* larvae collected from all habitats and had a high mean of 155.9 larvae per sample indicating high preference of water containers by this species (Table 4.4).

Table 4.4 Rift valley fever and other arboviral vector species larval mean distribution in all breeding habitats

Habitat Type	No. of samples	Total vectors	*Mansonia* spp	*Cx. quinquefasciatus*	*Cx.pipiens*	*Cx. univittatus*	*Ae.aegypti*	*Ae.africanus*
Concrete tank	7	1140	0(0.0)	38(5.4)	7(1.0)	4(0.6)	1091(155.9)	0(0.0)
Dam edge	25	125	0(0.0)	93(3.7)	32(1.3)	0(0.0)	0(0.0)	0(0.0)
Ditch	54	199	0(0.0)	164(3.0)	35(0.6)	0(0.0)	0(0.0)	0(0.0)
Lake margin	63	597	0(0.0)	526(8.3)	61(1.0)	2(0.0)	0(0.0)	8(0.1)
River bed	81	638	5(0.1)	325(4.0)	308(3.8)	0(0.0)	0(0.0)	0(0.0)
Swamp	35	296	4(0.1)	269(7.7)	23(0.6)	0(0.0)	0(0.0)	0(0.0)
Water pan	27	201	0(0.0)	198(7.3)	1(0.0)	0(0.0)	0(0.0)	2(0.1)
Water pit	50	307	18(0.4)	223(4.5)	60(1.2)	0(0.0)	6(0.1)	0(0.0)
Water spring	69	715	5(0.1)	331(4.8)	362(5.2)	0(0.0)	12(0.2)	5(0.1)
Total		4212	32	2167	889	6	1103	15

Statistical analysis was done only for *Cx. quinquefasciatus* which was the most abundant arboviral vector. Though there was a significant difference in habitat preference for *Cx. quinquefasciatus* ($F_{8, 401}$=2.132, p=0.032), it is only the ditch and river bed that were different from swamp (Appendix 9). The swamps had the highest mean of *Cx. quinquefasciatus* larvae while river beds and ditches had low means despite being sampled 81 and 54 times respectively compared to swamps which were collectively sampled 35 times (Table 4.4). The swamps were not found in all zones of the study hence total sampling effort was less than that of river beds which were common. All other breeding habitats were not significantly different from each other in terms of larval mean for *Cx. quinquefasciatus* (Figure 4.5).

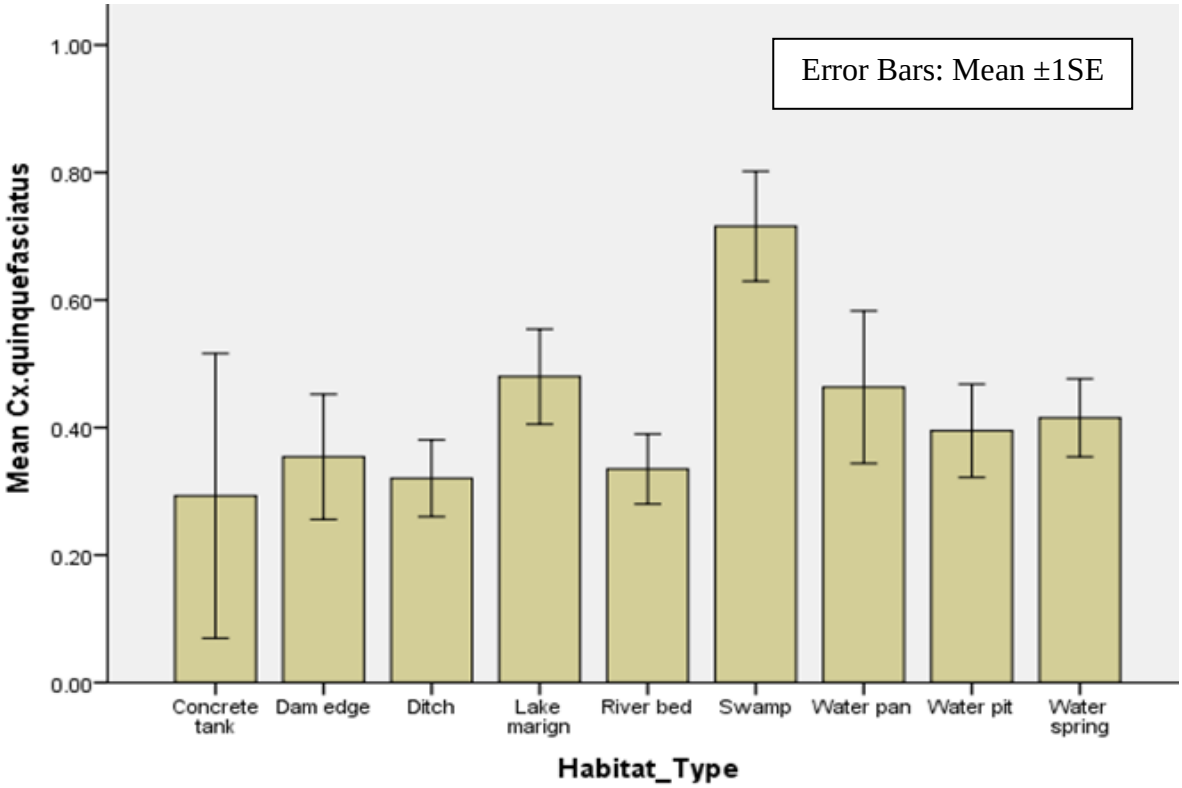

Figure 4.5 Mean of *Cx. quinquefasciatus* larvae in all sampled habitats in Baringo

4.5 Discussion

This study reveals a complex larval species composed of anophelines and culicines. All the larval *Culex* and *Mansonia* species identified in this study had not been documented in previous larval species studies. This might have been due the objectives of previous studies in Baringo that focussed on adult mosquitoes (Sang *et al.*, 2010; Lutomiah *et al.*, 2013; Omondi *et al.*, 2015).

Although some of the previous studies collected *Culex* larvae from Baringo, they did not identify them to species level (Reiter *et al.*, 1998; Arum *et al*, 2010; Mala *et al*., 2011a).

Three larval *Aedes* species, namely: *Ae. aegypti, Ae. taylori* and *Ae. africanus,* collected in this study, are all vectors of arboviruses (Gillett, 1972) but only *Ae. africanus* had previously been reported in high altitude woodlands in Baringo during a yellow fever outbreak of 1992-1993 (Reiter *et al*., 1998). Thus this study recorded *Culex, Aedes* and *Mansonia* larval species which had not been reported at larval stage. This information can be instrumental for integrated control strategies in view of the fact that control of immature stages would be more appropriate since they are confined in small aquatic habitats where they cannot escape as opposed to adults which are highly mobile (Killeen *et al.,* 2002; Mala *et al*., 2011a).

The current study identified *An. gambiae* s.l., *An. funestus*, *An. coustani*, and *An. pharoensis* as the predominant anopheline larval species found in Baringo. This is in agreement with previous studies (Arum *et al*., 2010; Mala *et al*., 2011a). In western Kenya more anopheline species, among them *An. squamosus, An. ziemanni, An*. *implexus, An. marshallii, An. maculipalpis, An. rufipes* and *An. rivorulum,* including the species identified in this study have been reported (Kweka *et al*., 2012; Omukunda *et al.,* 2012). This is a greater anopheline species diversity compared to Baringo which had only five anopheline species. Similarly, in central Kenya, a greater species diversity of up to seven different anophelines was found (Mwangangi *et al*., 2010). Worth noting is the nature of the ecology of Baringo that is semi- arid where breeding habitats for malaria vectors are permanent water sources mainly man-made such as irrigation canals and dams (Mala *et al*., 2011a) unlike western and central Kenya which are not arid.

Species diversity index was highest during the cold dry season an indication that the conditions were favourable for most species to survive. However, the cold dry season experienced more rainfall than the short rain season during the sampling period which could be a possible explanation for this outcome. *Culex quinquefasciatus* abundance was not affected by seasonal changes. Similarly there was no statistical significant difference in *An. gambiae* and *An. pharoensis* between the four seasons though seasonal abundance was observed. This is consistent with the findings of Kweka *et al.* in western Kenya where they found no difference in *Anopheles* larval abundance between seasons (Kweka *et al*., 2012). *Anopheles gambiae* was more abundant during the short rain season and lowest in the long rain season while *An. pharoensis* was more

abundant during dry season than wet season. In contrast, a study in Ghana found a significantly higher proportion of *Anopheles* larvae during the wet season than the dry season (Mattah *et al*., 2017). Therefore, it is advisable to control malaria vector larvae in all seasons by targeting all habitats.

The great diversity of *Culex* species some of which have been reported as secondary vectors of RVF could greatly enhance RVF spread during outbreaks. This is because RVF is transmitted by many mosquito species (CDC, 2013; Sang *et al*., 2017). *Aedes taylori* and *Ae. africanus* were collected in small numbers while *Ae. aegypti* was collected in large numbers during the rainy season. The great abundance of *Ae. aegypti* but not *Ae. taylori* and *Ae. africanus* suggests that species are affected differently by seasonal changes (Chuang *et al*., 2011).

This study shows variation in number and importance of habitats among the surveyed ecological zones. Unlike the species of *Anopheles, Aedes* and *Mansonia*, species of *Culex* were found in all habitats in all the four ecological zones. This finding is consistent with previous studies in which one or more *Culex* species were found in all types of habitats (Fillinger *et al.,* 2011). Very few pupae of *Mansonia* species were collected during this study and identification was only possible after they were left to emerge into adults. *Mansonia* spp larval stages are not easy to find in breeding habitats. This is probably because of their habit of attaching to aquatic plants (Rajendran *et al*., 1989; Chandra *et al*., 2006). However, adult mosquitoes of *Mansonia* species have been collected from Baringo in the previous studies and current study (Sang *et al*., 2010; Lutomiah *et al*., 2013; Omondi *et al*., 2015; Ondiba *et al*., 2017). The purpose of collecting pupae was to characterize the breeding habitats for *Mansonia* spp and the habitats were found to be mainly swampy.

Though this study identified eight larval *Culex* species, only two species, *Cx*. *quinquefasciatus* and *Cx. univittatus*, have previously tested positive for RVF virus in Baringo County (Sang *et al*., 2010). Whereas *Cx. quinquefasciatus* did not show preference for any particular habitat but instead was collected from all habitats across the four ecological zones, *Cx. univittatus* was only collected from lake margin and concrete tank. Since *Cx. Univittatus* was collected in small numbers, these findings may not be conclusive in determining its habitat preference.

The *Aedes* species incriminated in the transmission of RVF virus (*Ae. mcintoshi* and *Ae. ochraceus*) breed in flood waters after unusually heavy and persistent rainfall (CDC, 2013). However, the *Aedes* primary vectors of RVF have not been reported in Baringo even during the previous active epizootics. During the entire period of this study, there were no floods and this may explain why very few larval *Aedes* species were collected. Additionally, *Aedes aegypti* specifically prefer to breed in containers but this study sampling focused more on large and relatively permanent breeding habitats. Sampling during the few months when rain was heavy yielded a large number of *Ae. aegpti* larvae from two concrete tanks in the lowland zone. The abundance of *Ae. aegypti* during long rain season and its confinement to concrete tank makes it easier to control at larval stage. A previous survey of domestic and peridomestic water receptacles in Baringo found no *Aedes* larvae but one cistern within Marigat town was positive for *Ae. aegypti* (Reiter *et al.*, 1998). Similar results were reported from a study in Malaysia which revealed that indoor containers were more preferred breeding habitats for *Ae. aegypti* (Cheong, 1967).

The current study findings show that anopheline species were distributed in all surveyed ecological zones except larvae of *An. funestus* which were found only in the lowland. This is consistent with the findings of Minakawa *et al.* (2002) in western Kenya and findings of Protopopoff *et al*. (2007) in Burundi where they found that anopheline mosquitoes have a vertical distribution. The small and open, sunlit shallow pools of water preferred by *An. gambiae* (Gillies and Meillon, 1968; Gillies and Coetzee, 1987) were common at seasonal riverbeds in the midland zone. Consequently this species was more abundant in seasonal river beds than other habitats. This is similar to findings of studies conducted in Eritrea and Ethiopia where high larval productivity was recorded at stream bed pools (Shililu *et al.,* 2007). This implies that riverbed pools could sustain malaria vectors responsible for transmission during the dry season in Baringo.

Findings from other studies link *An. gambiae* complex to man-made environmentally disturbed habitats and small shallow habitats without emergent vegetation (Gimnig *et al*., 2001; Carlson *et al.,* 2004). However, a study conducted in Tanzania urban environment demonstrated that it was not clear to define larval habitats for *An. gambiae* as high densities were found in polluted water (Sattler *et al*., 2005). In the current study, *An. gambiae* and *An. pharoensis* were found to co-exist in the same breeding habitats such as riverbed pools, ditches and lake margins. On the other hand,

An. funestus prefers deeper and more persistent habitats with vegetation unlike those of *An. gambiae* s.l. (Gimnig *et al*., 2001). These species, *An. gambiae* s.l., *An. pharoensis* and *An. funestus* appear to prefer different types of habitats which are all present in Baringo County, a factor that would enhance malaria transmission throughout the year. This calls for larval source management as part of integrated approach towards vector control in Baringo County.

4.6 Conclusions

1. The findings on larval species diversity and habitat preference are instrumental for larval source reduction.

2. River bed pools, ditches, swamps and lake margins were the most prefered mosquito breeding habitats. These findings will guide interventions for the control of malaria and arboviral diseases in Baringo County by targeting these larval habitats.

3. *Anopheles gambiae* s. l. was more abundant during the short rain season and lowest in the long rain season while *An. pharoensis* was more abundant during dry season than wet season. This implies that transmission of malaria is possible in all seasons unlike previous assumptions that malaria transmission is seasonal in semi-arid areas.

CHAPTER FIVE

5.0 EFFECTS OF SEASONAL VARIATIONS IN RAINFALL AND TEMPERATURE ON THE DISTRIBUTION AND ABUNDANCE OF MOSQUITO VECTORS OF RIFT VALLEY FEVER AND MALARIA IN BARINGO COUNTY

5.1 Introduction

Several factors affect mosquito distribution and abundance either singly or synergistically. These include climatic variables (Khan *et al*., 1996; Lehmann *et al*., 2014 Umar *et al*., 2015) such as rainfall and temperature and altitudinal location (Githeko *et al*., 2000; Protopopoff *et al*., 2007; Animut *et al*., 2013; Gone *et al*., 2014), which is inversely related to temperature. For indoor resting mosquitoes, the type of materials used in house construction can also affect mosquito abundance (Ye *et al*., 2006; Temu *et al*., 2012). On the other hand, vector control tools such as ITNs/LLINs and IRS (Ferguson *et al*., 2010), which target indoor resting mosquitoes have significantly reduced indoor abundance; and led to change in indoor versus outdoor distribution of resting vectors (Mwangangi *et al*., 2013b; Yohannes and Boelee, 2012).

The variability in climatic factors is expected to alter the biology and ecology of mosquito vectors (Caminade *et al*., 2014). It has been established that temperature is inversely related to altitude thus the low ambient temperature in the highlands may restrict the development of vectors and the parasites they transmit (Afrane *et al*., 2011). In western Kenya, temperature was found to contribute to low density of *An. gambiae* and *An. funestus* in high elevation areas (Minakawa *et al*., 2002). The current longitudinal study was undertaken to assess adult mosquito distribution and abundance in four different ecological zones based on elevation in Baringo County.

Apart from temperature, a change in precipitation patterns can also have an effect on vector densities (Githeko *et al*., 2000). Although there is no immediate correlation between the amount of rainfall and number of mosquitoes, (Bashar and Tuno, 2014), it has been reported that seasonal increase in vector density is related to rainfall patterns (Oyewole *et al*., 2007). Seasonal rainfall influences persistence of standing water thus its pattern can affect mosquito abundance in water-limited environments (Bomblies, 2012).

Important mosquito vectors of human and animal disease-causing pathogens are found both indoors and outdoors with those transmitting malaria preferably resting indoors (Gillies and Meillon, 1968; Gillies and Coetzee, 1987). However, mosquitoes transmitting malaria and arboviruses have been found co-existing together hence exposing humans to a range of mosquito born diseases including yellow fever, rift valley fever, chikungunya and dengue fever (Mwangangi *et al*., 2012). For the indoor resting mosquitoes, house design and materials used for construction may have an impact on vector abundance. Although the relationship between vector density and house design has been reported by various authors (Konradsen *et al*., 2003; Lindsay *et al*., 2003; Kirby *et al*., 2008; Animut *et al*., 2013; Swai *et al*., 2016), a similar study has not been conducted in Baringo County.

Mosquito species which were previously known to exclusively rest indoors are now found outside probably due to control methods such as ITNs and IRS targeting indoor biting and resting mosquitoes only (Ferguson *et al*., 2010). Wide spread use of LLINs and IRS have altered the vector species composition with those resting outdoors taking a supplementary role in transmission of malaria (Mwangangi *et al*., 2013b). Alternatively, intensive use of these control measures may have altered vector mosquito behavior by biting outside or early before entering houses or going to bed respectively (Yohannes and Boelee, 2012). Therefore, understanding where and when persons are most likely to be exposed to bites of vector mosquitoes is necessary to decide which vector control method to scale up (Govella and Ferguson, 2012). Semi arid regions experience unstable malaria; they require a large population of vector mosquitoes for transmission to take place (Hay *et al*., 2001). In this study, the distribution and abundance of adult mosquito vector species collected from indoor and outdoor environments in different ecological zones in Baringo County were studied within seasons.

5.2 Literature review

5.2.1 Factors that affect mosquito abundance and distribution

Mosquito abundance and distribution may be affected by presence of suitable breeding habitats and source of blood meal (Kaddumukasa *et al*., 2013). Climatic factors may lead to seasonal changes in vector abundance and consequently influence the epidemiology of the vector borne diseases (Githeko *et al*., 2000). Evidence of this is seen in rift valley fever outbreaks which follow patterns of climatic anomalies particularly in semi arid areas of east Africa. For example,

previous outbreaks of RVF in Kenya reveal a close association with inter-annual climate variability (Anyamba *et al*., 2001). Disease emergence and transmission by mosquito vectors strongly depend on rainfall while spread to new areas depends on changes in temperature (Parham and Michael, 2010).

Research has shown that the influence of meteorological factors on vector densities varies from species to species (Khan *et al*., 1996). Therefore, knowledge of seasonal changes on mosquito vector species abundance is vital for effective control of vector borne diseases (Kigadye *et al*., 2010). The information on adult mosquito abundance and distribution is also fundamental for assessing risk of disease transmission and thus effective planning for the control activities (Coetzee *et al*., 2000; Cianci *et al*., 2013). Sivagnaname and Gunasekaran, (2012) have argued that larval indices can be used in surveillance of vector borne diseases but may not be accurate in assessing disease transmission risk. Larval collection is also considered labour intensive and difficult to standardize the procedures (Walton, 2005; Mendoza *et al*., 2008). Thus longitudinal surveillance of adult vector abundance gives a clearer picture on disease transmission dynamics.

(i) Altitudinal effect on mosquito abundance and distribution

Mosquito abundance and distribution in the heterogenous topography have a vertical distribution (Minakawa *et al.,* 2002; Protopopoff *et al.,* 2007; Asigau *et al*., 2017). The abundance of mosquito vectors in the lower altitude areas of western Kenya was found to be higher than high altitudes by Minakawa *et al*. (2002). Further, it was found that the densities of *An. gambiae* and *An. funestus* were less than one per house in the highlands compared to 12.6 and 10.4 respectively per house in the lowlands. Whereas *An. funestus* and *An. gambiae* s. s. were found above 1,700 m asl, *An. arabiensis* was only found below 1,400m asl in western Kenya. This is consistent with the observation that increases in altitude leads to a reduction in vector abundance (Bodker *et al*., 2003). In eastern Nepal, malaria vectors were confined to an altitude of below 1,200m asl (Dhimal *et al*., 2014). Similarly in Burundi highlands, up to 90% of the vectors were found at valley bottoms (Protopopoff *et al.,* 2007). The current study investigated mosquito vector distribution and abundance in four ecological zones based on elevation in Baringo County whose topography is highly heterogeneous.

