SPATIAL-TEMPORAL CHANGES OF MOSQUITOES DYNAMICS AND RISKS OF ARBOVIRUSES TO LIVESTOCK DURING A PERIOD OF EXTREME FLOODING OF LAKE BARINGO, KENYA

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DECLARATION

This thesis is my original work and has not been presented for degree or other award in any other University or institution of higher learning

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DEDICATION

This thesis is dedicated to my family; Mrs Miriam Waigwe Kabochi, Patrick Kamau, Susan Nungari and Maxwell Magonde, my parents Patrick Kamau Kabochi and Susan Watete for their understanding, support and sacrifice. It is also dedicated to all staff serving in the Division of Vector Regulatory and Zoological Services, Directorate of Veterinary Services, Kenya, who gave support in one way or another that led to the completion of the research work and the thesis.

Lastly, I dedicate this thesis in memory of the late Dr Samuel Muiruri Kinyanyui who encouraged me to pursue further studies and contribute to the advancement of entomology. He served ably in the Ministry of Health as a scientist and expert on vectors and vector-borne diseases.

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ACRONYMS AND ABBREVIATIONS

ABIF	Applied Biosystems binary Sanger sequencing **chromatogram** data file
BLASTIN	Basic Local Alignment Search Tool-Nucleotide
CHFIKV	Chikungunya Fever Virus
CHIKF	Chikungunya Fever
ClustalW	A General Purpose Multiple Sequence Alignment Program for DNA
CP	Contingency Plan
DNA	Deoxyribonucleic Acid
DVS	Director of Veterinary Services
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme Linked Immunosorbent Assay
FASTA	Fast All alignment file format
GPS	Global Positioning System
ILRI	International Livestock Research Institute
MEGA	Molecular Evolutionary Genetic Analysis
MIR	Minimum Infection Rates
O.I.E.	***O**ffice **I**nternational des **E**pizooties (*World Organization for Animal Health)
PCR	Polymerase Chain Reaction
RNA	Ribonucleic Acid
RVF	Rift Valley Fever
RVFV	Rift Valley Fever Virus
ViPR	Virus Pathogen Database and Analysis Resource
WNF	West Nile Fever

ABSTRACT

The ecology of mosquitoes and associated arboviruses are heavily influenced by precipitation and retention of water in the environment. In 2011 to 2014, unprecedented flooding occurred in Lake Baringo flooding expansive area and inundated over 88km^2 of the shoreline. The impact of this on animal health, livelihoods and ecology generated a lot of interest among stakeholders. The current study was aimed at assessing the spatial-temporal changes of mosquito abundance, diversity and associated risks to arboviruses in order to enrich control strategies for mosquitoes and zoonoses. This was a longitudinal study carried out for one year from October 2012 to October 2013. It analysed mosquito ecology, identified mosquitoes to species level where possible, characterized arboviruses and determined disease prevalence from livestock. Mosquitoes were trapped using Centre for Disease Control (CDC) light traps in three ecological zones namely swamp marshy habitat, flooded lake shoreline and dry rangeland. Blood was drawn from livestock to determine presence of arboviruses. A total of 386,624 individual mosquitoes were captured. The Anopheline mosquitoes constituted genus *Anopheles*, while the Culicinae constituted 10 genera namely *Aedes*, *Culex*, *Mansonia*, *Hodgesia*, *Uranotaenia*, *Coquillettidia*, *Aedeomyia*, *Ficalbia*, *Theobaldia* and *Orthodomyia*. Mosquitoes of genus *Mansonia* were the dominant species in the basin and accounted for 84.9% with 97.8% coming from flooded lake shoreline habitat. Genus *Aedes* formed 0.1% of total mosquito catches from the basin with 96.9% of the individuals caught coming from swamp marshy habitat. Isolated viruses were *Culex flavivirus* in mosquitoes, *Aedes flavivirus* and Rift Valley Fever virus in livestock blood. This study reports for the first time detection of nucleic acid for *Aedes flavivirus*, an insect specific flavivirus, from livestock blood even though it does not replicate in vertebrate cells. Of eight (8) sequences of isolated virus for Rift Valley Fever (RVF), only three (3) sequences were 100% similar and the other five (5) exhibited alterations at various nucleotide base positions. Results from Next Generation Sequencing produced nucleotide sequences identical to *Aedes albopictus* mosquito species for the first time in the area. The mean catches of mosquitoes between the three habitats from Lake Baringo basin was significantly different at ($F_{(2, 27)}$ =3.54; P=0.04). Also significantly different were mean catches of mosquitoes at night from communal grazing areas and homestead at ($F_{(11, 12)}$ =2.87; P=0.04). The flooded lake shoreline had the lowest diversity of Simpson's Index at 0.13 due to dominance by mosquitoes of genus *Mansonia*. The swamp marshy and dry rangeland areas were more diverse in mosquito genera with Simpson's Indices of 0.56 and 0.57 respectively. Overall infection prevalence in livestock with RVF virus was 10.4% with the highest risk encountered from swamp marshy habitat (7.8%), flooded shoreline (2.6%) and lowest at dry rangeland (0%). The risk of infection with arboviruses was significantly lower in the dry rangeland habitat than in flooded shoreline at ($t_{(6)}$ = 1.94; P=0.024). The findings indicate that unprecedented flooding altered the environmental tolerance range of different mosquito species and ecology and this affected the spatial-temporal parameters impacting on mosquitos' composition, abundance and diversity in the expansive lake shoreline habitat. This increased the risks of transmission and infection with mosquito-borne arboviruses to livestock. It is recommended that both the relevant departments and agencies for livestock and human health develop a strategic approach for the control and mitigation against RVFV in wetlands and floods prone areas.

CHAPTER 1: INTRODUCTION

1.1 Background to the study

Mosquitoes are well known vectors of human pathogens, including arboviruses and are considered to be the main vectors of killer diseases such as malaria, Dengue Haemorrhagic Fever, Yellow Fever, West Nile Fever and Chikungunya Fever (Tchankouo-Nguetcheu et al., 2012). Females mosquitoes suck animal blood and in the process act as primary or secondary transmitters of diseases. According to a report by WHO (2002), mosquitoes cause between 1.5 to 3 million human deaths each year. The main mosquito species responsible for zoonotic disease transmission include *Aedes mackintosh, Aedes excrucians, Aedes sollicitans, Aedes taeniorhynchus, Aedes triseriatus, Culex salinarius, Culex tarsalis, Culex territans,* and *Culex pipiens, and Mimomyia* spps, *Coquillettidia* spp, *Aedeomyia* spp, *Mansonia uniforms* and *Mansonia africana* species, (Bird et al., 2009). Many of these species have been reported to occur in the Lake Baringo Basin of Kenya, particularly during the wet season (Ajamma *et al*., 2016).

Mosquitoes belong to the family Culicidae which consists of three subfamilies Anopheline GRASSI 1818, Culicinae MEIGEN 1818 and Toxorhynchitinae. The subfamily Anopheline is composed of three genera: *Anopheles* MEIGEN 1818, *Bironella* THEOBALD 1905, and *Chagasia* CRUZ 1906. Mosquitoes of genus *Anopheles* consists of several species and complexes, with the most common in the tropics being *Anopheles darlingi, A. stephensi, A. funestus* and *A. gambie* s.s. complex (Harbach, 2007). There are 11 species in genus *Bironella* which are found in Australia but one species *B. obscura* found in New Guinea, while genus *Chagasia* has 3 species

and is found in tropics of South America (Edwards, 1941). The subfamily Culicinae is composed of genera: *Aedes* MEIGEN 1818, *Culex* LINNAEUS 1758, and *Mansonia* BLANCHARD 1901, among others that are of medical importance (Harbach, 2007). The other common genus is *Culex*, which include several species: *Culex pipens quinquefasciatus, Culex pipiens pipiens, Culex nigripalpus,* and *Culex tritaeniorhynchus.* Mosquitoes of genus *Aedes* include *Aedes notoscriptus, Ae. vexen, Ae. aegypti* and *Ae. albopictus.* There is also the genus *Mansonia* comprising of *Mansonia uniformis, Mn. Africana and Mn. dyari.* The subfamily Toxorhynchitinae occurs in tropics and includes only one genus *Toxorhynchites* THEOBALD 1901 (Harbach, 2007). Mosquitoes of genus *Toxorhynchites* feed on nectar and is therefore of no medical importance (Collins and Blackwell, 2000).

Arthropod-borne viruses are harboured and transmitted by arthropods. They are RNA viruses, double (dsDNA) or single (ssDNA) stranded, with a life cycle that requires both a host (birds or mammals) and a vector (Dash *et al*., 2013). Some of the mosquito transmitted viruses are West Nile Fever virus (WNFV) causing West Nile Fever, Rift Valley fever virus (RVFV) causing Rift Valley Fever disease (RVF), Yellow Fever and Dengue among many others. Most arboviruses of public health importance belong to one of three virus genera: *Flavivirus, Alphavirus* and *Bunyavirus* (Dash *et al.,* 2013). Early detection of the arboviruses from vector and animals is crucial in diseases prevention and control (Hall *et al.,* 2012). One of the most effective methods of detecting arboviruses is by use of Polymerase Chain Reaction (PCR) (Johnson *et al.,* 2012).