(ii) Temperature effects on mosquito abundance and distribution

Effect of temperature on mosquito abundance has been investigated in different parts of the world and either positive or negative association has been reported (Ganser and Wisely, 2013; Rezende *et al*., 2013). Gancer and Wisely found a positive relationship between mosquito abundance and temperature. This suggests that temperature directly influences vector density and this concurs with the findings of Yamana and Eltahir who observed that mosquito survival is affected by temperature as well as humidity (Yamana and Eltahir, 2013a). Laboratory experiments have also shown that temperature affects rate of embryo development during hatching of eggs and this may have implications on survival in the natural environment (Impoinvil *et al*., 2007). However, the study by Bashar and Tuno, (2014) established a relationship between temperature and mosquito abundance in Bangladesh. Furthermore, the increased temperatures at highlands have led to an increasing disease transmission in previously low endemic territories of highland regions (Ermert *et al*., 2012). In agreement with this, a recent analysis on malaria and temperature in Baringo County showed that high malaria cases were positively associated with minimum temperatures of 16-21°C (Amadi *et al.,* 2018a).

(iii) Rainfall effects on vector abundance and VBD transimission

Changing precipitation patterns can also have an effect on vector densities (Molineaux and Gramiccia, 1980; Githeko *et al*., 2000; El Mamy *et al*., 2011). For example unusually high rainfall in northern Mauritania favored high densities of *Culex* and *Anopheles* mosquitoes (El Mamy *et al*., 2011) followed by malaria and rift valley fever outbreaks a few weeks later. It has been reported that a time lag exists between environmental parameter change and the onset of effects (Khan *et al*., 1996) and this could explain the delayed occurrence of diseases after the heavy rains in northern Mauritania. Molineaux and Gramiccia also found a very large seasonal variation in vector density over a period of three years in northern Nigeria (Molineaux and Gramiccia, 1980). It was observed that the seasonal increases in vector density were related to rainfall patterns. Since rainfall affects vector density, it can be used as a form of early warning system to predict malaria cases (Thomson *et al*., 2005). In Baringo County, analysis showed that total monthly rainfall was significantly associated with malaria incident rates with a moderate rainfall leading to increase in malaria cases (Amadi *et al.,* 2018a).

5.2.2 Seasonal changes of adult mosquito vector abundance

Depending on the geographical locality, season can or cannot affect mosquito density (Shililu *et al*., 1998; Kabale *et al*., 2013). Separate studies conducted in western Kenya and Cameroon found out that malaria vector abundance was high during the dry season (Ndenga *et al*., 2006; Bigoga *et al*., 2012). Season was also found to have an effect on mosquito density in eastern Nepal (Dhimal *et al*., 2014). However, some studies found high abundance of *An. gambiae* during the rainy seasons (Minakawa *et al*., 2002; Shililu *et al*., 2004). Findings from southern Nigeria also showed that *An. gambiae* mosquitoes were mostly collected during the rains while none were collected during the dry season (Oyewole *et al*., 2007). It was also found that the density of *Anopheles* mosquitoes varied according to the season of the year (Oyewole *et al*., 2007). Rift valley fever vectors (flood water *Aedes* and *Culex* mosquitoes) increase quickly to large numbers at the beginning of the rain season and attain maximum population towards the end of the season (Chitnis *et al.,* 2013).

5.3 Materials and Methods

5.3.1 Sampling and house type selection

The study area was stratified into four ecological zones based on elevation namely; lowland, riverine, midland and highland. Indoor and outdoor resting mosquitoes were collected once every month for a period of 12 months from June 2015 to May 2016. Twenty four sites spread across the four ecological zones (six sites in each zone) were sampled. Houses for indoor collection were selected based on materials used for the construction of house wall and roof (Plate 5.1). A total of 100 houses categorized into six types were sampled. A unique traditional house made of grass thatched roof and mud wall but raised on stilts (locally known as "bororiet") was also sampled (Plate 5.1-B). The "bororiet" huts in Kerio valley are used as human dwellings or a store. Only those used for sleeping by humans were samples for mosquitoes. Before commencement of the entomologic sampling, it was important to determine the exact location of all houses in the 24 sites with a hand-held global positioning system (GPS) receiver (Garmin eTrex 10). A unique identification code was allocated to each of the selected houses from the four study zones.

5.3.3 Indoor sampling of adult mosquitoes by pyrethrum spray collection method

Pyrethrum spray collection was done in houses that had not used any form of insecticide or repellent during the previous two weeks and where no fire had been lit in the morning. During the actual collection, foodstuffs and utensils were removed from the houses. Horizontal surfaces were covered with white sheets of cotton which were spread over the floor and furniture from wall to wall. A pyrethrum-based insecticide aerosol (2mls of pure pyrethrin in 1 litre of paraffin) from a pressurized can was sprayed inside the house for 5-10 seconds and left with the windows and doors closed for 12minutes. The white sheets were removed carefully starting from the door by lifting them by the four corners. The sheets were then transferred outside for examination (WHO, 1975b; WHO, 2003). The dead and immobilized mosquitoes were collected from the white sheets into labeled plastic containers using entomological forceps. Anopheline and culicine mosquitoes were separated and placed in different labeled containers. Collections were done once every month between 0600 and 0830 hours for 12 months.

Plate 5.1 Types of houses sampled for mosquitoes in Baringo County

Key: Order of naming houses: Roof-Wall

A- Grass-mud, **B**- Grass-mud (bororiet), **C**- Iron-iron, **D**-Iron-mud, **E**-Iron-wood, **F**- Iron-stone

5.3.4 Outdoor sampling of adult mosquitoes by CDC light trap

Outdoor resting mosquitoes were sampled using CDC light trap the night preceding PSC collection once every month for a period of 12 months. The CDC light trap was operated outside one of the houses at each sampling site. A total of 24 traps spread throughout the study area were set during the sampling period once a month. The traps were set at about 1.5m above the ground (Plate 5.2G) and operated between 1800 hours and 0600 hours. The traps were collected between 0700 and 0800 hours and carried (Plate 5.2H) to the laboratory where the mosquitoes were sorted out from the other insects caught in the trap. The mosquitoes were further sorted into anophelines and culicines and placed in separate petri dishes for morphological identification (5.2J).

Plate 5.2 G-CDC light trap set under a tree, H-CDC light trap in the transport vehicle to the laboratory, J-Identifying mosquitoes under microscope

5.3.5 Identification of adult mosquitoes and preservation

Adult mosquitoes collected from indoors and outdoors were sorted with the aid of the dissecting microscope (Plate 5.2J) and identified morphologically to genera or species level using taxonomic keys (Edwards, 1941; Gillies and Coetzee, 1987). Identification was done at Marigat DVBD laboratory or Kabarnet hospital laboratory. The mosquito features used for identification included; wing, leg and abdomen markings (Edwards, 1941; Gillies & Meillon, 1968; Gillies and Coetzee, 1987). Anophelines were separated from culicines based on length of palps relative to proboscis. According to the taxonomic keys by Gillies and Coetzee (1987), the anophelines have almost same length of palps and proboscis whereas culicines have very short palps compared to proboscis. The culicines, mansonioides are heavily scaled and hairy with broad wing scales which are bi-colored. The *Aedes* mosquitoes are greatly ornamented and are the only ones with a sharp pointed abdomen and toothed claws. *Ficalbia* species have wings which are scantly scaled while *Culex* species lack any ornamentation (Edwards, 1941). *Anopheles gambiae s.l.* have speckled legs particularly the hind legs, the third pre-apical dark area on vein 1 has a pale interruption, and tarsi 1–4 have conspicuous pale bands. *Anopheles funestus* are characterized by absence of speckles on the legs which appear entirely dark, absence of pale interruption on the third pre-apical dark area of vein, a pale spot on the second dark area of vein 1, a light spot between the two dark spots on vein 6 and absence of fringes on vein 6. *Anopheles pharoensis* have shaggy palps, abdominal segments with laterally projecting tufts of scales on segments II – VII, hind tarsus 5 and about half of apical tarsus 4 pale. The three hind tarsus of *An. coustani* are entirely pale, wing scales are dark and very shaggy palps. Any other mosquito species not belonging to the above groups was also recorded.

All identified mosquitoes were counted and recorded separately for each species every time trapping was done. Female mosquitoes were categorized as fed or unfed, gravid or semi gravid. All mosquitoes were preserved in labeled vials placed in zip lock bags containing silica gel for moisture absorption. The labels on the vials included date, site of collection, name of species, sex, indoor or outdoor collection and abdominal status of females.

5.3.6 Statistical analyses

The relative abundance of a species was expressed as the percentage of the total number of mosquitoes collected.

5.3.6.1 Analysis of distribution and abundance of RVF and malaria vectors

Abundance data of mosquitoes were not normally distributed and showed a clumped distribution (variance > mean). Therefore, generalized linear models (GLM) were fitted assuming a negative binomial distribution and a log link function using the "Mass" package in R. Zone was used as abundance explanatory variables in R version 3.3.1 (Logan, 2011). Separate models were fitted for each species of RVF and malaria vectors. *Mansonia uniformis* and *Mansonia africana* were combined while *Culex pipiens* s.l., *Culex univittatus* and *An. gambiae* s. l. were analyzed separately. Variation in vector abundance between indoor and outdoor collections was compared by t-test.

5.3.6.2 Analysis of effect of house types on malaria vector abundance

The relative abundance of malaria vectors in house types was expressed as the percentage of the total number of mosquitoes collected from all houses. Based on the sample size per house type, only grass-mud, iron-iron, iron-mud and iron-wood houses were considered in statistical analysis by Generalized Estimating Equations (GEE).

Only *Anopheles gambiae* was used in analysis as it was the most abundant malaria vector species. *Anopheles funestus* and *Anopheles pharoensis* were not included in statistical analysis since they were very few. Likewise, only lowland and riverine zones, which had high population of malaria vectors, were included in the analysis. Midland and highland, which had negligible numbers of malaria vector mosquitoes, were not included in statistical analysis.

5.3.6.3 Analysis of effect of seasonal variations in rainfall and temperature on malaria vector abundance

Generalized linear model with negative binomial distribution was fitted to assess the effect of season on the abundance of malaria vectors while linear regression was used to assess the relationship between monthly rainfall and temperature with malaria vector abundance.

5.4 Results

5.4.1 Overall distribution and seasonal variations in abundance of RVF and malaria vectors in Baringo County

Out of the total collection of 12,204 mosquitoes, the highest number was collected in May 2016 accounting for 27.2% followed by December 2015 which accounted for 21.8%. The least proportion (1.4%) of mosquitoes was collected in March 2016. The lowland zone had the highest abundance of mosquitoes followed by riverine in all months except March and April 2016 when riverine had the largest number of mosquitoes compared to other zones. Midland had the lowest population of mosquitoes (0.8%) with none collected in February 2016 while highland also had a second low number of mosquitoes with none collected in November 2015. Season and zone were significant variables influencing mosquito abundance ($p=0.000$). When season was analyzed alone, only long rain season was significantly different from cold dry season which was used as reference category ($p=0.023$). The long rain season had the lowest abundance of mosquitoes. When season was modeled together with zone, short rain ($p=0.001$) and dry season ($p=0.021$) significantly influenced mosquito abundance.

Though mosquito populations peaked in December 2015 and May 2016, there was no overall clear trend between rainfall and mosquito abundance throughout the sampling period. Negative binomial modeling of mosquito abundance against rainfall alone showed that it significantly influenced abundance ($p=0.017$) but when minimum and maximum temperature were included in the model, rainfall was not significant ($p=0.785$). Similarly there was no clear trend between monthly average temperature and mosquito abundance. Whereas minimum temperature was a significant variable that influenced mosquito populations when considered alone ($p=0.000$) and with rainfall ($p=0.0175$), maximum temperature was not significant ($p=0.174$) in all cases either alone or combined with other factors.

Vectors of RVF and malaria were mainly collected from lowland zone (82.5%) and riverine zone (14.6%) but in low numbers from highland and midland zones. The lowland zone was the only significantly different zone from highland (reference category) in vector abundance ($P=0.029$ for *An. gambiae*, $p=0.018$ for *Mansonia* species and $p<0.000$ for *Culex pipiens* s.l.). Distribution of *Cx. univittatus* was not affected by zone ($p>0.05$).

5.4.2. Distribution and abundance of potential RVF vector species in Baringo County

Components of this section were published as:

Diversity, distribution and abundance of potential rift valley fever vectors in Baringo County, Kenya , International Journal of Mosquito Research 4(4):42-48 - ISSN: 2348-5906 CODEN: IJMRK2 by Ondiba *et al.* (2017) (Appendix 10).

Mosquito species abundance varied across zones with lowland having the highest abundance (81.3%) followed distantly by riverine (15.6%). Midland had the lowest population of mosquitoes which accounted for only 0.6% of the total collections (Table 5.1). The four vector species implicated in RVF virus transmission; *Mansonia africana, Ma. uniformis, Cx. pipiens* and *Cx. univittatus* were mainly collected from lowlands (85.9%) and riverine (9.1%) with only 5% collected from midland and highland zones combined.

Table 5.1 Culicine mosquito species collected in the study sites across the four ecological zones in Baringo County

Zone	**Site**	Secondary vectors				Other mosquito species																**Total**
		Cx. pip	Cx. uni	Ma. afr	Ma. unif	*Ae. spp	Aed Afr	An. cou	An. fun	An. gam	An. pha	Coq. spp	cx. mac	Cx. eth	Cx. van	Cx. ann	Cx. poi	Ere. spp	Fi. Spp	Hod. spp	Ort. spp	Ura. spp	
Highland	Borowonin	0	3	0	2	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	9
	Kapkenda	62	0	0	13	0	0	0	0	16	0	0	0	0	1	0	0	1	1	0	0	94
	Kaptimbor	128	2	0	24	1	0	0	0	0	0	0	12	4	4	0	0	0	1	2	0	178
	Kaptich	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Kiberege	6	0	2	0	0	0	0	0	2	0	0	0	0	0	0	0	0	1	0	0	11
	Talai	2	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	1	0	0	6
Subtotal		**198**	**5**	**2**	**39**	**1**	**0**	**0**	**0**	**25**	**0**	**0**	**12**	**4**	**5**	**0**	**0**	**1**	**4**	**2**	**0**	**298**
Spp %		**66**	**1.7**	**0.7**	**13.1**	**0.3**	**0**	**0**	**0**	**8.4**	**0**	**0**	**4.0**	**1.3**	**1.7**	**0**	**0**	**0.3**	**1.3**	**0.7**	**0**	
Lowland	Kapkuikui	2945	19	2	53	0	0	25	6	3197	74	1	0	1	0	0	0	0	168	0	0	2	6493
	Loboi	382	50	18	127	77	0	137	3	616	14	4	0	22	0	0	0	0	67	0	0	8	1525
	Nteppes	190	28	11	17	1	0	0	0	209	17	0	0	0	0	0	0	0	0	0	1	0	474
	Robert's	15	8	1	1	0	0	0	0	7	0	0	0	20	0	0	0	0	5	0	0	0	57
	Salabani	17	1	2	25	0	0	0	0	105	113	0	2	0	0	2	0	0	0	0	0	0	267
	Sirata	103	61	263	135	0	0	134	3	160	59	31	0	0	0	0	1	0	0	0	0	0	950
Subtotal		**3652**	**167**	**297**	**358**	**78**	**0**	**296**	**12**	**4294**	**277**	**36**	**2**	**43**	**0**	**2**	**1**	**0**	**240**	**0**	**1**	**10**	**9766**
Spp %		**37.4**	**1.7**	**3.0**	**3.7**	**0.8**	**0**	**3.0**	**0.1**	**44.0**	**2.8**	**0.4**	**0.0**	**0.4**	**0**	**0.0**	**0.0**	**0**	**2.5**	**0**	**0.0**	**0.1**	
Midland	Chebarsiat	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Kimalel	0	0	0	4	0	0	5	0	6	0	2	0	0	0	0	0	0	0	0	0	0	17
	Kimao	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	3
	Kipcherere	3	1	3	1	0	0	1	0	16	0	0	0	0	0	0	0	0	1	0	0	0	26
	Kabeswa	0	0	0	2	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	14
	Yomu	0	0	0	1	1	0	1	0	5	0	0	0	2	0	0	0	0	0	0	0	0	10
Subtotal		**4**	**2**	**3**	**8**	**1**	**0**	**7**	**0**	**40**	**0**	**2**	**0**	**2**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**70**
Spp %		**5.7**	**2.9**	**4.3**	**11.4**	**1.4**	**0**	**10**	**0**	**57.1**	**0**	**2.9**	**0**	**2.9**	**0**	**0**	**0**	**0**	**1.4**	**0**	**0**	**0**	
Riverine	Barwessa R	88	27	26	38	1	0	1	1	4	3	0	0	0	0	1	0	1	36	0	00	0	227
	Barwessa S	2	7	3	1	5	0	0	0	27	0	0	0	0	0	0	0	0	14	0	0	0	59
	Enot	13	1	6	51	0	4	3	0	66	0	1	0	0	0	0	0	0	0	0	2	0	147
	Kamnarok	12	64	60	43	0	142	14	55	912	11	0	0	0	0	0	2	0	12	0	0	0	1327
	Litein	16	5	1	4	0	0	4	1	28	0	0	0	0	0	0	0	0	0	0	29	0	88
	Salawa	1	5	1	0	0	0	0	0	16	0	0	0	0	0	0	0	0	3	0	0	0	26
Subtotal		**132**	**109**	**97**	**137**	**6**	**146**	**22**	**57**	**1053**	**14**	**1**	**0**	**0**	**0**	**1**	**2**	**1**	**65**	**0**	**31**	**0**	**1874**
Spp %		**7.0**	**5.8**	**5.2**	**7.3**	**0.3**	**7.8**	**1.2**	**3.0**	**56.2**	**0.7**	**0.1**	**0**	**0**	**0**	**0.1**	**0.1**	**0.1**	**3.5**	**0**	**1.6**	**0**	

*_Aedes_ species included: _Ae. hirsutus_, _Ae. vittatus_, _Ae. metallicus_, _Ae. aegypti_ and _Ae. tarsalis_; Spp % - Species percentage; Cx-Culex; pip-pipiens; uni-univittatus; Ma-Mansonia; afr-africana; unif-uniformis; Ae-Aedes; Spp-species; Aed-Aedeomyia; A.n-Anopheles; cou-coustani; fun-funestus; gam-gambiae; pha-pharoensis; Coq-Coquillettidia; mac-macfiei; eth-ethiopicus; van; vansomereni; ann-annulioris; poi-poicilipes; Ere-Eretmapodite; Fi-Ficalbia; Hod-Hodgesia; Ort-Orthopodomyia; Ura-Uranotaeni

Though RVF vector species were represented in all zones irrespective of sampling sites within the study zone, the distributional abundance varied for species in each zone. For *Mansonia africana,* 74.8%, 23.9%, 0.8% and 0.5% were collected at lowland, riverine, midland and highland zones respectively while *Ma. uniformis* was also collected in the lowland, riverine, midland and highland in the following proportions respectively: 66.1%, 25.2%, 1.5% and 7.2%. Proportions of *Cx. pipiens* s.l. collected were 91.6% in the lowland, 3.3% in the riverine, 0.1% in the midland and 5.0% in the highland while those for *Culex univittatus* were 59.0%, 38.5%, 0.7%, 1.8% respectively.

The abundance of *Mansonia* species combined was significantly different in lowland compared to highland which was used as a reference (p=0.018). Similarly, abundance of *Cx. pipiens* s.l. was significantly different in the lowland compared to highland (p=0.000). However, *Culex univittatus* abundance was not statistically different across zones (p>0.05).

5.4.3.1 Malaria vector species abundance and distribution across the four ecological zones in Baringo County

The components of this section were published as:

Malaria Vector Species Distribution and Seasonal Population Dynamics across Varied Ecological Zones in Baringo County, Kenya , Journal of Mosquito Research 7(21):174-183 (doi:10.537/jmr.2017.0021) by Ondiba *et al.* (2017) (Appendix 11).