Periods of heavy flooding caused by high rainfall are usually associated with outbreak of arboviral diseases transmitted by mosquitoes of genus *Aedes* (Sang *et al.,* 2010). Such rains seem to trigger some physiological conditions that results in hatching of infected eggs of *Aedes* mosquitoes. Any form of flooding, either artificially induced or unprecedented flooding results in creation of several ecotope layers in a habitat that promote the proliferation of mosquitoes, their distribution, breeding and eventual diseases transmission (Paula *at el*., 2012).

Lake Baringo basin has diversity of habitats whose composition of fauna and flora is influenced by human activities and environmental changes. The basin has severally experienced outbreaks of diseases such as RVF, mostly attributed to Elnino/Southern Oscillation phenomenon (ENSO) rains causing flooding (Nanyingi *et al.,* 2015). The notable floods caused by Elnino/Southern Oscillation phenomenon (ENSO) in Lake Baringo occurred in 1997-1998 (Okech *et al.,* 2019) and 2006-2007 (Sang *et al*., 2010). However, other than floods resulting from heavy rainfall, the unprecedented flooding that occurred in 2011-2014 in Lake Baringo basin was speculated to be associated with changes in earth geological factors resulting in rise of lake waters and submerging of the farmlands along the lake shores (Onywere *et al.,* 2013). This extreme flooding phenomena is rare and is said to be a 50 year cycle with previous recorded occurrence from the Lake Baringo drainage basin in 1901 and 1963 (Obando *et al.,* 2016; Okech *et al.,* 2019).

Studies on mosquito species infestations and their associated diseases in the Lake Baringo basin have generally been carried out during the dry or wet periods. However, there have been no studies on previous unprecedented extreme flooding of 1901 and

1963 on the mosquito species occurrence, diseases prevalence and potential risks of disease outbreaks. The current flooding offered an opportunity to study the impact it would have on ecology, mosquitos' dynamics and arboviral disease prevalence in livestock. This study commenced in October 2012 and ended in October 2013.

1.2 Statement of the problem

For a period of over 30 years, Lake Baringo has been experiencing a decreasing trend of the waters, from a depth of 8m in 1976 to 1.7m in 2001 (Okech *et al.,* 2019). The area covered by water along the shores of the lake followed a similar trend with submerged land shrinking from 219km^2 in 1976, 136km^2 in 1986, to 114km^2 in 2001 (Okech *et al.,* 2019). Between 2010 and 2014, within a period of three to four years, Lake Baringo experienced an unprecedented extreme flooding speculated to be caused by the earth's tectonic movements that resulted in water rising from a low of 143.6 km² in January 2010 to a high of 231.6 km² in September 2013, surpassing any previous flooded water marks on the land (Obando *et al.*, 2016). In addition, the rise in water level increased the area covered by water by 88 km², an increase of 61.3% (Onywere *et al*., 2016). Such flooding had only been witnessed in the area in 1901 and 1961 (Okech *et al.,* 2019). Studies have established that ecological disturbances and environmental changes influence vector populations that could result in increased disease incidences and risks (Ochieng *et al.*, 2016). Several reasons for the emergence or re-emergence of some of the vectors and diseases they transmit have been advanced and probably could be multi-factorial but in most cases are not understood well (Chevalier *et al*., 2004). It is therefore probable that extreme unprecedented floods could strongly affect the ecology, breeding sites and habitats of mosquitoes and impact on their distribution and transmission of zoonotic diseases.

1.3 Justification of the Study

Mosquitoes transmit zoonotic diseases causing approximate 2.3 billion human illness and 1.7 human million deaths (ILRI, 2012). The floods cause proliferation of the mosquitoes that transmit most of this arboviral diseases with one such as RVF, considered to be endemic in Lake Baringo basin (Tigoi *et al*., 2015).

In 2006/2007, an outbreak of RVF in Kenya resulted in deaths and loss of livelihoods estimated to have been in range of US $32 million based on losses from banned slaughters, transport and cost of livestock vaccinations (Karl and Wanyoike, 2010). The outbreak was caused by Elnino floods (Nanyingi *et al*., 2015).

It therefore became important to understand how the 2010-2014 extreme unprecedented floods impacted on mosquitos' dynamics and incidences of RVF inorder to develop policies towards mitigation measures for future use. Such study would help to zero in on the key drivers that lead to an outbreak for timely predictions and forecast.