A total of 6,113 anopheline mosquitoes belonging to four species were collected from both indoor and outdoor resting places across the four ecological zones. Among the four species, three were malaria vectors namely; *Anopheles gambiae* s.l., *An. pharoensis* and *An. funestus*. *Anopheles gambiae* s.l. (93.8%) was the most abundant species of the three malaria vectors and was collected from all four ecological zones. This species accounted for 88% and 90.8% of all anopheline mosquitoes collected in the lowland and riverine zones respectively. *Anopheles pharoensis* and *An. funestus* accounted for 4.8% and 1.1% respectively of the total anopheline species and were collected from lowland and riverine zones only. Another anopheline species, *Anopheles coustani,* was also collected in all study zones except highland. Most mosquitoes were collected from lowland and riverine zones which contributed 79.8% and 19.0% respectively

while midland and highland zones had 0.8% and 0.4% respectively of all malaria vectors collected (Table 5.2).

Table 5.2 Distribution and abundance of anopheline mosquitoes across the four ecological zones in Baringo County

Species	Lowland		Riverine		Midland		Highland		Totals	
	Total (N)		R.A. (%)	Total (N)	R.A. (%)	Total (N)	R.A. (%)	Total (N)	R.A. (%)	Total (N)
An. coustani	296	6.1	22	1.9	7	14.9	0	0.0	325	5.3
An. funestus[a]	12	0.2	57	4.9	0	0.0	0	0.0	69	1.1
An. gambiae s.l.[a]	4294	88.0	1053	90.8	40	85.1	25	96.2	5412	88.5
An. pharoensis[a]	277	5.7	14	1.2	0	0.0	0	0.0	291	4.8
Anopheles spp	1	0.02	14	1.2	0	0.0	1	3.8	16	0.3
Zone totals	4880 (79.8%)		1160 (19.0%)		47 (0.8%)		26 (0.4%)		6113 (100%)	

[a]vectors of malaria in Baringo County; N-Total number collected; R.A-Relative Abundance

5.4.3.2 Indoor and outdoor distribution of anopheline species in Baringo County

Indoor anophelines accounted for 80.8% of total collections compared to 19.2% collected outdoors (Table 5.3). *Anopheles gambiae* s.l. and *An. funestus* were mainly collected indoors; 89.5% and 89.9% respectively of total collections for individual species. On the other hand, *An. pharoensis* and *An. coustani* were mainly collected from outdoors; 87.6% and 99.7% respectively of total collections for individual species. The sixteen unidentified anopheline mosquitoes were all collected outdoors.

Table 5.3 Relative abundance of indoor and outdoor anopheline species in Baringo County

		Indoors		Outdoors	
Species	Overall total	Total number (N)	Relative abundance (%)	Total number(N)	Relative abundance (%)
An. coustani	325	1	0.3	324	99.7
An. funestus[a]	69	62	89.9	7	10.1
An. gambiae s.l[a]	5412	4842	89.5	570	10.5
An. pharoensis[a]	291	36	12.4	255	87.6
Anopheles spp	16	0	0	16	100
Totals	6113	4941	100.0	1172	100.0
Relative abundance of indoor and outdoor		80.8%		19.2%	

[a]vectors of malaria in Baringo County

A t-test was conducted to compare indoors and outdoor mosquito collections by species. *Anopheles gambiae* mean for indoor (24.5) and outdoor (8.0) collections were statistically different ($t_{266.9}$=3.379, p<0.000). Similarly mean for *An. pharoensis* mosquitoes collected indoors (0.18) and outdoors (3.6) were statistically different ($t_{71.0}$=2.885, p=0.005). However, indoor mean (0.3) and outdoor mean (0.1) collections for *An. funestus* were not statistically different ($t_{246.7}$=1.475, p=0.141).

5.4.3.3 Abundance of malaria vectors in different house types in Baringo County

The components of this section were published as:

Malaria vector abundance is associated with house structures in Baringo County, Kenya. *PLoS ONE*, 13(6):e0198970.https://doi.org/10.137/*journal.pone.019897* by Ondiba *et al.* (2018) (Appendix 12).

Three species of malaria vectors namely; *Anopheles funestus*, *Anopheles gambiae* and *Anopheles pharoensis* were identified in different houses in Baringo County. Based on cumulative monthly mosquito collections over a period of 12 months, corrugated iron roof mud walled houses had a

higher average number of malaria vectors per house per month (17.3) followed by grass thatched mud walled houses (11.2) in the lowland zone. A similar trend was observed in the riverine zone but midland and highland zones had very few mosquitoes to give any meaningful trend (Table 5.4). The few *An. gambiae* mosquitoes collected from the midland and highland zones were from iron-mud and iron-iron houses only. Whereas the corrugated iron roof and stone wall house had the least number of mosquito vectors of malaria, the grass thatched roof and stone wall house had a high cumulative number of malaria vectors (n=775). The grass thatched roof and stone wall house had both *An. gambiae* and *An. pharoensis* mosquitoes while corrugated iron roof and stone wall house had *An. gambiae* only (n=14). Vector abundance was highest in mud wall houses and those with open eaves. In Kamnarok village in riverine zone, a house made of grass thatched roof and mud wall but raised on stilts ("bororiet") had lower number of mosquito density (5.8 per collection) than ordinary houses made of same materials but at ground level (30.5 mosquitoes per collection).

Most of the malaria vector mosquitoes were collected in houses located in the lowland zone, 75.6% and riverine zone, 24.1%. *Anopheles gambiae* was the dominant species in all zones and accounted for 97.7% of the total malaria vectors. *Anopheles funestus* and *An. pharoensis* mosquitoes were collected in lowland and riverine zones but absent in the midlands and highlands where only *An. gambiae* was collected in small numbers. Both *An. funestus* and *An. pharoensis* were very few and accounted for only 1.5% and 0.9% respectively of the total malaria vectors collected in all zones.

Table 5.4 Malaria vector abundance over a period of 12 months in different house structures per zone in Baringo County

Zone	House types (n)	Vector species			Mosquitoes/
	Roof-Wall	*An. funestus*	*An. gambiae* s. l.	*An. pharoensis*	House/Month
Lowland	Grass-Mud (8)	4	1065	4	11.2
	Iron-Iron (6)	0	651	1	9.1
	Iron-Mud (6)	2	1246	0	17.3
	Iron-Wood (3)	0	104	16	3.3
	Iron-Stone (1)	0	0	0	0
	Grass-Stone (1)	0	774	1	64.6
Riverine	Grass-Mud (15)	25	723	0	4.1
	Iron-Iron (5)	0	1	0	0.02
	Iron-Mud (5)	31	253	14	4.9
	Iron-Stone (2)	0	14	0	0.6
Midland	Grass-Mud (6)	0	0	0	0
	Iron-Iron (11)	0	8	0	0.06
	Iron-Mud (5)	0	1	0	0.02
	Iron-Wood (1)	0	0	0	0
Highland	Grass-Mud (4)	0	0	0	0
	Iron-Iron (7)	0	2	0	0.02
	Iron-Mud (6)	0	0	0	0
	Iron-Wood (8)	0	0	0	0

n-number of houses sampled for the indicated house type in each zone

Lowland zone and riverine zone were different with regard to *An. gambiae* density (13.8 and 5.1 per sample per house respectively) and house type (iron roof-wooden wall houses were only found in lowland while "bororiet" houses were only found in riverine). This warranted separate analyses to be performed for each zone.

Houses in the riverine zone were significantly associated with *An. gambiae* abundance ($p<0.000$) while those in the lowland had no association with *An. gambiae* abundance ($p=0.662$). In the riverine zone, corrugated iron sheet roof-iron wall houses had significantly lower abundance of *An. gambiae* mosquitoes compared to grass thatched roof-mud walled houses which were used as a reference ($p<0.000$). The odds of finding *An. gambiae* in corrugated iron sheet roof-iron wall

houses in the riverine zone were less likely than in grass thatched roof-mud walled houses (OR=0.006, 95%CI=-7.013 to -3.062). When house effects were analyzed while adjusting for rainfall and temperature in the riverine zone, still there was an association between house type and *An. gambiae* mosquito abundance in the riverine zone (Table 5.5). However, these differences were not observed in the lowland zone where *An. gambiae* abundance was not significantly different in all house types.

Table 5.5 Effect of house type on mosquito abundance while controlling for rainfall and temperature in the riverine and lowland zones

Zone	Variable	Category	Odds Ratio	95%CI	P-value
Riverine	House type	Iron-mud (IM)	1.533	-1.374 ; 2.228	0.642
		Iron-iron (II)	0.006	-7.013 ; -3.062	0.000
		Grass-mud(GM)*			
	Rainfall		1.008	0.005 ; 0.011	0.000
	Temperature		1.231	-0.017 ; 0.434	0.071
Lowland	House type	Iron-wood (IW)	0.272	-2.826 ; 0.226	0.095
		Iron-mud (IM)	1.646	-1.287 ; 2.283	0.584
		Iron-iron (II)	0.762	-2.002 ; 1.458	0.758
		Grass-mud(GM)*			
	Rainfall		0.989	-0.017 ; 0.005	0.001
	Temperature		0.856	-0.321 ; 0.011	0.067

* Reference house

5.4.3.4 Effects of seasonal variations in rainfall and temperature on malaria vector abundance in Baringo County

The highest number of mosquitoes was collected in December 2015 accounting for 83.6% (N=1267) of the total anophelines collected in the lowland during the dry season. The lowland vector population reduced drastically to 181 in January and further down to 68 in February as the dry season progressed. On the other hand, highest number of vectors (N=309) in the riverine was collected in the month of April 2016 which accounted for 60% of the total collections during the long rain season in the riverine zone (Table 5.6). In terms of season, the cold dry season had the highest population of 1831 mosquitoes (31.7%) followed by dry season with 1798 mosquitoes

(31.2%) of total collections during the 12-month sampling period (Table 5.7). Generally higher proportions of malaria vectors were collected during the drier seasons than rainy seasons.

Table 5.6 Seasonal variations in malaria vector abundance in Baringo County

		Malaria vector numbers (N) across zones				
Season	Month	Lowland	Riverine	Midland	Highland	Total
Cold	June	446	186	9	6	647
dry	July	613	33	4	0	650
	August	487	46	1	0	534
Total		**1546**	**265**	**14**	**6**	**1831**
Short	September	588	24	15	12	639
rains	October	324	29	2	0	355
	November	368	18	1	0	387
Total		**1280**	**71**	**18**	**12**	**1381**
Dry	December	1267	71	1	5	1344
season	January	181	162	4	0	347
	February	68	39	0	0	107
Total		**1516**	**272**	**5**	**5**	**1798**
Long	March	38	48	1	0	87
rains	April	41	309	0	0	350
	May	163	158	2	0	323
Total		**242**	**515**	**3**	**0**	**760**

Negative binomial modeling of *An. gambiae* abundance against season showed a significant effect in the lowland. The long rain season in the lowland was significantly different from the cold dry season which was used as a reference ($p<0.000$). On the contrary, seasons were not significantly different in the riverine zone. When rainfall and temperature were combined in the same model, only rainfall was significant in influencing *An. gambiae* s.l. populations in the lowland ($p=0.044$) and riverine ($p=0.003$). However, the significance level for rainfall was weaker in the lowland than in the riverine zone. Therefore, further analysis was done to determine the association between rainfall and temperature (as constituents of season) and *An.*

gambiae s.l. populations in the lowland and riverine zones which had high numbers of mosquitoes.

When mosquito abundance was regressed against rainfall in the lowland, there was a weak negative correlation (r=-0.08) and r^2 = 0.007 meaning only 0.7% of mosquitoes collected could be explained by rainfall and this was not statistically significant (p=0.796). This contrasted results when vector abundance was modeled against rainfall and temperature in the lowland and rainfall was found to be significant (p=0.044). Regression of *An. gambiae* s.l. population against temperature in the lowland also showed weak negative correlation (r=-0.19) but higher than that of rainfall and only 3.8% of mosquitoes collected could be explained by temperature (r^2=0.038). The relationship between temperature and *An. gambiae* s.l. abundance was not statistically significant (p=0.544). Therefore, rainfall and temperature in the lowland may not be useful in predicting *An. gambiae* s.l. population dynamics in relation to the actual number of mosquitoes collected (Figure 5.1a and b). When mosquito abundance was regressed against rainfall and temperature, correlation improved (r=0.29) and 8.6% of mosquito population in the lowland could be explained by rainfall and temperature (r^2=0.086). This was, however, not significant overall (F=0.664) and both rainfall (p=0.505) and temperature (p=0.398) were not significant.

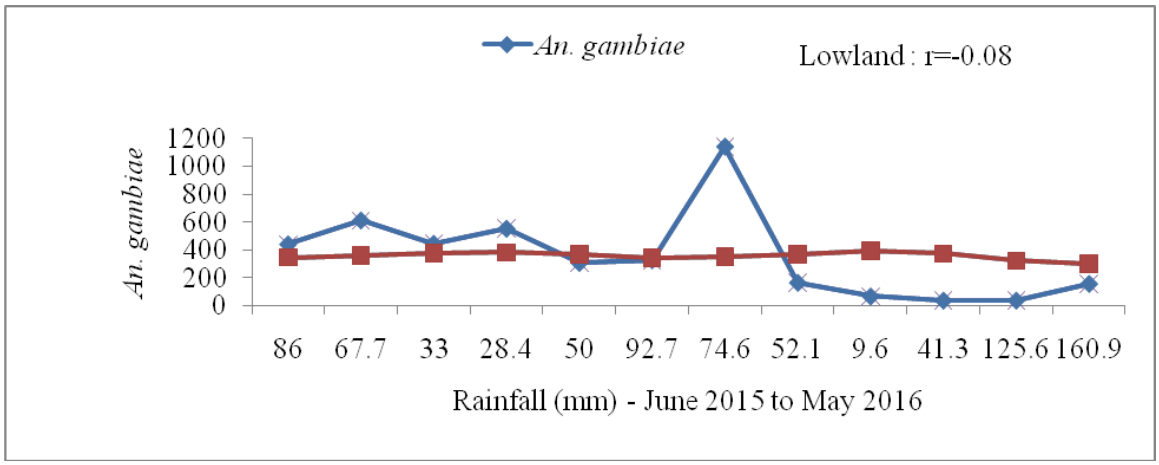

Figure 5.1a Correlation of *An. gambiae* s.l. population with rainfall in the lowland zone

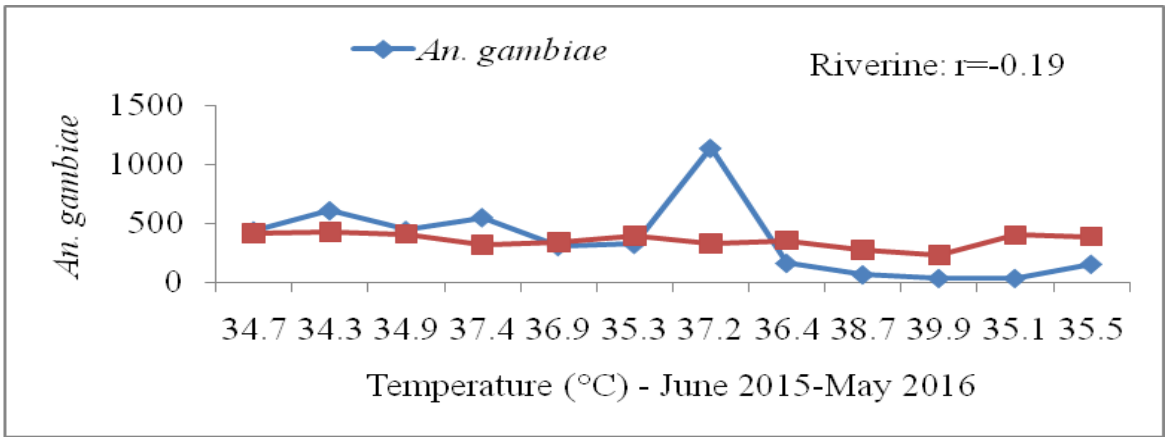

Figure 5.1b Correlation of *An. gambiae* s.l. population with temperature in the lowland zone

Regression of *An. gambiae* s.l. abundance against rainfall in the riverine showed a strong positive correlation (r=0.7) and 49.5% of mosquito population could be explained by rainfall (r^2=0.495) which was statistically significant (p=0.01). On the contrary, temperature in the riverine did not show any correlation with mosquito abundance (r=-0.01) and could not account for any change in vector population (r^2=0.00). Therefore, whereas rainfall pattern may be used to predict *An. gambiae* s.l. population in the riverine, temperature may not be useful (Figure 5.2a and b). However, when mosquito population was regressed against rainfall and temperature, correlation improved to r=0.72 and 52.6% of mosquito population in the riverine could be explained by rainfall and temperature collectively (r^2=0.526). This was significant overall (F=0.0346) and rainfall was significant (p=0.011) but temperature was not significant (p=0.460).

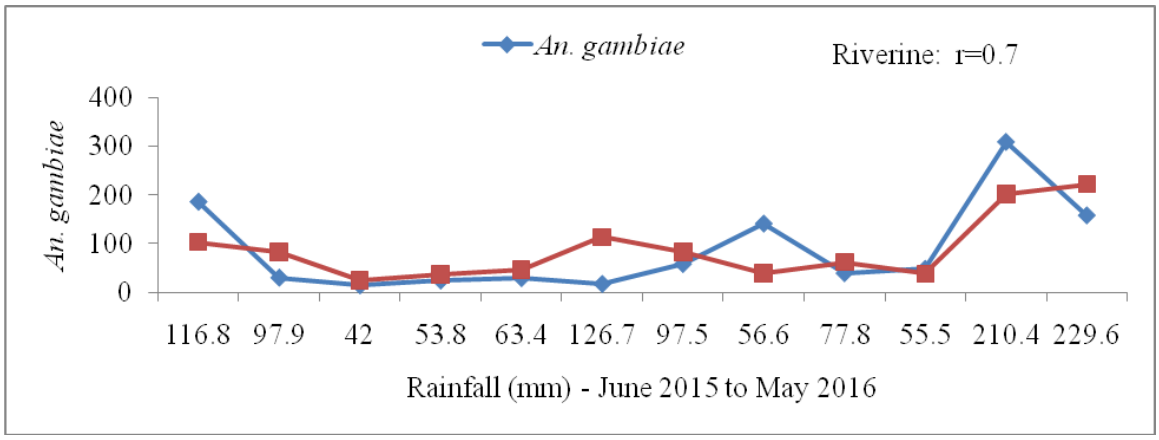

Figure 5.2a Correlation of *An. gambiae* s.l. population with rainfall in the riverine zone

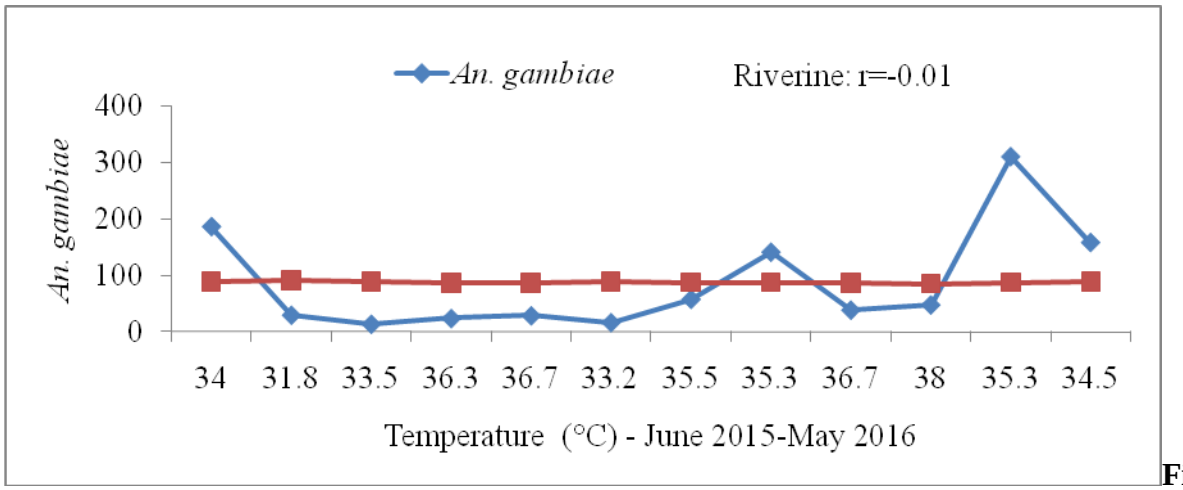

Figure 5.2b Correlation of *An. gambiae* s.l. population with temperature in the riverine zone

5.5 Discussion

The current study established distribution of rift valley fever and malaria vectors across the four ecological zones. Rift valley fever and malaria vectors, except *An. funestus*, were collected from all ecological zones in varying proportions. The greatest proportion of all vectors was collected from the lowland zone followed by riverine zone. Mosquitoes collected from midland and highland zones in all months were very few. Malaria vector *Anopheles gambiae* was mainly collected indoors while RVF vectors were collected outdoors. Drier seasons had more vectors than wet seasons. Temperature was not associated with mosquito abundance while rainfall had an influence on vector abundance in the riverine zone only.