1.4 Research questions

This study sought to answer the following questions:

i) What were the spatial-temporal changes on mosquito species composition and abundance during an unprecedented flooding of Lake Baringo?
ii) What arboviruses were harboured by mosquitoes and livestock from different habitats in Lake Baringo basin during an unprecedented flooding of the lake?
iii) What were the characteristics of arboviruses from livestock during the period of extreme flooding of Lake Baringo?

1.5 The hypothesis

It was hypothesized that:

i) There was no spatial-temporal changes in mosquito composition and abundance in the Lake Baringo basin during the period of unprecedented flooding.

ii) There was no spatial variation on arboviruses harboured in mosquito and livestock resident from different habitats in the Lake Baringo basin.

iii) There was no significant genetic variation of arboviruses harboured in livestock from Lake Baringo basin during the period of unprecedented flooding.

1.6 Objectives of the study

1.6.1 General objective

The general objective of the study was to determine spatial-temporal changes in mosquito dynamics and their potential risks for transmission of arboviruses to livestock during a period of unprecedented flooding of Lake Baringo, Kenya, 2012-2013.

1.6.2 Specific objectives

The specific objectives of the study were:-

i) To determine the spatial-temporal changes in mosquito species composition, abundance and diversity in Lake Baringo basin during the period of unprecedented flooding.

ii) To determine the arboviruses harboured in mosquitoes and livestock during the period of unprecedented flooding in Lake Baringo.

iii) To characterise arboviruses isolated from livestock resident in Lake Baringo basin during the period of unprecedented flooding.

1.7 Scope of the study

The impact of Elnino and artificial flooding on mosquitos' dynamics and disease prevalence have been well documented but the impact of flooding phenomena not associated with rainfall is poorly understood. The aim of this study was to report changes in mosquitos' ecology due to extreme unprecedented flooding and its impact on mosquito dynamics and arboviruses prevalence in Lake Baringo basin. The data was limited to mosquitoes of genus *Culicinae* and arboviruses prevalence in livestock. A total of 77 herd of livestock were recruited based on their health status for identification of arboviruses and characterization in the laboratory. This study lasted for 12 months from October 2012 – October 2013. The study was a part of an ongoing collaborative research project between Directorate of Veterinary Services and ILRI (Appendix I). A research approval was granted by the Kenyatta University (Appendix II, III).

1.8 Significance of the study

Previous outbreak of arboviruses have left Governments exposed on preparedness of containing the spread and controlling of diseases such as Rift Valley fever. It has been demonstrated that climate change has resulted in changes of vectors dynamics with new pests invading new areas and introducing diseases. These has led to new explosions of zoonotic diseases. Adequate public health measures are therefore required to be put in place for mitigation against the upsurge of vector-borne viral related diseases as demonstrated by outbreak of emergence of Chikungunya disease

in Asian and African Countries, upsurge of dengue, sporadic outbreaks of Rift Valley Fever diseases.

Rift Valley lakes have been experiencing changes in their water levels over the recent years. Families have been displaced and exposed to water and vector borne diseases. Sound and informed decisions by the stakeholders responsible for public health prevention and control can only act and put in place proper mitigation measures by use of available and archived data. This research will help the Governments, institutions and health officials to come up with protocols and policies to guide on mitigation measures against RVF and other zoonotic diseases in future in case of a recurring extreme flooding caused by natural factors or from overflowing dams. In Kenya, the findings of this study can be used to plan and put in place mitigations for other Rift Valley lakes experiencing similar changes in their water levels.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

The biology and life cycle of mosquitos' life cycle are central in its efficiency as a vector of several devastating diseases. The male mosquitoes feed on plant nectar while the females feed on vertebrate blood, a behaviour that triggers the vitellogenic stage of oogenesis leading into oviposition of mature eggs (Valzania *et al.,* 2019). *Anopheles* lay their eggs singly forming exochronic floats while Culicinae eggs are laid in rafts. Contact with water triggers hatching into larvae and eventual pupae stage before developing into adults. Eggs of *Aedes* mosquitoes are resistant to desiccation and can overstay for several years before hatching while those of *Uranotaenia* are deposited directly to flood water (Wayne, 2004).

2.2 Theoretical review

Agricultural activities, landscaping, dam construction including natural flooding affect the vector population and vector – borne diseases by altering the exposure of the host and disease transmission dynamics (Lambin *et al.*, 2010). The breeding sites, resting places, and the extent of maturation of the vector is therefore dependent on the season and prevailing environmental factors in a an ecology (Ellis and Wilcox, 2009). This exerts genetic pressure on the pathogen and vector which may influence the virulence of the diseases and the spread (Kilpatrick and Randolph, 2016). The resultant infect are changes in vector composition, distribution, abundance, diversity, and pathogen distribution characterize the ecosystem and its spatiotemporal dynamics (Keesing *et al*., 2010). Lyapunov models then suggests that these may lead into a loss of a virus within a community or its amplification in the host population (Pedro, 2018)

2.3 Conceptual Framework

The conceptual framework of this study explores the independent variables, climatic, environmental and time impacted on mosquitos' species composition, abundance and diversity over time and space.

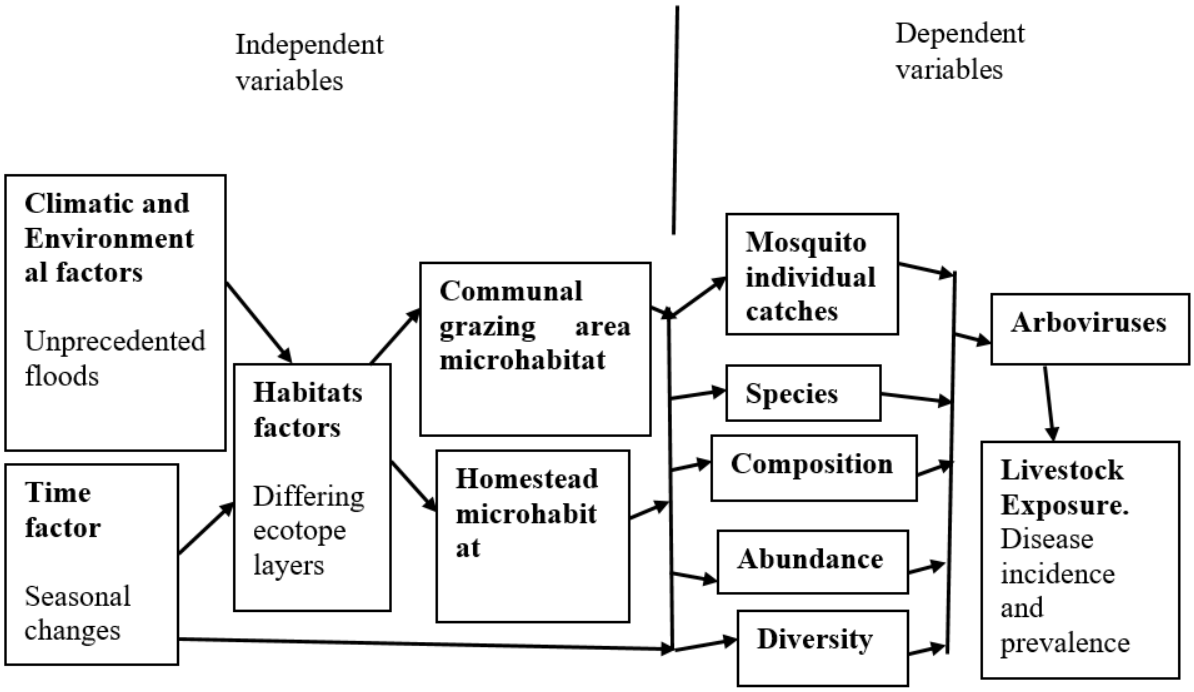

Figure 2.1: Conceptual framework illustration for the variables on exposure of livestock to mosquitoes and arboviruses prevalence

2.4 Impact of flooding on mosquito ecology and dynamics

The Rift Valley lakes have had a history of water fluctuations since 1860 (Richardson, 1966). These fluctuations are attributed to reduced rain water and inflows to the lakes and at times due to flooding from the heavy rains experienced in the upper escarpments of the valley. Some of fluctuations and flooding are caused by Elnino rains. However another uncommon and unprecedented form of flooding is experienced after every 50 years, a rare cycle caused by geological disturbances of the Rift Valley (Obando *et al.,* 2016; Okech *et al.,* 2019).

According to Onywere (2013), the flooding of Lake Baringo between the years 2010-2014, which resulted in farmlands along the lake shoreline to be submerged, was a 50 years cycle recurring phenomena. There was an increase of the submerged land from 143.6 km² in January 2010 to a high of 231.6 km² in September 2013, an increase of 88 km² (61.3%). Families were displaced, submerged homesteads abandoned, and vegetation destroyed with some drying up, and invasive weeds emerging along the shorelines (Plates 2.1, 2.2, 2.3). This form of extreme flooding phenomenon, in accordance to the community, was witnessed lastly 50 years ago. Other reviews supports these and states that the unprecedented flooding of Lake Baringo occurred in 1901 and 1963 (Obando *et al.*, 2016; Okech *et al.*, 2019).