The findings on the distribution and abundance of mosquitoes in the current study are in agreement with the studies of Minakawa *et al*. (2002) who found higher density of mosquitoes at lowlands than highlands in western Kenya. Mosquito abundance was also found to fluctuate in northern Tanzania over small geographical areas (Kigadye *et al*., 2011). The large number of indoor resting *An. gambiae*, principal vector of malaria, in the lowland and riverine zones could be an indication of greater risk of malaria in those two zones compared to midland and highland zones. This is a possible explanation for the high prevalence of malaria cases found recently in the lowland and riverine areas of Baringo County (Omondi *et al*., 2017). The dominance of malaria vectors resting indoors implies a higher exposer of humans to indoor transmission of malaria. This scenario requires up scaling of indoor control interventions such as ITNs and IRS. However, it has been noted that these methods remain effective where majority transmission occur indoors (Russell *et al*., 2011). The outdoor species were mainly those responsible for arbovirus transmission to both humans and animals and it has been observed that indoor control methods are ineffective for outdoor resting mosquitoes (Pates and Curtis, 2005). In addition, two species of genus *Anopheles* namely; *An. pharoensis* and *An. coustani*, which have been incriminated in malaria transmission were exclusively collected outdoors. Therefore, additional control methods are required for outdoor resting species (Russell *et al*., 2011).

Mosquito abundance in this study varied per month and zone with midland and highland zones recording no mosquitoes during some months of the year. These results are similar to the findings of a study in Senegal in which higher mosquito densities were documented during some months of the year (Ndiath *et al.,* 2012). This study collected more mosquitoes during the months which followed heavy rainfall. This is consistent with previous studies which have shown that a time lag exists between onset of rainfall and mosquito abundance (Khan *et al*., 1996; El Mamy *et al*., 2011). However, even if a lag phase exists, meteorological data can be used to model mosquito population dynamics and to predict population spikes of vector species to prevent imminent disease outbreaks (Shone *et al*., 2006). During this study, sharp population spikes for *An. gambiae* s.l. and *Cx. pipiens* s.l. were observed in the month of December 2015 and May 2016 respectively. Whereas increase in mosquito abundance in December 2015 corresponded to a small peak in temperature, this was not the case in May 2016 when population spike of mosquitoes corresponded with high rainfall. The increase in *An. gambiae* s.l. and *Cx. pipiens* s.l. in the two months is in agreement with previous findings which reported that weather conditions

affect species differently (Chuang *et al*., 2011). Besides, warmest temperatures have been associated with high mosquito population while low temperature and very high rainfall lead to reduction in mosquito population (Yoo *et al.,* 2016). Therefore, variations in mosquito abundance may be correlated to local weather conditions either simultaneously for temperature or after a time lag for rainfall. Variations in weather patterns and mosquito population dynamics are related to disease trends hence may be used to predict disease outbreaks. Disease resurgence has also been attributed to warming trends due to climate change (Patz *et al*., 2008). For example, increased temperature has been shown to be a good predictor of mosquito infections hence higher human disease incidence (Ruiz *et al*., 2010; Chen *et al*., 2012). This study found minimum temperature to be more influential to mosquito abundance than maximum temperature.

Presence of RVF potential vectors in all ecological zones though in small numbers points out an expansion of areas at risk. This information has been modeled by Ochieng *et al.* (2016) and predicts that future climatic conditions will lead to increase in spatial distribution of RVF vectors. *Mansonia* species which are the principal vectors of RVF in Baringo (Sang *et al*., 2010; Lutomiah *et al*., 2013), were collected in relatively large numbers from sites which were near swamps in each ecological zone. In particular, Loboi and Sirata have large permanent swamps with the latter having the largest number of *Mansonia* mosquitoes collected in this study and was one of the hotspots of RVF during the 2006/2007 outbreak in Baringo. The large number of *Mansonia* species collected from sites near a swamp is similar to findings of Arum *et al.* in which more mosquitoes of this genus were collected from marshy areas (Arum *et al*., 2015). An entomological survey in diverse regions of Kenya also indicated that *Mansonia* species are adapted to large swampy areas around lakes which provide suitable breeding habitats (Lutomiah *et al*., 2013).

Mansonia uniformis, *Ma. africana*, *Culex univittatus* and *Cx. pipiens* s.l. which are vectors of RVF were the most abundant species consistent with a previous study in Baringo (Lutomiah *et al*., 2013). A study in the northeastern and coastal regions of Kenya also found similar results in which *Mansonia* species were the most abundant followed by RVF primary vectors belonging to *Aedes* species (Arum *et al*., 2015). Species of *Aedes* genus were the least abundant throughout the sampling period. Overall, few adults of *Aedes* species were collected from all zones probably because they bite outdoors during the day (Haddow, 1960) yet this study used CDC light traps at

night to collect outdoor resting mosquitoes. This would have minimized the chances of capturing *Aedes* mosquitoes. Besides using the CDC light trap during the night, few *Aedes* mosquitoes were collected in this study probably due to lack of Carbon dioxide bait normally used with CDC light traps (Newhouse *et al*., 1966).

Anopheles gambiae s.l. was the most abundant species of the three malaria vectors while *An. pharoensis* and *An. funestus* were less abundant. This result corroborates findings of a previous study in the lowland zone of Baringo (Aniedu, 1992) and western Kenya where *An. gambiae* was found to be the predominant vector of the total anophelines collected (Shililu *et al*., 1998). Most mosquitoes were collected from lowland and riverine zones while midland and highland zones had few malaria vectors. A study in western Kenya highlands also found high density of malaria vectors at valley bottom compared to mid hill and hill tops (Githeko *et al*., 2006). The findings of this study on distribution and abundance of malaria vectors are important in guiding on areas where vector control efforts should be scaled up. Therefore, bed net coverage and sensitization of the community to optimize net utilization should be focused in the riverine and lowland which had high populations of vector mosquitoes and thus high malaria risk zones. The proportion of *An. gambiae* s.l. in relation to other malaria vectors in the zone was higher in riverine compared to lowland and this may explain the higher rates of malaria incidence found in riverine compared to lowland (Omondi *et al*., 2017) during the same period of this study.

Anopheles coustani and *An. pharoensis* were mostly collected outdoors while *An. gambiae* s.l. and *An. funestus* were mainly collected indoors. This would suggest that *An. gambiae* and *An. funestus* are the main vectors of malaria in Baringo County. Aniedu (1993) also found similar resting pattern for the four species in Baringo. Contrary to general perception that malaria transmission occurs indoors, presence of *An. pharoensis* and *An. coustani* which have been implicated in malaria transmission would possibly propagate outdoor transmission of malaria in Baringo County. Aniedu (1993) found high biting activity by *An. pharoensis* in Baringo while Mwangangi *et al*. (2013a) found high outdoor infectious biting rates by *An. coustani* in Taita District. Most research on malaria vector ecology is skewed towards the most important primary vector *An. gambiae*. However, the little studied less efficient secondary vectors like *An. pharoensis* and *An. coustani* might replace the primary vectors due to changing environmental conditions (Rejmáková *et al*., 2013). The observed occurrence of both indoor and outdoor places

by malaria vector species in Baringo implies that risk of malaria transmission is possible both indoors and outdoors in this pastoral community. Studies by Mboera *et al*. (2005) in Tanzania and Chinwe *et al*. (2014) in Nigeria found that herders in semi arid areas occasionally spend nights outside hence exposed to outdoor malaria transmission.

Grass thatched-mud walled houses had the highest number of vector mosquitoes and corrugated iron roofed stone-walled houses had the least. Based on mosquito counts, this observation is in agreement with a study in the Gambia which showed that mosquito numbers were high when walls were made of mud (Kirby *et al*., 2008). A similar study in Sri Lanka also found that houses with thatched roofs and mud walls had more mosquitoes compared to those constructed well with plastered walls and tiled roofs (Konradsen *et al*., 2003). It has also been reproted that houses that are ventilated and poorly lit provide ideal indoor resting places for mosquitoes (RBM, 2014). The findings of the current study attest to this since the grass thatched mud walled houses which had high abundance of malaria vectors did not have windows to allow light in but had open eaves for ventilation. An investigation conducted in western Kenya by Zhou *et al.* also found that house structure variables significantly affected abundance of indoor resting mosquitoes among other environmental factors (Zhou *et al*., 2007). Similar observations were made in Thailand and Puerto Rico where adult mosquitoes were usually collected in large numbers from some houses but not others (Scott *et al*., 2000).

In this study, malaria vector abundance association with different house structures was greater in the riverine zone compared to the lowland zone. This is probably because riverine zone had more varied house designs made of same materials. Of particular interest is the finding that houses built on stilts (locally known as "bororiet") had relatively fewer malaria vectors than similar houses built on ground level in the riverine zone. Studies conducted in São Tomé, Trinidad and the Dominican Republic also showed that houses raised by stilts had fewer vector mosquitoes than houses built on the ground (Charlwood *et al*., 2003; Howell and Chadee, 2007).

Malaria vectors *An. gambiae* and *An. funestus* feed inside houses at night implying that most of the malaria transmission occurs indoors. Therefore, malaria transmission may be affected by house design due to entry rates of the vectors (Charlwood *et al*., 2003). Gamage-Mendis *et al.* in their study attributed malaria incidence variation with type of housing construction in which poorly–constructed houses had higher malaria cases (Gamage-Mendis *et al*., 1991). In Burkina

Faso, roof type was found to have an effect on *Plasmodium. falciparum* infection whereby prevalence among children who lived in iron-sheet roofed houses was less than those who lived in mud roofed houses (Ye *et al*., 2006). Further evidence on effects of materials used for construction of houses show that living in non-earth floors and non-thatched roofs reduced malaria incidence by half compared to living in traditional houses in Uganda (Snyman *et al*., 2015).

The burden of malaria is highest among the poorest households in the community (Chuma *et al.,* 2010) who live in poorly constructed rural houses likely to expose them to mosquito bites hence increased malaria transmission (Charlwood *et al.,* 2003). Generally, modification of house design has been suggested as an intervention for reduction of malaria vectors resting indoors (Lindsay *et al*., 2003; Walker, 2010; Haines *et al.,* 2012; Swai *et al.,* 2016) and this should be encouraged in Baringo County.

Generally higher proportions of malaria vectors were collected during the drier seasons than rainy seasons. Similar results have been obtained by a study in western Kenya (Ndenga *et al*., 2006) and south region of Cameroon (Bigoga *et al*., 2012) where *Anopheles* mosquito densities increased during the period of the dry season. This is also in agreement with results obtained in a study conducted in a Sahelian village in which higher mosquito densities were found in houses during the dry season than expected (Lehmann *et al.,* 2014). Contrary to findings of some studies in which *An. gambiae* s.l. mosquito abundance was high during rainy seasons (Minakawa *et al*., 2002; Shililu *et al*., 2004; Mwangangi *et al*., 2009), the proportion of malaria vectors collected during the long rain season was the lowest in this study. This is not surprising because long and heavy rain affects breeding sites by flushing out larvae and killing them (WHO, 1975a; Paaijmans *et al.,* 2007). Hence, the breeding of a vector population is greatly reduced with repeated rains which cause flooding and wash away larvae. High population of *An. gambiae* s.l. was observed during the dry season in the current study. This could explain high prevalence of malaria cases recorded in school children in in Baringo during the dry season (Omondi *et al*., 2017).

The present study found a high correlation between monthly average rainfall and *An. gambiae* s.l. population in the riverine zone but not in the lowland zone. The lack of correlation between malaria vector abundance and rainfall in the lowland zone of Baringo is consistent with the

findings of Aniedu (Aniedu, 1992). Most breeding habitats in the lowland zone are permanent water bodies whose productivity may not be affected by rainfall trends hence low correlation between rainfall and vector abundance. Moreover, it has been shown through modeling simulations that rainfall alone does not account for mosquito populations (Bomblies, 2012) in water-limited semi-arid environments. The simulated values are still greater than actual values reported for correlation between rainfall and vector abundance (Koenraadt *et al*., 2004; Kelly-Hope *et al*., 2009). In the current study, correlation between vector abundance and rainfall in the riverine zone was less than the simulated value. A study conducted in western Kenya highlands showed no correlation between mosquito abundance and monthly rainfall though population appeared to increase with rainfall or shortly after rainfall (Shililu *et al*., 1998). However, a later study in east African highlands showed a linear relationship between *An. gambiae* density and a 2-month lag in rainfall peak (Kristan *et al*., 2008). This suggests that the relationship between rainfall and vector abundance is not the same therefore surveillance should be carried out more frequently at localized areas.

There was no correlation between vector abundance and temperature in the riverine compared to a very low correlation in the lowland which was not statistically significant. This implies that temperature may not be an important climatic factor regulating malaria vector abundance in semi arid Baringo. This is supported by findings of a study in Bangladesh where temperature did not have an effect on abundance of anophelines (Bashar and Tuno, 2014). However, Minakawa *et al*. found a significant influence of temperature on malaria vector abundance in Kenya (Minakawa *et al*., 2002) but their analysis included highlands and non arid areas.

5.6 Conclusions

1. Rift Valley Fever and malaria vector abundance were high in the lowlands (lakes region) and riverine zone (Kerio valley).

2. The known vectors of RVF (*Mansonia* and *Culex* species) were mainly collected outdoors mostly in swampy areas. The less common vector of malaria, *An. pharoensis*, was also collected outdoors while *An. gambiae* was mainly found indoors.

3. Lowest number of malaria vectors was recorded during the long rain season while highest number was collected during the dry seasons. This implies that malaria cases are expected to be

high during the dry rather than wet seasons. A positive correlation between rainfall and *An. gambiae* abundance was observed in the riverine zone. This could be important in predicting malaria vector abundance and hence malaria outbreak.

4. Houses with open eaves, made of mud walls and grass thatched roofs had high abundance of malaria vectors. Stilted houses houses had fewer malaria vectors than than those build at ground level.

CHAPTER SIX

6.0 IMPACT OF SEASONAL VARIABILITY IN RAINFALL AND TEMPERATURE ON INFECTION STATUS OF MALARIA VECTORS IN BARINGO COUNTY

6.1 Introduction

Malaria is one of the diseases that have been linked to climate change. The impact of malaria is likely to be altered under different climatic conditions as the mosquitoes' vector potential, abundance, blood feeding behaviour, survival and ability to support parasites also changes (Gage *et al.,* 2008). Climatic factors directly associated with occurrence of malaria are temperature and rainfall. High temperature increases blood digestion and feeding frequency. This leads to more host contact hence increase in population of infective mosquitoes (Afrane, 2005). Rainfall on the other hand has been associated with high infection rates in malaria vectors and transmission of malaria (Ngom *et al*., 2014; Imbahale *et al*., 2012). A retrospective study of malaria cases in Baringo from 2004 to 2014 revealed a time lag of two months between rainfall and increase in malaria cases (Kipruto *et al*., 2017).

Malaria transmission patterns follow seasonal changes in some places. A previous study in western Kenya highlands found perennial malaria with transmission occurring in wet (July-September) and dry (December-February) seasons (Shililu *et al*., 1998). This is in agreement with a recent study in Baringo that found perennial transmission in the low lying regions (Omondi *et al.,* 2017). In Cameroon, malaria transmission was found to occur both during the dry and rainy seasons but peaks during dry season (Bigoga *et al*., 2012). This is similar to recent reports by Amadi *et al.* (2018a) which showed that malaria cases followed a seasonal pattern whereby some parts of Baringo experienced high cases of malaria in cold dry (June to August) and short rain (September to November) seasons. A study by Mala *et al.* in lowland area of Baringo County revealed that transmission of malaria is low and seasonal with peak transmission occurring during the wet season (Mala *et al*., 2011b). The findings from previous studies in Baringo suggest inter-annual variability in seasonal malaria transmission patterns and hence need for regular surveillance to guide control efforts. In Baringo County, Only lowland region has been investigated for malaria vector infections with *Plasmodium falciparum* parasites (Mala *et al.,*

2011b). The current study investigated seasonal changes in reservoir patterns of *Plasmodium falciparum* parasites in vectors of malaria in the four ecological zones in Baringo County.

6.2 Literature review

6.2.1Malaria Burden in Kenya

In Kenya, malaria still remains a significant public health problem that occurs throughout the year and about 70% of the population is at risk of the disease (NMCP, 2016). The disease continues to be the cause of morbidity and mortality. In 2010, clinically diagnosed malaria accounted for 34% of outpatient hospital visits (KMIS, 2011). Nationally malaria cases in children under 14 years old decreased from 11% in 2010 to 8% in 2015 (KMIS, 2016). The burden of malaria in Kenya is not homogeneous but arid and semi arid areas such as Baringo County experience seasonal variations of disease occurrence (Aniedu, 1997; Mala *et al.,* 2011b).

Kenya is divided into four malaria epidemiological zones namely; highland epidemic prone zone where transmission is seasonal, endemic areas of stable malaria, seasonal malaria transmission areas with arid and semi arid conditions and low risk areas with very low temperatures (KMIS, 2016). In western Kenya highlands malaria transmission is low but perennial (Shililu *et al.,* 1998). In the coastal region of Kenya, high incidence of severe disease occurred under conditions of very low levels of transmission by vector populations (Mbogo *et al*., 1995). According to the Ministry of Health, a recent survey indicated that *P. falciparum* parasite prevalence in low risk areas increased from 35.1% in 2000 to 53.6% in 2015 while largest reductions were observed in the endemic lake and coastal regions (MoH, 2016).

6.2.2 Vectors of malaria in Kenya

Mosquito species transmitting malaria in Kenya are *An. gambiae* s. s., *An. arabiensis* and *An. funestus* s. s. (Taylor *et al*., 1990; Mbogo *et al*., 1995). In addition, *Anopheles coustani* which is not a common vector of malaria has been reported as a vector of malaria parasites in Taveta district of Kenya (Mwangangi *et al*., 2013a). In Baringo County, common malaria vector species are *An. gambiae* complex, *An. funestus* and *An. pharoensis* (Aniedu, 1992). The reporting of *An. pharoensis* as a vector of malaria parasites has only been done in Baringo and Mwea irrigation scheme in central Kenya (Mukiama and Mwangi, 1989). Thus, the role of *An. pharoensis* and *An.*

coustani in malaria transmission could be secondary or incidental in the areas where they have been incriminated.

6.2.3 Malaria control and challenges

Malaria can be controlled by avoiding the bite of the anopheline mosquitoes (through protective measures against the vector), by administration of anti-malarial drugs (chemotherapy) and by use of prophylactic drugs to prevent development of the disease in case of an infective bite when a person visits malaria endemic areas (chemoprophylaxis), (WHO, 1997). However, due to widespread resistance to drugs by parasites and evidence of the emergence of resistance to insecticides by vectors, malaria control calls for the development of new methods and improved utilization of available control strategies (Hemingway *et al*., 2016).

6.2.4 Malaria vector species and insecticide resistance

Anopheles funestus is highly susceptible to insecticides hence is easily eliminated and is slow to re-colonize the same place (Gillies and Meillon, 1968). In western Kenya, this species was greatly reduced in ITN intervention areas (Gimnig *et al*., 2003) while in Mwea area of Central Kenya, Kamau *et al.* found no *An. funestus* mosquitoes and attributed this to the use of pesticides in agriculture and / or the use of insecticides in the form of aerosols or mosquito coils (Kamau *et al.*, 2003). A recent study in western Kenya revealed reemergence of *An. funestus* with low mortality to bioassays; a finding which is of great concern (McCann *et al*., 2014). Similar results have been obtained in coastal Kenya where *An. funestus* was more abundant than *An. gambiae* s.l. and showed low susceptibility to insecticides (Kiuru *et al*., 2018). In addition, more malaria vectors were collected outdoor in the coastal Kenya corresponding to higher sporozoite rates outdoors. This change in behavior poses a challenge to malaria control as the strategies used (ITNs/LLINs and IRS) target indoor biting and resting mosquitoes.

6.2.5 Vectorial capacity of malaria vectors

The mosquito species which have been incriminated in the transmission of malaria parasites in Baringo are *An. gambiae* s.l., *An. funestus* and *An. pharoensis* (Aniedu, 1992, 1993; Mala *et al*,. 2011b). The sporozoite rates from previous studies in these vectors in western Kenya were found to be 6.3% and 9.5% in *An. gambiae* and *An. funestus* respectively (Shililu *et al*., 1998); 2.6% and 4.5% in *An. gambiae* s. l. and *An. funestus* respectively (McCann *et al.*, 2014) while infection

rate for *An. pharoensis* was 1.3% (Mukiama and Mwangi, 1989) and 0.1% (Ijumba *et al.,* 1990) in Mwea irrigation scheme.

Different vectors of malaria differ in sporozoite rates in different ecological areas. Previous studies in western Kenya obtained *P. falciparum* sporozoite rates of 9.6%, 6.4% and 0.4% for *An. gambiae* s. s., *An. funestus* and *An. arabiensis* respectively (Taylor *et al*., 1990). *Anopheles arabiensis* has a lower sporozoite rate probably because of its less longevity in nature (Gillies and Coetzee, 1987) and its zoophilic and exophilic tendencies (Molineaux and Gramiccia, 1980). *An. funestus* is responsible for the major part of malaria transmission mostly in rice fields. In Madagascar, more than 95% of *An. funestus* breeds in rice fields (Laventure *et al*., 1996). *Anopheles funestus* was also found to have higher sporozoite rates than *An. arabiensis* in central Kenya where there is planned rice irrigation program (Muturi *et al.*, 2008). Recent studies in Ahero and coastal Kenya also reported higher sporozoite rates in *An. funestus* than in *An. arabiensis* (Degefa *et al*., 2017; Kiuru *et al*., 2018). Contrary to these findings, a recent study in three islands of Lake Victoria found no infections in *An. funestus* but instead found highest infection rates at 18.5% in *An. coustani* for the first time (Ogola *et al*., 2017).

6.2.6 Effect of seasonal variability in rainfall and temperature on malaria vector infection status

Rainfall and temperature are the climatic factors which have been linked to infections with malaria (Githeko *et al*., 2000). A study conducted in northern Nigeria by Molineaux and Gramiccia (1980) observed an increase in sporozoite rates during the wet season. This implies that infection status of malaria vectors varies with seasonal changes in rainfall and transmission dynamics may follow similar pattern. In semi arid Eritrea, significant seasonal variations were recorded in *An. arabiensis* which is the major malaria vector (Shililu *et al.,* 2003). In western Kenya, findings of a study showed that temporal variation of *P. falciparum* malaria prevalence in the highlands is determined by meteorological variables such as rainfall, temperature and humidity (Wanjala *et al.,* 2011). Rainfall has been positively associated with the occurrence of *P. falciparum* infections in different sites in western Kenya (Imbahale *et al*., 2012).

Development of *Anopheles* mosquitoes and the malaria parasites they transmit depend on temperature (Beck-Johnson *et al.,* 2013). The transmission intensity of malaria parasites was found to be high at cooler temperatures but drops off rapidly at higher temperatures. Experiments

by Paaijmans *et al*. (2012) have found that warmer temperatures reduce vectorial capacity of malaria vectors though it is assumed that development rate of parasites in mosquitoes increase with temperature. This implies that there is an optimum temperature for mosquito vectors to transmit parasites. Shapiro *et al*. (2017) have suggested an optimum transmission temperature of 29°C, minimum temperature of 12°C and maximum temperature of 38°C from their experiments. This is consistent with the findings of Paaijmans *et al.* (2012) that higher temperatures reduce vectorial capacity.

6.3 Materials and Methods

6.3.1 Identification of *Anopheles gambiae* complex to sub species by PCR

After morphological identification of mosquito species according to the taxonomic keys by Gillies and Coetzee (1987), *Anopheles gambiae* complex was further identified to sub species level by species diagnostic polymerase chain reaction (PCR).

6.3.2.1 Extraction of DNA from individual mosquitoes

Genomic DNA was extracted from the single field collected mosquitoes using the alcohol precipitation method according to (Collins *et al.,* 1987). Briefly, individual adult mosquito legs and wings were placed in a 1.5 ml microfuge tube containing 100µl of grinding buffer (4 parts of homogenization buffer and 1 part of lysis buffer) then ground. The triturated homogenized specimen was incubated in a water bath at 65°C for 30 minutes to denature the nucleases. Fourteen microlitres of Potassium acetate (8M) were then added and the mixture cooled on ice for 30 minutes to precipitate the mosquito parts, other insoluble substances and denatured proteins. The mixture was spun in a cold centrifuge (4°C) at 14,000 revolutions per minute (rpm) for 15 minutes and the resulting supernatant was transferred to a new sterile microfuge tube. Two hundred microlitres of cold 100% ethanol were then added and the mixture was chilled overnight at -20°C to precipitate DNA. The following day the mixture was centrifuged for 20 minutes to pellet the DNA. The excess ethanol was poured out and the DNA pellet was rinsed with 200µl of 70% ethanol. The pellet was rinsed again with 200µl of absolute ethanol (100%) and air-dried for 12 hours at room temperature after which the DNA was resolubilized in 100µl of sterile deionized water and stored at -20°C. One microlitre of this DNA was used for the polymerase

chain reaction (PCR) to distinguish between *An. gambiae* complex sibling species; *Anopheles gambiae* s.s. and *An. arabiensis*.

6.3.2.2 DNA amplification

Anopheles gambiae complex sibling species were distinguished by PCR using the method of Scott *et al.* (Scott *et al*., 1993). The DNA was amplified in a GeneAmp PCR system 9700 machine supplied by Applied Biosystems. For a single 15µl reaction, the following were added to the appropriate 0.2 ml PCR tube: 1.5µl of 10x $MgCl_2$-free buffer, 1.8µl of 25mM $MgCl_2$, 0.6µl of 10mM dNTP, 0.52µl of 0.02µg/µl of each diagnostic primers, 0.06µl of amplitaq polymerase, 1µl of template DNA and sterile deionized water added to final volume of 15µl. The PCR was performed for 30 cycles at a denaturation temperature of 94°C for 30 seconds, an annealing temperature of 60°C for 30 seconds and an extension temperature of 72°C for 30 seconds. The program included a pre-denaturation step of 5 minutes at 94°C and a final extension step at 72°C for 5 minutes. The amplified DNA was then analyzed by electrophoresis on agarose gel or stored at 4°C awaiting electrophoresis.

6.3.2.3 Agarose gel electrophoresis of the amplified products

To analyze the PCR products, 15µl of each amplified sample was mixed with a standard agarose gel loading buffer containing bromophenol blue dye and electrophoresis was performed in 3% agarose Tri-Borate-EDTA (TBE) gels containing ethidium bromide for staining. The gels were run for 20-30 minutes at 5 to 10 v/cm for sufficient separation of PCR products. DNA of known specimens, which give characteristic bands (*An. gambiae* and *An. arabiensis*), was included on all gels for comparison. The amplified fragments (DNA bands) were visualized by illumination with short wave ultraviolet light.

6.3.3. 1 Determination of malaria vector infections with *Plasmodium falciparum*

Nine hundred and twelve *Anopheles* mosquitoes (*An. arabiensis*, *An. gambiae* s. s. and *An. funestus*) were screened for presence of malaria parasites (*Plasmodium falciparum*) by enzyme-linked immunosorbent assay (ELISA) technique. Infection rate was estimated from the number of field mosquitoes found infected with sporozoites.

6.3.3.2 Screening mosquitoes for *P. falciparum* infections by ELISA

Head and thorax of a whole dried mosquito were cut and placed in labeled PVC microfuge tube. Fifty microlitres of blocking buffer + NP40 was added to each PVC tube containing head and thorax of mosquito to be tested. The mixture was ground with pestle attached to mortar driven grinder. The ground mosquitoes were stored overnight at -20^{0}C or at -70^{0}C if longer storage was required. Fifty microlitres of monoclonal antibody (MAb) was placed into each well of the ELISA plate: 0.20µg/50µl phosphate buffered saline (PBS) per well for *P. falciparum*. The plate was covered and incubated for 30 minutes at room temperature. The MAb solution was dumped from the wells by banging on the paper towels. The wells were filled with approximately 200µl/well blocking buffer and incubated for one hour. Blocking buffer was dumped and 50µl of mosquito triturate added to each well. Positive and negative controls were added in appropriate wells and the plate was incubated for two hours. The mosquito triturate was dumped and wells washed twice with PBS-Tween. Fifty microlitres of MAb-peroxidase conjugate was placed in each well: 0.05µg/50µl blocking buffer per well for *P. falciparum* then incubated for one hour. The MAb-peroxidase conjugate was dumped and wells washed four times with PBS-Tween. Hundred microlitres of fresh peroxidase substrate was added per well followed by incubation for 30minutes. The results were read at 414 nm using ELISA plate reader (Multiskan FC supplied by Thermo Scientific). Positive results were determined by mean of negative controls times two (Wirtz *et al.,* 1987). Any sample that had a higher value than the mean of negative controls times two was considered positive for *P. falciparum*.

6.3.4 Statistical analysis

The infection rate was expressed as a percentage of the total number of mosquitoes tested. A t-test was used to compare the mean difference between infections with *P. falciparum* in *An. arabiensis* and *An. funestus*. One way ANOVA was used to compare mean infections with *P. falciparum* between the months during which sampling was done. Post hoc Tukey test was used to separate means when ANOVA test was significant at 95%CI. Regression analysis was used to determine correlation between monthly rainfall and mosquito infections with *P. falciparum*.

6.4 Results

6.4.1 Distribution of malaria vectors and *Anopheles gambiae* s.l. sibling species composition

A total of 5772 anopheline mosquito vectors of malaria in Baringo were identified. They included *An. gambiae, An. funestus* and *An. pharoensis*. *Anopheles gambiae* was the most abundant malaria vector species while *An. funestus* was the least abundant. Both *An. gambiae* and *An. funestus* were collected indoors while *An. pharoensis* was mainly collected outdoors. *Anopheles funestus* and *An. pharoensis* were collected from lowland and riverine zones only while *An. gambiae* was collected from all the four ecological zones (Table 6.1).

Table 6.1 Proportions of *An. gambiae* s. l. sibling species and other malaria vectors in the four ecological zones in Baringo County

Species	Lowland		Riverine		Midland		Highland		Overall totals	
	Total (N)	R.A. (%)	Total (N)	R.A. (%)	Total (N)	R.A. (%)	Total (N)	R.A. (%)	Total (N)	R.A. (%)
An. funestus	12	0.2	57	5.0	0	0.0	0	0.0	69	1.2
An. pharoensis	277	5.7	14	1.2	0	0.0	0	0.0	291	5.0
An. gambiae s.l.	4294	88.0	1053	91.9	40	85.1	25	100	5412	93.8
Zone totals	4583 (79.4%)		1124 (19.5%)		40 (0.7%)		25 (0.4%)		5772 (100%)	
An. arabiensis	458	73.2%	145	23.2%	13	2.1%	10	1.6%	626	
An. gambiae s.s	7	77.8%	2	22.2%	0	0	0	0	9	

N-Total number collected; R.A-Relative Abundance

Overall, 880 *An. gambiae* s. l. mosquitoes were subjected to genetic characterization to identify them further to sibling species by PCR and 635 were amplified. *Anopheles arabiensis* was the most abundant sibling species (98.6%) of *An. gambiae* complex in Baringo County while the remaining percentage was *An. gambiae* s. s. (Plate 6.1)

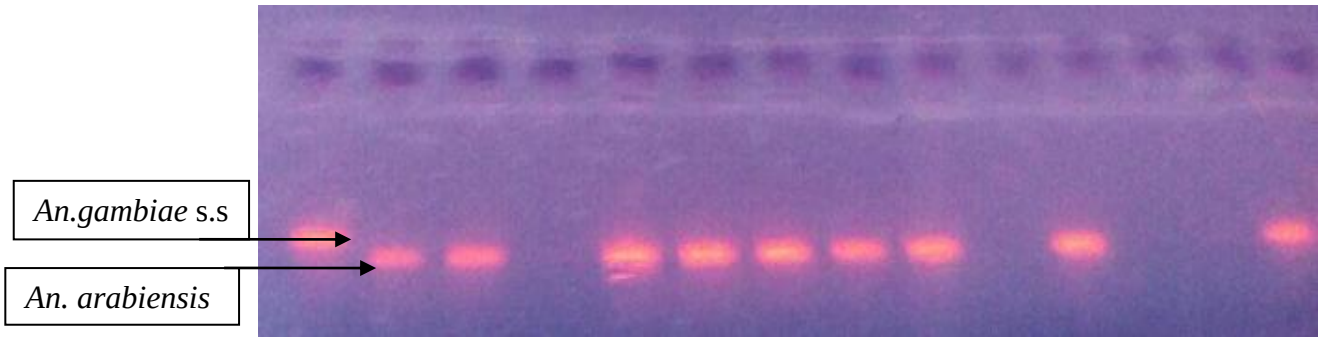

Plate 6.1 Agarose gel showing amplification bands for *An.gambiae* s.s. and *An.arabiensis*

6.4.2 Sporozoite rates in *An. arabiensis* and *An. funestus* in Baringo across the ecological zones

Out of the 852 *An. gambiae* mosquitoes screened for *Plasmodium falciparum* parasites, only 3 were positive representing a sporozoite rate of 0.35% (Table 6.2). The three *An. arabiensis* mosquitoes that tested positive for *P. falciparum* were all from the lowland zone. *Plasmodium falciparum* parasites were not detected in the only nine *An. gambiae* s. s. mosquitoes that were tested. On the other hand, 6 of the 60 *An. funestus* mosquitoes tested for *P. falciparum* were positive representing a sporozoite rate of 10% and all of them were from the riverine zone. Of the nine mosquitoes positive for *P. falciparum*, six (66.7%) were *An. funestus* though they were fewer than *An. arabiensis* and constituted only 6.6% of the tested mosquitoes. No sporozoites were detected in the 26 mosquitoes tested from the midland zone and 13 mosquitoes from the highland zone of the study area. The difference between the mean *P. falciparum* sporozoite rates for *An. funestus* (10%) and *An. arabiensis* (0.35%) over the 12-month period was significantly different (t=-2.470, df=59.299, p=0.016).

Table 6.2 Monthly sporozoite rates of *An. arabiensis* and *An. funestus* alongside mean rainfall and temperature in Baringo County

	Climate factors*		*An. gambiae (arabiensis)*		*An. funestus*		Overall sporozoite
Month	Rainfall (mm)	Mean Temp(°C)	No. tested	Sporozoite Rate (%)	No. tested	Sporozoite Rate (%)	rate (%)
June	168	27.7	113	0	4	0	0
July	37.5	26.5	88	0	0	0	0
Aug	86.1	28.5	72	0	21	23.8	5.4
Sep	195.3	29.1	97	3.1	2	0	3.0
Oct	54.4	27.3	72	0	5	0	0
Nov	86.1	25.1	50	0	2	0	0
Dec	101.4	26.5	97	0	13	7.7	0.9
Jan	48.4	30.1	62	0	0	0	0
Feb	8.7	27.3	58	0	1	0	0
March	109.7	26.5	52	0	12	0	0
April	56.8	28.7	37	0	0	0	0
May	41.1	28.5	54	0	0	0	0
Total			852	n=3	60	n=6	

* Average rainfall and temperature for lowland and riverine zones where sporozoite infections were detected

6.4.3 Effects of seasonal variability in climatic factors and sporozoite rates in lowland and riverine zones of Baringo

Sporozoite rate was significantly different between months ($F_{11, 928}$=2.76, p=0.002). Post hoc test showed that the month of August was particularly different from all other months. August recorded a sporozoite rate of 23.8 % for *An. funestus* only but 5.4% when *An. arabiensis* was included. However, there was no difference in sporozoite rates between seasons ($F_{3, 936}$=1.46, p=0.224) and zones ($F_{3, 936}$=2.45, p=0.062).

Infections of malaria vectors with *P. falciparum* parasites were detected in the months of August, September and December. The highest sporozoite rate was recorded in the riverine zone in August at 23.8% for *An. funestus* only followed by lowland zone in September at 3.1% for *An. arabiensis* only. One *An. funestus* mosquito from the riverine zone also tested positive in December. The months of August and December when *P. falciparum* sporozoites were detected in *An. funestus,* fall in cold dry and dry season respectively but moderate rainfall was experienced

during the two month (Appendix 13). On the other hand, the month of September when sporozoites were detected in *An. arabiensis* falls in the short rain season but unexpectedly high amount of rainfall was received. When rainfall was compared between months, there was a significant difference ($F_{11, 928}$=6.219, p<0.000) and there were no two months that were similar in amount of rainfall. The average temperature ranging from 25.1° to 30.1° C was also different between all months of the sampling period. Infections were significantly correlated to rainfall (p=0.032) but not temperature (p=0.654). However, there was no correlation between seasons and infections (p=0.514).

6.5 Discussion

Anopheles arabiensis was the main sibling species of the *An. gambiae* complex in Baringo County. This is in agreement with the findings of Mala *et al*. in earlier studies conducted in the lowland zone (Mala *et al*., 2011b). Presence of *An. arabiensis* in the highland zone with cooler temperatures (KMIS, 2016) is an indication that climate change has created favourable conditions where this species is not expected to thrive. However, it should be noted that this was the first time highland zone was surveyed for malaria vectors. Baringo County is semi arid and according to Shililu *et al*. (2003), *Anopheles arabiensis* is the predominant sibling species *An. gambiae* complex in semi arid areas. *Anopheles arabiensis* is also zoophilic and its high abundance in Baringo requires consideration of two aspects of animals in vector control; animals can either be used for zooprophylaxis or serve as alternative source of blood meal for mosquitoes whose population will increase.

Anopheles funestus was collected from low lying areas but not highlands in this study. This is similar to results obtained in western Kenya where it was collected from valley bottom but not hill tops (Githeko *et al*., 2006). This would suggest that topography affects distribution of this particular species. Whereas *P. falciparum* was detected only in *Anopheles arabiensis* in the lowland zone, *An. funestus* was the only malaria vector infected with *P. falciparum* in the riverine zone. The *An. arabiensis* and *An. funestus* mosquitoes which were positive for *P. falciparum* were all collected indoors and were blood fed. This finding differs from a study conducted in the coastal Kenya where sporozoite infectivity was found both indoors and outdoors with the outside infection rates being higher than those for indoors (Mwangangi *et al*., 2013a). This implies that the vectors transmitting malaria behave differently in localized areas.

Though *An. funestus* constituted only 6.6% of all malaria vectors, it had the highest sporozoite rate of 10%. This is similar to the findings of a study in western Kenya highlands where they found higher sporozoite rates in *An. funestus,* which constituted only 16% of test mosquitoes compared to *An. gambiae* (Shililu *et al*., 1998). Similarly, it was also found recently in Tanzania that *An. funestus,* though less abundant than *An. arabiensis,* had higher sporozoite rates (Kaindoa *et al*., 2017). All these findings suggest that *An. funestus* significantly contributes to malaria transmission even in areas where *An. arabiensis* is the predominant vector species. In some areas, *An. funestus* is considered the most efficient vector of malaria because of its strong anthropophily and high sporozoite rates (McCann *et al*., 2014). Further support of this is from the coastal Kenya where high sporozoite rates were recently obtained from *An. funestus* compared to *An. gambiae* s. l. (Kiuru *et al*., 2018).

All the *Anopheles funestus* mosquitoes that were positive for *P. falciparum* were collected from the riverine zone. This is the first time high malaria *Plasmodium falciparum* infections in mosquitoes has been detected in riverine zone of Baringo and the most infected vector found to be *An. funestus* though outnumbered by *An. arabiensis.* Coincidentally, a concurrent study in Baringo also found high cases of malaria in the riverine zone compared to lowland zone (Omondi *et al*., 2017). This is a major finding from this study because previous studies in Baringo only reported findings in the lowland zone only around the lakes region. Therefore, up scaling indoor interventions targeting malaria vectors could greatly reduce malaria transmission in focalized areas such as riverine zone in Baringo County.