Plate 2.1: Flooded farmlands in lake shoreline habitat of Lake Baringo by rising waters of Lake Baringo

Plate 2.2: Submerged infrastructure and trees along the flooded lake shoreline habitat of Lake Baringo.

Plate 2.3: Submerging homestead from lake shoreline habitat of Lake Baringo. The homestead was eventually submerged and waters polluted by animal and human wastes including other decaying organic matters

The Lake Baringo basin forms a part of Great Rift Valley lakes. Within the basin lies Lake Baringo which is fresh water and L. Bogoria a salt water lake. Lake Baringo located between 0_300–0_450N and 36_000–36_100 E, (Reuben Omondi et al., 2016) is a part of tropical lake ecosystems whose sustainable management remains a pressing priority on account of impact from degraded catchment due to intensive cultivation, subsistence farming and ever expanding urban settlements (Obando *et al*., 2016). It is characterised by semi-arid environment and faces challenges of soil erosion and water pollution that affects human and livestock health (Obando *et al*., 2016). The Loboi wetland is an important part of the ecosystem because of the papyrus harvesting, water springs, and as grazing lands (Terer *et al.,* 2012). The L. Baringo basin is the home of Perkerra Irrigation Scheme where vegetables and fruits are grown using ground water canal irrigation system.

Studies from other areas, have shown that the physio-chemical parameters of a habitat, which includes low levels of alkalinity, turbidity, depth of the pond or well, temperature, level of organic matters, salinity and shade are important in eliciting successful oviposition of mosquitoes (Salit, *et al*,, 1996). This is not different to Lake Baringo. The ecology of Lake Baringo basin is complex ranging from an even lake shoreline with vegetation, swamps that are fed by underground streams, to dry rangeland located in upper escapements. It is home to numerous mosquitoes that breed in differing sites with the adults thriving in different habitats (Ondiba *et al.,* 2018).

Adults of Mansonia mosquitoes lay their eggs under surface of the floating leaves of aquatic vegetation and onto the roots of which immatures attach themselves for respiration, with adults dispersing to other resting areas (Rajendran and Panicker,

1994). Female mosquitoes of the family Culicidae and Mansonia oviposit in several types and dimensions of reservoir waters where they are associated with plant matter while larva and pupae affix themselves to the roots of aquatic macrophytes, from which they derive oxygen accumulated in the aerenchyma of the plant floating organs (Paula *et al*., 2012).

Aedes mosquitoes can withstand long periods of dry weather with the eggs surviving for several seasons awaiting wet weather or flooding to dampen them, before they hatch (Tantely *et al.*, 2015). There is transovarian transmission of RVF virus in the eggs and in instances where they are not infected, the adult gets lifetime infection once they feed on the infected host (Tantely *et al*., 2015). The adult species carry diseases, and can be vicious biters, found in large numbers within homesteads and in field prone flooding areas (Sang *et al.*, 2010).

The breeding sites of Culex is in the natural habitat such as river edges, riverbed pools, rain pools, stream edges, grasslands, marshes, and hoof-prints, rice fields, irrigation channels and wells. The genus also breed in polluted water with low dissolved oxygen, while others breed in saline areas (Azari-Hamidian, 2007).

Similarly, in the Lake Baringo basin, physical and biotic factors play a significant role in influencing the population and species harboured in an ecology. Various studies shows that the basin is infested with several species of mosquitoes such as *Anopheles, Culex, Aedes,* and *Mansonia* breeding in different diverse habitats and microhabitats (Ondiba *et al.*, 2019). Factors such as habitat and season (wet or dry), are stated to influence the mosquito infestation and species richness and evenness, with dry season

having highest diversity and lowest during the long wet season (Ondiba *et al.,* 2019; Ajamma *et al.*, 2016). Study also by Ochieng, (2016) showed that *Mansonia africana, Mansonia uniformis* and *Culex pipiens* have been the dominant species at various points between 2007 -2012. These species had been previously implicated in transmission of Rift Valley Fever virus in Lake Baringo basin (Ondiba *et al.*, 2017). Predication models of habitat suitability to mosquito infestation under normal climatic conditions and landscape variables, the lowlands were found to be highly suitable for all the species such as *Culex univitattus*, *M. africana,* and *M. uniformis* and the highlands having few spots that are highly suitable (Ochieng *et al.*, 2016).