Infection of *An. arabiensis* with *P. falciparum* sporozoites was detected only in September 2015. Similarly, *Anopheles arabiensis* sporozoite infections were found in the month of September during a study conducted in Senegal (Ngom *et al*., 2014). A previous study in the lowland zone of Baringo also found that vector infections with malaria parasites were undetectable during much of the investigation period (Mala *et al*., 2011b). This finding confirms that malaria transmission in arid areas is seasonal (Shililu *et al*., 2003).

The current study suggests a potential correlation between rainfall and *P. falciparum* occurrence and no correlation with temperature. This is in agreement with a study conducted in western Kenya where an association was established between rainfall and malaria parasites (Imbahale *et al*., 2012). There was no positive association of sporozoite infection with season unlike the study

of Mwangangi *et al*. where sporozoite positivity was associated with season (Mwangangi *et al*., 2013b). Though season was not significantly associated with sporozoite rates in the current study, coincidentally the month of August which had the highest infection rate is categorized under dry season. The study of Mwangangi *et al*. also found high infection rates in the dry seasons. A survey to check *P. falciparum* infection rates in school children in Baringo County found high infection rates during the dry season (Omondi *et al.,* 2017). This is not surprising because dry seasons are associated with high temperatures which increase blood digestion rate and feeding frequency hence more host contact leading to increase in population of infective mosquitoes (Afrane, 2005). Dry season, therefore, could be a high risk season for malaria infections in Baringo.

6.6 Conclusions

1. This is the first time a pocket of high *Plasmodium falciparum* infections in mosquitoes has been reported in riverine zone of Baringo and the most infected vector found to be *An. funestus.* This is a major finding from this study because previous studies in Baringo only reported findings in the lowland zone around the lakes region.

2. Though *An. arabiensis* was the dominant species, *An. funestus* could be contributing significantly to malaria transmission in Baringo County.

3. Sporozoite rates were detected during the months that are dry and hot. Thus the high temperatures experienced in dry seasons with presence of moderate rainfall could be an indicator of potential increase in infections with *P. falciparum.*

CHAPTER SEVEN

7.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General Discussion

The current study surveyed the larger area (3 out out of 6 Sub Counties) of Baringo to investigate RVF and malaria vector species diversity, larval habitat preference, distribution, seasonal abundance and infection status in four varied ecological zones. Baringo County is topographically heterogeneous but despite several studies having been conducted on RVF and malaria vectors (Aniedu, 1992; 1997; Arum *et al*., 2010; Sang *et al*., 2010; Mala *et al*., 2011a, b; Lutomiah *et al*., 2013; Omondi *et al*., 2015), localized information on the distribution and variations of mosquito vector abundance is still not sufficient. The said studies largely focused on entomological surveys around the lakes in the lowland (Marigat Sub County) but this study has collected information on vector abundance in the riverine, midland and highland regions. Infection status of malaria vector species has not been established in the rarely surveyed regions though recent analyses showed that malaria cases were spread up to the highlands (Kipruto *et al*., 2017; Amadi *et al*., 2018a).

The current study design in which the study area was stratified into ecological zones then random sites selected from each zone; ensured that every unique location was sampled. This was useful in determining the mosquito species diversity and how they were distributed across the four varied ecological zones. Walton (2005) recommends stratified random sampling as compared to simple random sampling because habitat preference by mosquitoes differs. The coverage of a large area of Baringo, identification of culicine larval species, duration of the study and involving community members in the current study is an additional value compared to previous studies. This was an extensive study describing large-scale distribution and abundance of species by sampling different life stages. Silver (2008) proposes that sampling same site repeatedly is appropriate for prediction of outbreaks.

Sampling of larvae and adults from same sites ensured that all species present were captured. This is because some species like *Mansonia* spp are not easy to capture at larval stage because they attach to the aquatic plants (Rajendran *et al*., 1989; Chandra *et al*., 2006) while others are not easy to get at adult stage depending on method of collection. For example, *Aedes* species

which bite during the day outdoors (Haddow, 1960; Ajamma *et al*., 2016) could not be captured by the CDC light traps which were set over-night in the current study. Therefore, most of the *Mansonia* species were collected at adult stage while *Aedes* species were collected at larval stage. Sampling every month ensured that all seasons of the year were sampled while indoor and outdoor adult collections ensured all species with different resting behavior were collected (WHO, 1975b).

This study identified mosquito species of culicine and anopheline larvae that can lead to emergence of adults responsible for transmission of arboviral diseases and malaria in Baringo. Information on species diversity of mosquitoes of medical importance can help identify areas of high risk of disease transmission (Selvan *et al*., 2015). Seventeen larval species and twenty six adult species were identified in the current study. Most of the species were culicines while only five were anophelines. Four of the anophelines were malaria vectors, two of which are the main vectors (*An. gambiae* and *An. funestus*) while the other two are secondary vectors (*An. pharoensis* and *An. coustani*). Presence of *An. gambiae, An. funestus, An. coustani*, and *An. pharoensis*, previously found in Baringo (Arum *et al*., 2010; Mala *et al*., 2011a) and confirmed by this study, shows that they are the most predominant anopheline species in Baringo. Molecular analysis by PCR showed that *An. arabiensis* was the dominant sibling species (over 98%) of *An. gambiae* complex mosquitoes. This is slightly different from the findings of previous studies in Baringo (Mala *et al*., 2011a; Ajamma *et al*., 2016) which documented *An. arabiensis* as the only sibling species in Baringo. Out of the twenty six adult species, only four culicine species have been confirmed as vectors of RVF virus in Baringo and these are *Mansonia africana, Mansonia uniformis, Culex pipiens* and *Culex univittatus* (Sang *et al*., 2010; Lutomia *et al*., 2013). Overall species diversity was highest in the lowland zone followed by riverine zone (Ondiba *et al*. 2017b). The findings of this study are important in developing control strategies for RVF and malaria vectors in Baringo where reservoir pathogens of these two diseases occur.

This study established general distribution of various mosquito species that have been implicated in the spread of vector borne diseases. *Anopheles gambiae*, which is the principal vector of malaria, was found in all the four ecological zones. *Anopheles funestus* and the secondary vector of malaria, *An. pharoensis*, were only found in the lowland and riverine zones (Ondiba *et al*., 2017a). *Anopheles coustani*, though not confirmed as a vector of malaria in Baringo, was not

found in the highland zone. On the contrary, potential vectors of RVF were found in all ecological zones though with varied abundance (Ondiba *et al*., 2017b). Similar mosquito distribution was found in eastern Nepal where malaria vectors were collected up to 1,800m asl while other vectors (culicines) existed above 2,300m asl (Dhimal *et al*., 2014).

Mosquitoes infected with *Plasmodium falciparum* were detected only from low lying ecological zones (lowland and riverine zones). This could explain findings of a recent concurrent study in which highest malaria cases were reported in riverine zone followed by lowland zone (Omondi *et al*., 2017). Similarly, high prevalence of RVF virus sero-positivity in animals was detected in animals from lowland zone followed by highland zone (Juma *et al*., unpublished data). This shows that variations exist in mosquito vector distribution and infections they transmit across ecological zones in Baringo County with the greatest risk being in the low lying areas.

Majority of the culicine mosquitoes responsible for transmission of arboviral diseases were collected outdoors than indoors unlike anophelines. *Anopheles gambiae* and *An. funestus* which are the main vectors of malaria were mainly collected indoors (over 89%) while over 90% of *An. pharoensis* and *An. coustani* which are secondary vectors were collected outdoors (Ondiba *et al*., 2017a). This shows that outdoor transmission of malaria could be going on in Baringo since the outdoor anophelines, *An. pharoensis* and *An. coustani*, have been implicated in malaria transmission (Aniedu, 1993; Mwangangi *et al*., 2013a). Contrary to findings in Baringo, majority of anophelines in western Kenya were collected outdoors an indication that outdoor transmission was considerably high (Degefa *et al*., 2017). This calls for additional interventions to complement ITNs and IRS which target indoor resting mosquitoes.

Several factors affect occurrence of mosquito larvae in breeding habitats. These factors include presence of predators (Kroeger *et al*., 2014)), physico-chemical factors (Nikookar *et al*., 2017), depth and water surface area (Reiskind and Zarrabi, 2012). Climatic and environmental variables can also influence distribution of mosquito larvae (Amadi *et al*., 2018b). A recent study in Baringo found that most mosquito larval species were linked to habitats with larger surface area of the water bodies and inceasing pH value (Loye *et. al*., unpublished data). These species included *An. gambiae, An. pharoensis* and *Mansonia* spp. *Anopheles gambiae* and *An. pharoensis* were found in the ditch, river bed pools and swamp in high density compared to other breeding habitats. The ditch was the most productive habitat for *An. gambiae* consistent with the findings

of Arum *et al*. in the lowland Baringo (Arum *et al.,* 2010). Similarly the study of Arum *et al*., found that the swamp/marsh produced larvae all through the wet and dry seasons an indication that it is a major source of malaria vectors. Seasonal riverbeds have been found to be highly productive for *An. gambiae* larvae in semi arid areas (Shililu *et al*., 2007) consistent with this study. Thus management and maintenance of habitats such as ditches, swamp and seasonal riverbed pools could greatly reduce malaria vectors in Baringo. However, *Culex* mosquitoes were found in all types of habitats that were sampled in Baringo. This is consistent with the findings of other studies (Muturi *et al*., 2007; Mwangangi *et al*., 2009; Fillinger *et al*., 2011). *Aedes aegypti* was mainly found in concrete tanks since it prefers breeding in containers (Cheong, 1967; Ngugi *et al*., 2017). These findings are important for targeted larval control.

Rift valley fever vectors (*Mansonia* spp, *Cx. pipiens* s.l., *Cx. univittatus* and other culicines) were in high abundance in the lowland followed by riverine zone. *Culex pipiens* s. l. was the most abundant species followed by *Mansonia uniformis*. Lutomiah *et al.* also found *Cx. pipiens* to be the most abundant culicine mosquito in Baringo during a previous survey (Lutomiah *et al*., 2013). Similarly, the highest number of indoor resting anophelines (most abundant species being *An. gambiae*) was collected from the lowland zone and least from midland zone. These findings are similar to those reported by Githeko *et al.* (Githeko *et al*., 2000) in which more malaria vectors were collected in houses at valley bottom than mid hill and hilltop, an indication that altitude also influences mosquito abundance (Githeko *et al*., 2006). Minakawa *et al.* also found that vector abundance was higher in the lower parts of western Kenya than highland regions (Minakawa *et al*., 2002).

Midland zone with many seasonal rivers had high abundance of malaria vector larvae but this did not give rise to high population of adult mosquitoes. Instead, more anopheline adults were collected from the highland zone which had fewer larvae than midland zone. These findings show that larval abundance is a poor estimate of adult population size (Sivagnaname and Gunasekaran, 2012) and hence not suitable for prediction of disease risk in an area.

More vectors of malaria were collected during the drier seasons than the wet seasons (Ondiba *et al*., 2017a). This is similar to findings of previous studies in which *Anopheles* mosquito abundance increased during the dry season (Ndenga *et al*., 2006; Bigoga *et al*., 2012). The high

abundance of *An. gambiae* observed during the dry season may partly explain the high malaria cases in Baringo during the same season (Omondi *et al*., 2017). There was a high correlation between average monthly rainfall and *An. gambiae* abundance in the riverine zone but not the lowland zone (Ondiba *et al*, 2017a). This shows that prediction of vector abundance using rainfall should be interpreted locally as climatic conditions in the ecological zones are different. A recent study on malaria risk in Baringo between 2009 and 2012 indicated that moderate rainfall was necessary for increase in malaria risk (Amadi *et al.,* 2018a). The study of Amadi *et al*. found that malaria risk decreased when monthly rainfall was above 181mm and that rainfall had no effect on malaria risk when it as less than 94mm. This is consistent with the findings of this study because vector abundance was lowest during the rainy season thus explaining the low risk of malaria.

Grass thatched-mud walled houses had the highest number of vector mosquitoes and corrugated iron roofed stone-walled houses had the least (Ondiba *et al*., 2018). This supports several other findings that the burden of malaria is highest among the poorest households in the community (Chuma *et al.,* 2010; Charlwood *et al.,* 2003). Malaria vector abundance association with different house structures was greater in the riverine zone compared to the lowland zone. This is probably because riverine zone had more varied house designs made of same materials. A unique stilted traditional hut locally known as "bororiet" in riverine had a low number of mosquitoes compared to similar houses at ground level. This is consistent with findings of studies conducted in São Tomé, Trinidad and the Dominican Republic which showed that houses raised by stilts had fewer vector mosquitoes than houses built on the ground (Charlwood *et al*., 2003; Howell and Chadee, 2007). House modifications such as raising houses on stilts where possible, improvement of construction material and screening open eaves would complement existing protection methods like use of bed nets to protect against endophagic and endophilic malaria vectors in Baringo County.

The current study revealed that infections with *P. falciparum* are higher in *An. funestus* than *An. arabiensis* which is the dominant species in Baringo County. Similar results were obtained in a study conducted in Ahero where infection rates were higher in *An. funestus* than *An. arabiensis* which was more abundant (Degefa *et al*., 2017). This is in agreement with findings of studies conducted elsewhere (Shililu *et al*., 1998; McCann *et al*., 2014; Kaindoa *et al*., 2017; Kiuru *et al*., 2018). It should be noted that though *An. funestus* was less abundant than *An. arabiensis*, this

species could be making a significant contribution towards malaria transmission since it was the most infected species in Baringo. This is because *An. funestus* is highly anthropophilic while *An. arabiensis* is zoophilic and can obtain blood meal from either animals or humans (Gillies& Meillon, 1968; McCann *et al*., 2004).

In midland and highland zones, the absence of *Plasmodium falciparum*-infected mosquitoes could mean lower tansmission of malaria in comparison to riverine and lowland zones. Surprisingly, analysis of previous health records in Baringo has shown that malaria cases exist in midland and highland (Kipruto *et al*., 2017; Amadi *et al*., 2018a). This could imply that the inhabitants of the midland and highland zones might have picked infections elsewhere since few malaria vectors were collected in the two zones and none tested positive for *P. falciparum.* A study in the Sahelian area of Senegal also found heterogeneity in infected mosquitoes across landscapes (Ngom *et al*., 2014). In Cameroon, *Plasmodium* infections decreased with increase in altitude indicating that low altitude areas bore the heaviest burden of malaria infections (Tchuinkam *et al*., 2015). The findings of Tchuinkam *et al.* revealed that the scarcity of malaria in the highlands is not because of absence of parasites in humans but due to scarcity of vectors. The presence of malaria cases found in the highlands of Baringo though vectors were few is an indication that malaria parasite reservoirs are present in humans and outbreaks occur at the slightest incease in vector abundance. This is similar to the finding of a study conducted in Usambara Tanzania where it was deduced that increase in altitude decreased proportion of infected mosquitoes and that highland malaria was maintained at extra ordinary low vector densities (Bødker *et al*., 2003). The Baringo malaria control programs should, therefore, consider other measures of malaria control strategies like chemoprophylaxis for the highlands which are usually neglected when it comes to distribution of bed nets since vectors are few but parasites exist in humans.

Anopheles arabiensis infections were detected in September in lowland Baringo consistent with the findings of Ngom *et al*. which suggested that malaria transmission could be high in September (Ngom *et al*., 2014). A previous study in lowland Baringo also demonstrated that infections of mosquitoes with malaria parasite *P. falciparum* were seasonal with most most of the months of the year recording zero infected mosquitoes (Mala *et al*., 2011).

7.2 Conclusions

1. The study generated evidence to show that area-specific actions are needed to achieve VBD control in Baringo County by taking into account the heterogenous nature of the county with unique climatic, environmental and socio-economic status among the community members.

2. The river bed pools and the ditch were the most productive habitats for *An. gambiae* and *An. pharoensis* compared to other breeding habitats. However, culicine larval species were not very specific in habitat preference and the primary RVF vectors which transmit the rift valley virus trans-ovarially were not detected. The findings on larval species diversity and habitat preference are instrumental for integrated control strategies in view of the fact that control of immature stages would be more appropriate since they are confined in small aquatic habitats where they cannot escape.

3. This study collected more mosquitoes during dry seasons than wet seasons an indication of changing patterns probably due to climate change. This implies that transmission of malaria is possible in all seasons unlike previous assumptions that malaria transmission is seasonal in semi-arid areas.

4. Mosquitoes infected with *Plasmodium falciparum* were detected only from low lying ecological zones (lowland and riverine zones) where vector abundance was highest. This is an indication that inhabitants in the low lying areas of Baringo and their livestock are at greater risk of mosquito borne diseases than those occupying midland and highland regions.

5. Though *An. arabiensis* was the most abundant vector in Baringo, infection rates with *P. falciparum* were higher in *An. funestus* which was distantly second in indoor vector abundance, an indication that this vector plays a great role in malaria transmission particularly in the riverine zone where it was most abundant.

6. All mosquitoes positive for *P. falciparum* were collected indoors and this calls for scaling up of indoor control strategies. Since majority of malaria vectors were found indoors, house design which hinders mosquito entry and resting should be encouraged as a possible intervention in Baringo County. House modifications such as raising houses on stilts where possible, screening of eaves and improvement of construction material would complement existing protection

methods like ITNs to protect against endophagic and endophilic malaria vectors in Baringo County. New vector control methods that will target outdoor vectors (*An. pharoensis*, *An. coustani* and RVF vectors) should also be considered during control programs.

7.3 Recommendations and future research prospects

1. Larval control should be incorporated in vector management to reduce outdoor resting vector species such as the known vectors of RVF (*Mansonia* and *Culex* species) and *An. pharoensis*, a possible malaria vector. Particular breeding habitats such as ditches and seasonal riverbed pools which were more productive for malaria vectors should be targeted for larval control.

2. Vector control efforts should be enhanced in the low altitude areas (lowland and riverine zones) where vector abundance and infections were highest particularly in the dry season. The County government should support the community, particularly those in the riverine zone (Kerio valley), to acquire LLINs. This would be in line with one of the commitments of Kenya Health Policy (2014-2030), which is to ensure use of appropriate malaria preventions by at least 80% of all people living in high risk areas.

3. Infection rates in mosquitoes were high during the dry seasons thus Ministry of Health in Baringo County should test for malaria parasites during all seasons of the year.

4. The Baringo County government is encouraged to incorporate area-specific vector predictive maps of Baringo (Ochieng *et al*., 2016) in the existing national RVF contingency plan (Rift Valley Fever Contingency Plan, 2014 and RVF Decision Support Tools, 2010) by the veterinary department as part of an improved local preparedness and early response to RVF outbreaks. In addition, the county veterinary department should maintain adequate stockpiles of RVF vaccines to facilitate strategic prevention and control programs.

5. Most of the malaria vectors were found indoors and therefore house design which hinders mosquito entry and resting should be encouraged as a possible intervention in Baringo County. House modifications such as raising houses on stilts where possible, improvement of construction material and screening open eaves would complement existing protection methods against endophagic and endophilic vectors in malaria high risk areas of Baringo County.

6. Continuous education of communities to improve their knowledge on RVF and malaria causes, symptoms, risk factors and prevention should be carried out by the public health and veterinary departments in collaboration with relevant stakeholders. Furthermore, the community members should be sensitized to use bednets consistently during wet and dry seasons.

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APPENDICES

APPENDIX 1: **Ethical Clearance Letters**

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:www.uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/81

15th April 2013

Prof. Benson B.A. Estambale
Deputy Vice-Chancellor
Jaramogi Oginga Odinga University of Science and Technology
Bondo

Dear Prof. Estambale

Research proposal: Early warning Systems for Improved Human Health and Resilience to Climate Sensitive Vector-Borne Diseases in Dryland areas of Kenya (P70/02/2013)

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above cited proposal. The approval periods are 15th April 2013 to 14th April 2014.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website **www.uonbi.ac.ke/activities/KNHUoN**

Protect to Discover

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P O BOX 19676 Code 00202
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(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: www.uonbi.ac.ke
Link:uonbi.ac.ke/activities/KNHUoN

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref. No.KNH/ERC/R/68

21st May 2014

Prof. Benson Estamblae
Principal Investigator
Research, Innovation and Outreach
Jaramogi Oginga Odinga University of Science and Technology

Dear Prof.Estambale

Re: Approval of annual renewal – Early warning systems for improved Human Health and Resilience to climate sensitive Vector-Borne Disease in Dryland Areas of Kenya (P70/02/2013)

Refer your communication of May 4, 2014.

This is to acknowledge receipt of the study progress report and hereby grant you annual extension of approval for ethical research Protocol **P70/02/2013.**

The approval dates are 15th April 2014 to 14th April 2015.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.

g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

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COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH/UON-ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/ R/75

4th May, 2015

Prof. Benson B.A. Estambale
Deputy Vice-Chancellor
Principal Investigator
Jaramogi Oginga Odinga University of Science and Technology
Bondo

Dear Prof. Benson

Re: Approval of annual study renewal - Early Warming Systems for Improved Human Health and Resilience to Climate Change Sensitive Vector-Borne Diseases in Dryland Areas of Kenya (P70/02/2013)

Your communication of 10th April 2015 refers.

This is to acknowledge receipt of the study progress report and hereby grant you annual extension of approval for ethical research protocol.

The study renewal dates are 15th April, 2015 to 14th April 2016.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
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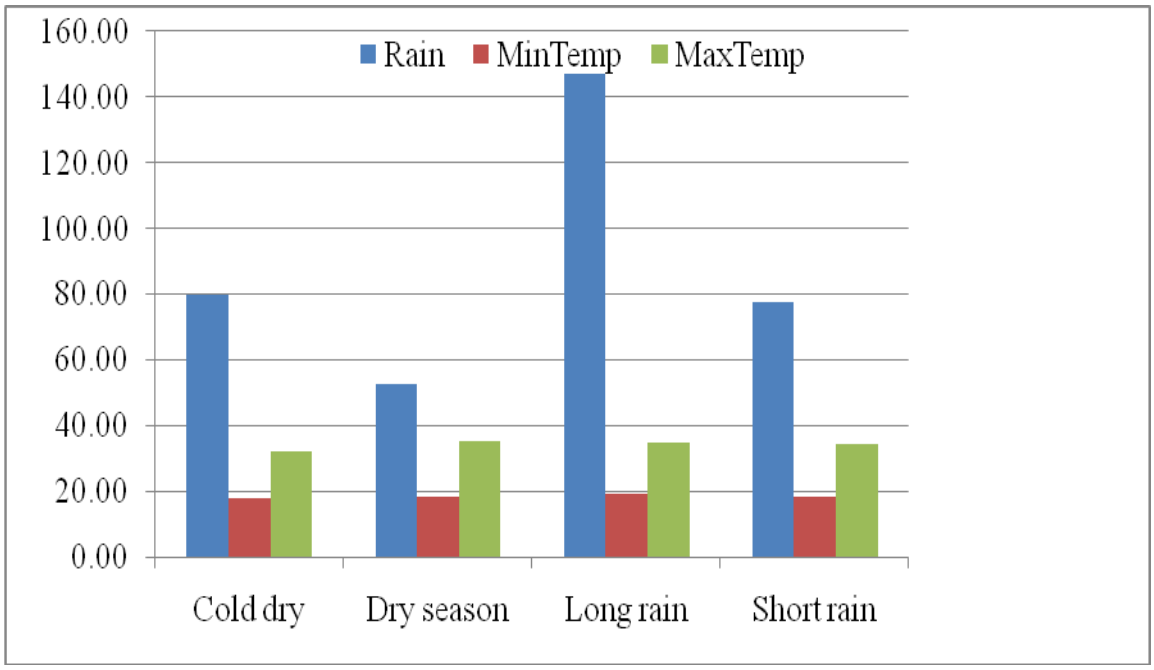
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

APPENDIX 2: **Community Involvement**

Dissemination at Local Community Level

Publicity materials with sensitization message

APPENDIX 3: Seasonal variation in rainfall, minimum and maximum temperature in Baringo County during the sampling period (2014-2016)



APPENDIX 4: Multiple Comparisons of the mean of all larval species in all sampled breeding habitats

Dependent Variable: Lg10_meanlarvae20dips : Tukey HSD

(I) Habitat Type	(J) Habitat Type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Concrete tank	Dam edge	.66218*	.19951	.028	.0393	1.2851
	Ditch	.57603	.18675	.056	-.0070	1.1591
	Lake margin	.64544*	.18699	.018	.0616	1.2293
	River bed	.61872*	.18346	.023	.0459	1.1915
	Swamp	.47771	.19232	.244	-.1228	1.0782
	Water pan	.64934*	.20756	.049	.0013	1.2974
	Water pit	.61526*	.19232	.040	.0148	1.2157
	Water spring	.51235	.18385	.123	-.0617	1.0864
Dam edge	Concrete tank	-.66218*	.19951	.028	-1.2851	-.0393
	Ditch	-.08615	.11682	.998	-.4509	.2786
	Lake margin	-.01674	.11721	1.000	-.3827	.3492
	River bed	-.04346	.11148	1.000	-.3915	.3046
	Swamp	-.18447	.12554	.869	-.5764	.2075
	Water pan	-.01284	.14783	1.000	-.4744	.4487
	Water pit	-.04692	.12554	1.000	-.4389	.3450
	Water spring	-.14983	.11213	.920	-.4999	.2003
Ditch	Concrete tank	-.57603	.18675	.056	-1.1591	.0070
	Dam edge	.08615	.11682	.998	-.2786	.4509
	Lake margin	.06941	.09386	.998	-.2236	.3625
	River bed	.04269	.08661	1.000	-.2277	.3131
	Swamp	-.09832	.10408	.990	-.4233	.2266
	Water pan	.07331	.13009	1.000	-.3329	.4795
	Water pit	.03923	.10408	1.000	-.2857	.3642
	Water spring	-.06368	.08744	.998	-.3367	.2093
Lake margin	Concrete tank	-.64544*	.18699	.018	-1.2293	-.0616
	Dam edge	.01674	.11721	1.000	-.3492	.3827
	Ditch	-.06941	.09386	.998	-.3625	.2236
	River bed	-.02671	.08713	1.000	-.2988	.2453
	Swamp	-.16772	.10451	.802	-.4940	.1586
	Water pan	.00390	.13044	1.000	-.4034	.4112
	Water pit	-.03018	.10451	1.000	-.3565	.2961
	Water spring	-.13309	.08796	.849	-.4077	.1415
River bed	Concrete tank	-.61872*	.18346	.023	-1.1915	-.0459
	Dam edge	.04346	.11148	1.000	-.3046	.3915

	Ditch	-.04269	.08661	1.000	-.3131	.2277
	Lake margin	.02671	.08713	1.000	-.2453	.2988
	Swamp	-.14101	.09805	.882	-.4472	.1651
	Water pan	.03062	.12533	1.000	-.3607	.4219
	Water pit	-.00347	.09805	1.000	-.3096	.3027
	Water spring	-.10638	.08017	.923	-.3567	.1439
Swamp	Concrete tank	-.47771	.19232	.244	-1.0782	.1228
	Dam edge	.18447	.12554	.869	-.2075	.5764
	Ditch	.09832	.10408	.990	-.2266	.4233
	Lake margin	.16772	.10451	.802	-.1586	.4940
	River bed	.14101	.09805	.882	-.1651	.4472
	Water pan	.17163	.13798	.946	-.2592	.6024
	Water pit	.13754	.11378	.954	-.2177	.4928
	Water spring	.03464	.09879	1.000	-.2738	.3431
Water pan	Concrete tank	-.64934*	.20756	.049	-1.2974	-.0013
	Dam edge	.01284	.14783	1.000	-.4487	.4744
	Ditch	-.07331	.13009	1.000	-.4795	.3329
	Lake margin	-.00390	.13044	1.000	-.4112	.4034
	River bed	-.03062	.12533	1.000	-.4219	.3607
	Swamp	-.17163	.13798	.946	-.6024	.2592
	Water pit	-.03408	.13798	1.000	-.4649	.3967
	Water spring	-.13699	.12590	.976	-.5301	.2561
Water pit	Concrete tank	-.61526*	.19232	.040	-1.2157	-.0148
	Dam edge	.04692	.12554	1.000	-.3450	.4389
	Ditch	-.03923	.10408	1.000	-.3642	.2857
	Lake margin	.03018	.10451	1.000	-.2961	.3565
	River bed	.00347	.09805	1.000	-.3027	.3096
	Swamp	-.13754	.11378	.954	-.4928	.2177
	Water pan	.03408	.13798	1.000	-.3967	.4649
	Water spring	-.10291	.09879	.981	-.4114	.2055
Water spring	Concrete tank	-.51235	.18385	.123	-1.0864	.0617
	Dam edge	.14983	.11213	.920	-.2003	.4999
	Ditch	.06368	.08744	.998	-.2093	.3367
	Lake margin	.13309	.08796	.849	-.1415	.4077
	River bed	.10638	.08017	.923	-.1439	.3567
	Swamp	-.03464	.09879	1.000	-.3431	.2738
	Water pan	.13699	.12590	.976	-.2561	.5301
	Water pit	.10291	.09879	.981	-.2055	.4114

*. The mean difference is significant at the 0.05 level.

APPENDIX 5: **Multiple Comparisons of anopheline larvae mean in all breeding habitats**

Dependent Variable: Lg_10 Anopheline mean : Tukey HSD

(I) Hab_Code	(J) Hab_Code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Concrete tank	Dam edge	-.36891	.24098	.840	-1.1205	.3827
	Ditch	-.60460	.22639	.162	-1.3107	.1015
	Lake margin	-.14683	.22452	.999	-.8471	.5534
	River bed	-.20954	.22202	.990	-.9020	.4829
	Swamp	-.29571	.23390	.941	-1.0252	.4338
	Water pan	.21132	.23902	.994	-.5342	.9568
	Water pit	.17349	.22742	.998	-.5358	.8828
	Water spring	.02429	.22355	1.000	-.6729	.7215
Dam edge	Concrete tank	.36891	.24098	.840	-.3827	1.1205
	Ditch	-.23569	.13633	.728	-.6609	.1895
	Lake margin	.22209	.13321	.766	-.1934	.6375
	River bed	.15937	.12894	.948	-.2428	.5615
	Swamp	.07320	.14847	1.000	-.3899	.5363
	Water pan	.58024*	.15642	.007	.0924	1.0681
	Water pit	.54240*	.13804	.003	.1119	.9729
	Water spring	.39320	.13155	.072	-.0171	.8035
Ditch	Concrete tank	.60460	.22639	.162	-.1015	1.3107
	Dam edge	.23569	.13633	.728	-.1895	.6609
	Lake margin	.45778*	.10451	.001	.1318	.7837
	River bed	.39506*	.09901	.003	.0863	.7038
	Swamp	.30889	.12338	.233	-.0759	.6937
	Water pan	.81593*	.13283	.000	.4017	1.2302
	Water pit	.77809*	.11060	.000	.4331	1.1230
	Water spring	.62889*	.10239	.000	.3095	.9482
Lake margin	Concrete tank	.14683	.22452	.999	-.5534	.8471
	Dam edge	-.22209	.13321	.766	-.6375	.1934
	Ditch	-.45778*	.10451	.001	-.7837	-.1318
	River bed	-.06272	.09467	.999	-.3580	.2325
	Swamp	-.14889	.11993	.947	-.5229	.2251
	Water pan	.35815	.12963	.130	-.0461	.7624
	Water pit	.32031	.10674	.070	-.0126	.6532
	Water spring	.17111	.09820	.720	-.1352	.4774
River bed	Concrete tank	.20954	.22202	.990	-.4829	.9020
	Dam edge	-.15937	.12894	.948	-.5615	.2428
	Ditch	-.39506*	.09901	.003	-.7038	-.0863

	Lake margin	.06272	.09467	.999	-.2325	.3580
	Swamp	-.08617	.11516	.998	-.4453	.2730
	Water pan	.42086*	.12523	.024	.0303	.8114
	Water pit	.38303*	.10135	.006	.0669	.6991
	Water spring	.23383	.09232	.220	-.0541	.5218
Swamp	Concrete tank	.29571	.23390	.941	-.4338	1.0252
	Dam edge	-.07320	.14847	1.000	-.5363	.3899
	Ditch	-.30889	.12338	.233	-.6937	.0759
	Lake margin	.14889	.11993	.947	-.2251	.5229
	River bed	.08617	.11516	.998	-.2730	.4453
	Water pan	.50704*	.14527	.016	.0540	.9601
	Water pit	.46920*	.12527	.006	.0785	.8599
	Water spring	.32000	.11808	.148	-.0483	.6883
Water pan	Concrete tank	-.21132	.23902	.994	-.9568	.5342
	Dam edge	-.58024*	.15642	.007	-1.0681	-.0924
	Ditch	-.81593*	.13283	.000	-1.2302	-.4017
	Lake margin	-.35815	.12963	.130	-.7624	.0461
	River bed	-.42086*	.12523	.024	-.8114	-.0303
	Swamp	-.50704*	.14527	.016	-.9601	-.0540
	Water pit	-.03784	.13459	1.000	-.4576	.3819
	Water spring	-.18704	.12793	.872	-.5860	.2119
Water pit	Concrete tank	-.17349	.22742	.998	-.8828	.5358
	Dam edge	-.54240*	.13804	.003	-.9729	-.1119
	Ditch	-.77809*	.11060	.000	-1.1230	-.4331
	Lake margin	-.32031	.10674	.070	-.6532	.0126
	River bed	-.38303*	.10135	.006	-.6991	-.0669
	Swamp	-.46920*	.12527	.006	-.8599	-.0785
	Water pan	.03784	.13459	1.000	-.3819	.4576
	Water spring	-.14920	.10466	.888	-.4756	.1772
Water spring	Concrete tank	-.02429	.22355	1.000	-.7215	.6729
	Dam edge	-.39320	.13155	.072	-.8035	.0171
	Ditch	-.62889*	.10239	.000	-.9482	-.3095
	Lake margin	-.17111	.09820	.720	-.4774	.1352
	River bed	-.23383	.09232	.220	-.5218	.0541
	Swamp	-.32000	.11808	.148	-.6883	.0483
	Water pan	.18704	.12793	.872	-.2119	.5860
	Water pit	.14920	.10466	.888	-.1772	.4756

*. The mean difference is significant at the 0.05 level.

APPENDIX 6: **Multiple Comparisons of culicine larvae mean in all breeding habitats**

Dependent Variable: Culicines : Tukey HSD

(I) Habitat Type	(J) Habitat Type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Concrete tank	Dam edge	-.00229	.35949	1.000	-1.1235	1.1189
	Ditch	.19608	.33771	1.000	-.8572	1.2494
	Lake margin	.15460	.33493	1.000	-.8900	1.1992
	River bed	.04460	.33119	1.000	-.9883	1.0775
	Swamp	-.43252	.34892	.947	-1.5208	.6557
	Water pan	.06127	.35656	1.000	-1.0508	1.1733
	Water pit	-.03969	.33926	1.000	-1.0978	1.0184
	Water spring	-.52892	.33347	.812	-1.5690	.5111
Dam edge	Concrete tank	.00229	.35949	1.000	-1.1189	1.1235
	Ditch	.19837	.20336	.988	-.4359	.8326
	Lake margin	.15689	.19871	.997	-.4629	.7766
	River bed	.04689	.19234	1.000	-.5530	.6468
	Swamp	-.43024	.22149	.585	-1.1210	.2605
	Water pan	.06356	.23333	1.000	-.6642	.7913
	Water pit	-.03740	.20592	1.000	-.6796	.6048
	Water spring	-.52664	.19624	.157	-1.1387	.0854
Ditch	Concrete tank	-.19608	.33771	1.000	-1.2494	.8572
	Dam edge	-.19837	.20336	.988	-.8326	.4359
	Lake margin	-.04148	.15590	1.000	-.5277	.4448
	River bed	-.15148	.14769	.983	-.6121	.3091
	Swamp	-.62861*	.18405	.020	-1.2026	-.0546
	Water pan	-.13481	.19815	.999	-.7528	.4832
	Water pit	-.23577	.16499	.886	-.7504	.2788
	Water spring	-.72501*	.15274	.000	-1.2014	-.2486
Lake marign	Concrete tank	-.15460	.33493	1.000	-1.1992	.8900
	Dam edge	-.15689	.19871	.997	-.7766	.4629
	Ditch	.04148	.15590	1.000	-.4448	.5277
	River bed	-.11000	.14122	.997	-.5504	.3304
	Swamp	-.58712*	.17890	.031	-1.1451	-.0292
	Water pan	-.09333	.19337	1.000	-.6964	.5098
	Water pit	-.19429	.15923	.952	-.6909	.3023
	Water spring	-.68353*	.14649	.000	-1.1404	-.2266
River bed	Concrete tank	-.04460	.33119	1.000	-1.0775	.9883
	Dam edge	-.04689	.19234	1.000	-.6468	.5530
	Ditch	.15148	.14769	.983	-.3091	.6121

	Lake margin	.11000	.14122	.997	-.3304	.5504
	Swamp	-.47712	.17179	.125	-1.0129	.0587
	Water pan	.01667	.18682	1.000	-.5660	.5993
	Water pit	-.08429	.15119	1.000	-.5558	.3873
	Water spring	-.57353*	.13772	.001	-1.0031	-.1440
Swamp	Concrete tank	.43252	.34892	.947	-.6557	1.5208
	Dam edge	.43024	.22149	.585	-.2605	1.1210
	Ditch	.62861*	.18405	.020	.0546	1.2026
	Lake margin	.58712*	.17890	.031	.0292	1.1451
	River bed	.47712	.17179	.125	-.0587	1.0129
	Water pan	.49379	.21671	.358	-.1821	1.1697
	Water pit	.39284	.18687	.473	-.1900	.9757
	Water spring	-.09640	.17615	1.000	-.6458	.4530
Water pan	Concrete tank	-.06127	.35656	1.000	-1.1733	1.0508
	Dam edge	-.06356	.23333	1.000	-.7913	.6642
	Ditch	.13481	.19815	.999	-.4832	.7528
	Lake margin	.09333	.19337	1.000	-.5098	.6964
	River bed	-.01667	.18682	1.000	-.5993	.5660
	Swamp	-.49379	.21671	.358	-1.1697	.1821
	Water pit	-.10096	.20077	1.000	-.7271	.5252
	Water spring	-.59019	.19083	.054	-1.1854	.0050
Water pit	Concrete tank	.03969	.33926	1.000	-1.0184	1.0978
	Dam edge	.03740	.20592	1.000	-.6048	.6796
	Ditch	.23577	.16499	.886	-.2788	.7504
	Lake margin	.19429	.15923	.952	-.3023	.6909
	River bed	.08429	.15119	1.000	-.3873	.5558
	Swamp	-.39284	.18687	.473	-.9757	.1900
	Water pan	.10096	.20077	1.000	-.5252	.7271
	Water spring	-.48924*	.15613	.048	-.9762	-.0023
Water spring	Concrete tank	.52892	.33347	.812	-.5111	1.5690
	Dam edge	.52664	.19624	.157	-.0854	1.1387
	Ditch	.72501*	.15274	.000	.2486	1.2014
	Lake margin	.68353*	.14649	.000	.2266	1.1404
	River bed	.57353*	.13772	.001	.1440	1.0031
	Swamp	.09640	.17615	1.000	-.4530	.6458
	Water pan	.59019	.19083	.054	-.0050	1.1854
	Water pit	.48924*	.15613	.048	.0023	.9762

*. The mean difference is significant at the 0.05 level.