2.5 Arboviruses and epidemiology of mosquito-borne zoonosis

2.5.1 Rift Valley Fever

Mosquitoes remain the only important biological vectors of some of viruses such as Rift Valley Fever virus (RVF), West Nile Fever virus and Chikungunya among others (Davies, 2010). The viruses have been isolated from several species of mosquitoes with a high vector competence among *Aedes albopictus, Ae. canadensis, Ae. cantator, Ae. excrucians, Ae. sollicitans, Ae. taeniorhynchus, Ae. triseriatus, Culex salinarius, Cx. tarsalis, Cx. territans,* and *Cx. pipiens* species (Bird *et al*., 2009).

Rift Valley fever (RVF) virus belongs to order Bunyavirales, family *Phenuiviridae* and genus *Phlebovirus* (Abudurexiti *et al*., 2019). It was first isolated in 1930 from sheep (Sang *et al*., 2010) but was first described in Kenya in 1931 (Daubney *et al.*, 1931). Since then, the disease has also been reported outside of sub- Saharan and Southern Africa with an outbreak occurring in Egypt in 1977 (Imam and Medhat,

1979). The disease primarily affects cattle, sheep, and goats, but also affects people and Wildlife (Jost *et al.*, 2010). Transmission to humans is thought to occur through direct contact with tissues, blood of infected animals, and by the bite from an infected mosquito (Sang *et al.,* 2010). In humans, the disease may manifest itself as a mild fever, but a small percentage (1%) does experience the fatal haemorrhagic stage of the disease (Jost *et al.,* 2010), with liver damage and occasionally encephalitis (Tchankouo-Nguetcheu *et al.,* 2012).

The outbreak of RVF disease occurs after one (1) month or more of heavy rainfall and especially when topographical depressions called damboos suddenly flood causing the eggs of dormant RVFV that are infected float on water and hatch (Linthicum *et al.,* 1988). The presence of disease in a new geographical area can be attributed to movement of livestock during trade and presence of vector mosquitoes during the heavy rains that lead to floods (Chevalier *et al.,* 2004). For instance, in Saudi Arabia, it is hypothesized that the disease found its way into the region from Kenya through livestock trade and this is supported by genetic matching of the strains of RVF that were isolated in the area (Chevalier *et al.,* 2004). Similarly, trade of infected sheep and camels between Sudan and Egypt is believed to be responsible for the 1977 RVF outbreak near Lake Nasser in Egypt (Chevalier *et al.,* 2004). Heavy rainfall and floods such as Elnino rains are also indicated as responsible for increased number of breeding sites and number of RVF vectors, hence intensifying virus transmission and circulation (Anyamba *et al.,* 2001).

According to the World Organization for Animal Health (OIE, 2009), the RVF disease in livestock affects ruminants with new-born animals being highly susceptible and

most of the pregnant ones aborting. The incubation period ranges from one to three days followed by fever, recumbency, and haemorrhagic diarrhoea (OIE, 2009; Chevalier *et al*., 2004). Mortality rate can reach 70% with high rates of abortion associated with epidemics of Rift Valley haemorrhagic fever (RVHF) which is referred to as abortion storms, and in humans an influenza-like illness that may end up haemorrhagic fever (OIE, 2009). The RVF disease epidemics usually occur after every 10 years with the virus able to invade diverse ecological systems (Jost *et al*., 2010). In South Africa, the disease was reported first in 1950, Namibia in 1955 and again in South Africa in 1952-1953, 1955-1959, 1969-1971, 1974-1976, 1981, 1999; Egypt in 1977-1978, Senegal, Kenya, Tanzania and Somalia in 1987-1988, and Saudi Arabia in 2000 where it caused death of 224 people (Chevalier *et al*., 2004).

Kenya has experienced several outbreaks of RVF in the past. Between 1951 and 2007, 11 national outbreaks of RVF were recorded with an average inter-epizootic period of 3-6 years (Nanyingi *et al.,* 2015). The 2006/2007 the outbreak of RVF in Kenya was reported in Garissa, Ijara, Maragwa, Thika, Baringo, Marigat and Kilifi (Breiman *et al.,* 2010; Munyua *et al*., 2010). The highest livestock morbidity and mortality rates, including cases of human infections were recorded in Garissa and Baringo Counties (Breiman *et al.,* 2010). Studies on infections to humans with RVF virus indicate that males are more prone to infections than females, and adults more than children (LaBeaud, *et al.,* 2015). This is attributed to high exposure of males and adults to mosquito vectors than females and children. Those people who have direct contact with infected livestock such as butcher men, herders and clinicians and animal health professionals are regarded as on high risk of contracting the disease (LaBeaud, *et al*., 2015).