APPENDIX 7: **Multiple Comparisons of *An. gambiae* larvae mean in all breeding habitats**

Dependent Variable: Lg10_An.gambiae ; Tukey HSD

(I) Hab_Code	(J) Hab_Code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Concrete tank	Dam edge	-.24908	.14343	.723	-.6964	.1982
	Ditch	-.24972	.13474	.646	-.6699	.1705
	Lake margin	-.13036	.13363	.988	-.5471	.2864
	River bed	-.18850	.13214	.887	-.6006	.2236
	Swamp	-.17878	.13921	.936	-.6130	.2554
	Water pan	.00056	.14226	1.000	-.4431	.4442
	Water pit	.02744	.13536	1.000	-.3947	.4496
	Water spring	-.07648	.13305	1.000	-.4914	.3385
Dam edge	Concrete tank	.24908	.14343	.723	-.1982	.6964
	Ditch	-.00063	.08114	1.000	-.2537	.2524
	Lake margin	.11872	.07928	.856	-.1286	.3660
	River bed	.06059	.07674	.997	-.1788	.2999
	Swamp	.07030	.08837	.997	-.2053	.3459
	Water pan	.24964	.09310	.158	-.0407	.5400
	Water pit	.27653*	.08216	.023	.0203	.5328
	Water spring	.17261	.07830	.405	-.0716	.4168
Ditch	Concrete tank	.24972	.13474	.646	-.1705	.6699
	Dam edge	.00063	.08114	1.000	-.2524	.2537
	Lake margin	.11935	.06220	.601	-.0746	.3133
	River bed	.06122	.05893	.982	-.1226	.2450
	Swamp	.07094	.07343	.989	-.1581	.3000
	Water pan	.25027*	.07906	.044	.0037	.4968
	Water pit	.27716*	.06583	.001	.0718	.4825
	Water spring	.17324	.06094	.106	-.0168	.3633
Lake margin	Concrete tank	.13036	.13363	.988	-.2864	.5471
	Dam edge	-.11872	.07928	.856	-.3660	.1286
	Ditch	-.11935	.06220	.601	-.3133	.0746
	River bed	-.05813	.05634	.983	-.2339	.1176
	Swamp	-.04842	.07138	.999	-.2710	.1742
	Water pan	.13092	.07715	.748	-.1097	.3715
	Water pit	.15781	.06353	.243	-.0403	.3559
	Water spring	.05389	.05845	.992	-.1284	.2362
River bed	Concrete tank	.18850	.13214	.887	-.2236	.6006
	Dam edge	-.06059	.07674	.997	-.2999	.1788
	Ditch	-.06122	.05893	.982	-.2450	.1226
	Lake margin	.05813	.05634	.983	-.1176	.2339

	Swamp	.00972	.06854	1.000	-.2040	.2235
	Water pan	.18905	.07454	.218	-.0434	.4215
	Water pit	.21594*	.06032	.011	.0278	.4041
	Water spring	.11202	.05495	.517	-.0594	.2834
Swamp	Concrete tank	.17878	.13921	.936	-.2554	.6130
	Dam edge	-.07030	.08837	.997	-.3459	.2053
	Ditch	-.07094	.07343	.989	-.3000	.1581
	Lake margin	.04842	.07138	.999	-.1742	.2710
	River bed	-.00972	.06854	1.000	-.2235	.2040
	Water pan	.17934	.08646	.493	-.0903	.4490
	Water pit	.20622	.07456	.129	-.0263	.4388
	Water spring	.10230	.07028	.875	-.1169	.3215
Water pan	Concrete tank	-.00056	.14226	1.000	-.4442	.4431
	Dam edge	-.24964	.09310	.158	-.5400	.0407
	Ditch	-.25027*	.07906	.044	-.4968	-.0037
	Lake margin	-.13092	.07715	.748	-.3715	.1097
	River bed	-.18905	.07454	.218	-.4215	.0434
	Swamp	-.17934	.08646	.493	-.4490	.0903
	Water pit	.02689	.08010	1.000	-.2229	.2767
	Water spring	-.07703	.07614	.985	-.3145	.1604
Water pit	Concrete tank	-.02744	.13536	1.000	-.4496	.3947
	Dam edge	-.27653*	.08216	.023	-.5328	-.0203
	Ditch	-.27716*	.06583	.001	-.4825	-.0718
	Lake margin	-.15781	.06353	.243	-.3559	.0403
	River bed	-.21594*	.06032	.011	-.4041	-.0278
	Swamp	-.20622	.07456	.129	-.4388	.0263
	Water pan	-.02689	.08010	1.000	-.2767	.2229
	Water spring	-.10392	.06229	.766	-.2982	.0904
Water spring	Concrete tank	.07648	.13305	1.000	-.3385	.4914
	Dam edge	-.17261	.07830	.405	-.4168	.0716
	Ditch	-.17324	.06094	.106	-.3633	.0168
	Lake margin	-.05389	.05845	.992	-.2362	.1284
	River bed	-.11202	.05495	.517	-.2834	.0594
	Swamp	-.10230	.07028	.875	-.3215	.1169
	Water pan	.07703	.07614	.985	-.1604	.3145
	Water pit	.10392	.06229	.766	-.0904	.2982

*. The mean difference is significant at the 0.05 level.

APPENDIX 8: **Multiple Comparisons of *An. pharoensis* larvae mean in all breeding habitats**

Dependent Variable: Lg10_An.Pharoensis : Tukey HSD

(I) Hab_Code	(J) Hab_Code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Concrete tank	Dam edge	-.14034	.18017	.997	-.7023	.4216
	Ditch	-.41282	.16926	.266	-.9407	.1151
	Lake margin	-.13568	.16786	.997	-.6592	.3879
	River bed	-.13297	.16599	.997	-.6507	.3847
	Swamp	-.24569	.17488	.896	-.7911	.2997
	Water pan	.06889	.17870	1.000	-.4885	.6262
	Water pit	.04817	.17003	1.000	-.4821	.5785
	Water spring	-.01462	.16713	1.000	-.5359	.5066
Dam edge	Concrete tank	.14034	.18017	.997	-.4216	.7023
	Ditch	-.27248	.10192	.161	-.5904	.0454
	Lake margin	.00466	.09959	1.000	-.3060	.3153
	River bed	.00737	.09640	1.000	-.2933	.3080
	Swamp	-.10534	.11101	.990	-.4516	.2409
	Water pan	.20924	.11694	.689	-.1555	.5740
	Water pit	.18852	.10321	.664	-.1334	.5104
	Water spring	.12572	.09835	.937	-.1810	.4325
Ditch	Concrete tank	.41282	.16926	.266	-.1151	.9407
	Dam edge	.27248	.10192	.161	-.0454	.5904
	Lake margin	.27714*	.07814	.013	.0334	.5208
	River bed	.27985*	.07402	.006	.0490	.5107
	Swamp	.16713	.09224	.674	-.1206	.4548
	Water pan	.48172*	.09931	.000	.1720	.7914
	Water pit	.46100*	.08269	.000	.2031	.7189
	Water spring	.39820*	.07655	.000	.1594	.6370
Lake margin	Concrete tank	.13568	.16786	.997	-.3879	.6592
	Dam edge	-.00466	.09959	1.000	-.3153	.3060
	Ditch	-.27714*	.07814	.013	-.5208	-.0334
	River bed	.00271	.07078	1.000	-.2180	.2235
	Swamp	-.11000	.08966	.950	-.3896	.1696
	Water pan	.20458	.09692	.467	-.0977	.5068
	Water pit	.18386	.07980	.342	-.0650	.4327
	Water spring	.12106	.07342	.777	-.1079	.3500
River bed	Concrete tank	.13297	.16599	.997	-.3847	.6507
	Dam edge	-.00737	.09640	1.000	-.3080	.2933

	Ditch	-.27985*	.07402	.006	-.5107	-.0490
	Lake margin	-.00271	.07078	1.000	-.2235	.2180
	Swamp	-.11272	.08610	.928	-.3812	.1558
	Water pan	.20187	.09363	.437	-.0901	.4939
	Water pit	.18115	.07578	.292	-.0552	.4175
	Water spring	.11835	.06902	.737	-.0969	.3336
Swamp	Concrete tank	.24569	.17488	.896	-.2997	.7911
	Dam edge	.10534	.11101	.990	-.2409	.4516
	Ditch	-.16713	.09224	.674	-.4548	.1206
	Lake margin	.11000	.08966	.950	-.1696	.3896
	River bed	.11272	.08610	.928	-.1558	.3812
	Water pan	.31458	.10861	.093	-.0242	.6533
	Water pit	.29386*	.09366	.047	.0018	.5860
	Water spring	.23107	.08828	.183	-.0443	.5064
Water pan	Concrete tank	-.06889	.17870	1.000	-.6262	.4885
	Dam edge	-.20924	.11694	.689	-.5740	.1555
	Ditch	-.48172*	.09931	.000	-.7914	-.1720
	Lake margin	-.20458	.09692	.467	-.5068	.0977
	River bed	-.20187	.09363	.437	-.4939	.0901
	Swamp	-.31458	.10861	.093	-.6533	.0242
	Water pit	-.02072	.10062	1.000	-.3346	.2931
	Water spring	-.08351	.09564	.994	-.3818	.2148
Water pit	Concrete tank	-.04817	.17003	1.000	-.5785	.4821
	Dam edge	-.18852	.10321	.664	-.5104	.1334
	Ditch	-.46100*	.08269	.000	-.7189	-.2031
	Lake margin	-.18386	.07980	.342	-.4327	.0650
	River bed	-.18115	.07578	.292	-.4175	.0552
	Swamp	-.29386*	.09366	.047	-.5860	-.0018
	Water pan	.02072	.10062	1.000	-.2931	.3346
	Water spring	-.06279	.07825	.997	-.3068	.1813
Water spring	Concrete tank	.01462	.16713	1.000	-.5066	.5359
	Dam edge	-.12572	.09835	.937	-.4325	.1810
	Ditch	-.39820*	.07655	.000	-.6370	-.1594
	Lake margin	-.12106	.07342	.777	-.3500	.1079
	River bed	-.11835	.06902	.737	-.3336	.0969
	Swamp	-.23107	.08828	.183	-.5064	.0443
	Water pan	.08351	.09564	.994	-.2148	.3818
	Water pit	.06279	.07825	.997	-.1813	.3068

*. The mean difference is significant at the 0.05 level.

APPENDIX 9: **Multiple Comparisons of *Cx. quinquefasciatus* larvae mean in all breeding habitats**

Dependent Variable: Lg10_Cx.quinquefasciatus : Tukey HSD

(I) Habitat Type	(J) Habitat Type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Concrete tank	Dam edge	-.06175	.22256	1.000	-.7559	.6324
	Ditch	-.02805	.20907	1.000	-.6801	.6240
	Lake margin	-.18791	.20735	.993	-.8346	.4588
	River bed	-.04283	.20504	1.000	-.6823	.5966
	Swamp	-.42329	.21602	.573	-1.0970	.2504
	Water pan	-.17139	.22075	.997	-.8599	.5171
	Water pit	-.10336	.21003	1.000	-.7584	.5517
	Water spring	-.12324	.20645	1.000	-.7671	.5206
Dam edge	Concrete tank	.06175	.22256	1.000	-.6324	.7559
	Ditch	.03370	.12590	1.000	-.3590	.4264
	Lake margin	-.12616	.12302	.983	-.5098	.2575
	River bed	.01892	.11908	1.000	-.3525	.3903
	Swamp	-.36154	.13712	.175	-.7892	.0661
	Water pan	-.10964	.14445	.998	-.5602	.3409
	Water pit	-.04161	.12748	1.000	-.4392	.3560
	Water spring	-.06149	.12149	1.000	-.4404	.3174
Ditch	Concrete tank	.02805	.20907	1.000	-.6240	.6801
	Dam edge	-.03370	.12590	1.000	-.4264	.3590
	Lake margin	-.15986	.09652	.773	-.4609	.1412
	River bed	-.01478	.09143	1.000	-.2999	.2704
	Swamp	-.39524*	.11394	.017	-.7506	-.0399
	Water pan	-.14334	.12267	.963	-.5259	.2393
	Water pit	-.07531	.10215	.998	-.3939	.2433
	Water spring	-.09519	.09456	.985	-.3901	.1997
Lake margin	Concrete tank	.18791	.20735	.993	-.4588	.8346
	Dam edge	.12616	.12302	.983	-.2575	.5098
	Ditch	.15986	.09652	.773	-.1412	.4609
	River bed	.14508	.08743	.771	-.1276	.4178
	Swamp	-.23538	.11075	.458	-.5808	.1100
	Water pan	.01652	.11972	1.000	-.3569	.3899
	Water pit	.08455	.09857	.995	-.2229	.3920
	Water spring	.06467	.09069	.999	-.2182	.3475
River bed	Concrete tank	.04283	.20504	1.000	-.5966	.6823
	Dam edge	-.01892	.11908	1.000	-.3903	.3525

	Ditch	.01478	.09143	1.000	-.2704	.2999
	Lake margin	-.14508	.08743	.771	-.4178	.1276
	Swamp	-.38046*	.10635	.012	-.7122	-.0488
	Water pan	-.12856	.11566	.972	-.4893	.2322
	Water pit	-.06053	.09360	.999	-.3525	.2314
	Water spring	-.08041	.08526	.990	-.3463	.1855
Swamp	Concrete tank	.42329	.21602	.573	-.2504	1.0970
	Dam edge	.36154	.13712	.175	-.0661	.7892
	Ditch	.39524*	.11394	.017	.0399	.7506
	Lake margin	.23538	.11075	.458	-.1100	.5808
	River bed	.38046*	.10635	.012	.0488	.7122
	Water pan	.25190	.13416	.630	-.1665	.6703
	Water pit	.31993	.11569	.129	-.0409	.6808
	Water spring	.30005	.10905	.134	-.0401	.6402
Water pan	Concrete tank	.17139	.22075	.997	-.5171	.8599
	Dam edge	.10964	.14445	.998	-.3409	.5602
	Ditch	.14334	.12267	.963	-.2393	.5259
	Lake margin	-.01652	.11972	1.000	-.3899	.3569
	River bed	.12856	.11566	.972	-.2322	.4893
	Swamp	-.25190	.13416	.630	-.6703	.1665
	Water pit	.06803	.12430	1.000	-.3196	.4557
	Water spring	.04815	.11814	1.000	-.3203	.4166
Water pit	Concrete tank	.10336	.21003	1.000	-.5517	.7584
	Dam edge	.04161	.12748	1.000	-.3560	.4392
	Ditch	.07531	.10215	.998	-.2433	.3939
	Lake margin	-.08455	.09857	.995	-.3920	.2229
	River bed	.06053	.09360	.999	-.2314	.3525
	Swamp	-.31993	.11569	.129	-.6808	.0409
	Water pan	-.06803	.12430	1.000	-.4557	.3196
	Water spring	-.01988	.09666	1.000	-.3213	.2816
Water spring	Concrete tank	.12324	.20645	1.000	-.5206	.7671
	Dam edge	.06149	.12149	1.000	-.3174	.4404
	Ditch	.09519	.09456	.985	-.1997	.3901
	Lake margin	-.06467	.09069	.999	-.3475	.2182
	River bed	.08041	.08526	.990	-.1855	.3463
	Swamp	-.30005	.10905	.134	-.6402	.0401
	Water pan	-.04815	.11814	1.000	-.4166	.3203
	Water pit	.01988	.09666	1.000	-.2816	.3213

*The mean difference is significant at 0.05 level

APPENDIX 10: **PUBLISHED PAPER 1**

International Journal of Mosquito Research 2017; 4(4): 42-48**earch Article Open**

Diversity, distribution and abundance of potential rift valley fever vectors in Baringo County, Kenya

Isabella M Ondiba, Florence A Oyieke, Isaac K Nyamongo and Benson BA Estambale

Abstract

Rift Valley fever (RVF) is a zoonotic disease that occurs sporadically in form of outbreaks and is transmitted by diverse mosquito species in different geographic regions. Knowledge on diversity,distribution and abundance of RVF vectors is useful for risk assessment of RVF outbreaks. Diversity,distribution and abundance of RVF vectors from four ecological zones in Baringo County were studied.Four potential RVF vectors, namely *Mansonia uniformis; Mansonia africana; Culex pipiens* and *Culex univittatus* were among the 26 species identified. Rift Valley Fever vectors were most abundant inlowlands (85.9%); riverine (9.1%); midland and highland combined (5%). Diversity indices were higher in the riverine (H'=1.65) and midland (H'=1.64) than lowland (H'=1.429) and highland (H'=1.229). Area–specific vector distribution and abundance data generated from this study can be incorporated into thenational RVF contingency Plan as part of an improved preparedness and early response to RVF outbreaks.

Keywords: RVF, Vectors, Diversity, Distribution, Abundance, Baringo

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1. Isabella M Ondiba
School of Biological Sciences, University of Nairobi, P.O. Box 30197-00100, Nairobi Kenya

2. Florence A Oyieke
School of Biological Sciences, University of Nairobi, P.O. Box 30197-00100, Nairobi Kenya

3. Isaac K Nyamongo

4.Benson BA Estambale

	Cooperative Development, Research and Innovation, The Cooperative University of Kenya, P.O Box 24814-00502, Nairobi, Kenya	Division of Research Innovation and Outreach, Jaramogi Oginga Odinga University of Science andTechnology, P.O. Box 210-40601, Bondo, Kenya

APPENDIX 11: **PUBLISHED PAPER 2**

Malaria Vector Species Distribution and Seasonal Population Dynamics across Varied Ecological Zones in Baringo County, Kenya

Isabella M. Ondiba[1] , Florence A. Oyieke[1], Alfred O. Ochieng[2], Douglas N. Anyona[2], Isaac K. Nyamongo[3], Benson B.A.Estambale[2]

1 University of Nairobi, Nairobi, Kenya

2 Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya

3 The Cooperative University of Kenya, Nairobi, Kenya

Corresponding author email: bellamoraa@yahoo.co.uk

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Abstract Vector populations fluctuate on a seasonal basis annually. Knowledge on seasonal abundance and distribution of vector species at the local level would improve vector control programmes and contribute to malaria prevention. Despite this, information on malaria vector species distribution and seasonal fluctuations in Baringo County is scarce. This study examined distribution and seasonal abundance of malaria vector species in Baringo. The study area was stratified into four ecological zones namely; lowland,riverine, midland and highland. Monthly collection of outdoor and indoor mosquitoes was conducted between June 2015 and May2016 using CDC light traps and pyrethrum spray collection respectively. A total of 6,113 anopheline mosquitoes belonging to four species were collected across the four ecological zones. *Anopheles*

gambiae was the most abundant malaria vector species accounting for (93.8%) while *An. pharoensis* and *An. funestus* accounted for 4.8% and 1.1% respectively. Mosquitoes were mainly collected from lowlands (79.8%) and riverine (19.0%) zones. Malaria vector abundance was higher in the dry season compared to the rainy season. *Anopheles gambiae* abundance showed high positive correlation with rainfall in the riverine zone only (r=0.7). Knowledge gained from this study, on malaria vector species distribution and seasonal abundance at local level, is important in implementation of control strategies against malaria by the Baringo County Health Department. The findings highlight the seasons when malaria cases are likely to be higher due to vector abundance and also inform specific areas to target for intervention.

Keywords Malaria; Vectors; Abundance; Distribution; Season; Baringo

APPENDIX 12: **PUBLISHED PAPER 3**

RESEARCH ARTICLE

Malaria vector abundance is associated with house structures in Baringo County, Kenya

Isabella M. Ondiba[1]*, Florence A. Oyieke[1], George O. Ong'amo[1], Macrae M. Olumula[2], Isaac K. Nyamongo[3], Benson B. A. Estambale[2]

1 School of Biological Sciences, University of Nairobi, Kenya, **2** Division of Research Innovation and Outreach, Jaramogi Oginga Odinga University of Science and Technology, Bondo, Kenya, **3** Cooperative Development, Research and Innovation, The Cooperative University of Kenya, Nairobi, Kenya

* bellamoraa@yahoo.co.uk

Abstract

Malaria, a major cause of morbidity and mortality, is the most prevalent vector borne disease in Baringo County; a region which has varied house designs in arid and semi-arid areas. This study investigated the association between house structures and indoor-malaria vector abundance in Baringo County. The density of malaria vectors in houses with open eaves was higher than that for houses with closed eaves. Grass thatched roof houses had higher density of malaria vectors than corrugated iron sheet roofs. Similarly, mud walled houses had higher vector density than other wall types. Houses in the riverine zone were significantly associated with malaria vector abundance ($p<0.000$) possibly due to more varied house structures. In Kamnarok village within riverine zone, a house made of grass thatched roof and mud wall but raised on stilts with domestic animals (sheep/goats) kept at the lower level had lower mosquito density (5.8 per collection) than ordinary houses made of same materials but at ground level (30.5 mosquitoes per collection), suggestive of a change in behavior of mosquito feeding and resting. House modifications such as screening of eaves, improvement of construction material and building stilted houses can be incorporated in the integrated vector management (IVM) strategy to complement insecticide treated bed nets and indoor residual spray to reduce indoor malaria vector density.

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Data Availability Statement: This study is part of a larger research project on [a]Early warning systems for improved human health and resilience to climate-sensitive vector borne diseases in Kenya[o]. The data can be requested from the project's scientific committee through the email address: climatechange@jooust.ac.ke.

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Appendix 13: **Average monthly rainfall and temperature in Baringo County during sampling period (2015 June-2016 May)**