2.5.2 West Nile Fever virus

The mosquito species that transmit West Nile Fever (WNF) virus are *Culex modestus, Cx. univittatus, Coquillettidia richiardii, Ochlerotatus cantans, Aedes rossicus, Anopheles maculipennis s.s.* and *Anopheles atroparvus* (Reusken *et al*., 2011) and (Tchankouo-Nguetcheu *et al*., 2012). The ornithophilic members of *Culex pipiens s.l.* species complex are the most common vector for West Nile Fever Virus, with maintenance of virus in birds and horses (Reusken *et al*., 2011). In Kenya, WNV isolates have been isolated from *Cx univittatus* in Garissa and Turkana (Ochieng *et al.*, 2013).

The West Nile Fever arboviruses were first isolated in 1937 from blood of a febrile woman in West Nile District of Uganda (OIE, 2009). The first cases of West Nile virus in its lethal encephalitic form were reported in Algeria in 1994 (Filette *et al*., 2012). In horses, the disease manifests itself in form of fever with subsequent neurological conditions that include ataxia, paresis and limb paralysis (Tchankouo-Nguetcheu *et al*., 2012). Vertical and horizontal transmissions do occur in some instances as reported by LaBeaud *et al*. (2015). The West Nile virus has been isolated from at least 326 species of birds among them, crows, tufted titmice, blue jays, American robins, and eastern bluebirds. Chickens and turkeys are said to be resistant to the disease (Filette *et al*., 2012). However, in humans, the virus can be spread between individuals through blood transfusion and organ transplantation (Charatan, 2002). The West Nile Fever Virus (WNV) documentation in Kenya is scanty with a few human cases reported in Garissa (Sang *et al*., 2010) and Trans Nzoia County (Ngoi, 2012). A cross-sectional study carried out in three Counties of Busia, Malindi

and Samburu showed a human infection standing at 4.36%, 18.81%, and 2.16% respectively (Luke *et al*., 2011).

2.5.3 Chikungunya

Chikungunya (CHIK) virus is a member of the *Alphavirus* genus in the family *Togaviridae* (Ann *et al*., 2000). It was first isolated from the serum of a febrile human in Tanzania in 1953 (Karabatsos, 1985). In the 1960s and 1980s, it was isolated from most of central, southern and western African countries as well as Asia where it caused outbreaks (Rao, 1966). The main vectors of chikungunya virus in Africa are *Aedes furcifer, Ae. vittatus, Ae. fulgens, Ae. luteocephalus, Ae. dalzieli, Ae. vigilax, Ae. camptorhynchites, Cx. annulirostris, Mansonia uniformis* (Gilles *et al*., 2007). In Asia and India *Ae. aegypti* and *Ae. albopictus* are the main urban vectors of chikungunya virus (Reiter, Fontenille, and Paupy, 2006). The virus has been found to circulate in a sylvatic cycle between forest *Aedes* species of mosquitoes and primates (Bird, Nichol, and MacLachlan, 2009). Within the urban areas of Africa and Asia, the virus is reported to circulate between mosquitoes and human hosts (Bird *et al.*, 2009).

The largest documented outbreak of Chikungunya virus (CHIKV) disease occurred in the Indian Ocean islands and India during 2004–2007 (Staples, Breiman, and Powers, 2009). In La Reunion Island's, a genetic mutation of *Aedes albopictus* was responsible for an epidemic that occurred in the year 2006 affecting over one third of the entire population (Bird *et al*., 2009). In India, the re-emergence of the disease is reported to be due to the emergence of a new genotype with an A226V mutation in the membrane fusion of glycoprotein E1 during the recent outbreak in the Indian Ocean islands (Schuffenecker *et al.*, 2006). In Italy an outbreak of the disease in 2007 resulted in

around 205 human cases of infection with CHIKV identified in Ravenna. The source of the outbreak is thought to have been a visitor from India who had visited relatives in a village in Italy (Rezza, Nicoletti, and Angelini, 2007).

In Kenya, a major outbreak was reported in Lamu in the year 2004 (Staples et al., 2009) where an estimated 13,500 cases representing 70% of the population of the island, were infected (Sergon and Kalani, 2008). New infections have taken place in Kenya especially in some parts of north eastern and coastal regions since then between 2011 -2014 (Konongoi *et al*., 2016).

Use of molecular diagnostic techniques have helped in discoveries and reporting of new arboviruses. Novel arboviruses within the families *Togaviridae*, *Flaviviridae* and *Bunyaviridae* are reported globally. However, in Africa, only a fraction of existing arboviruses have been identified (Junglen *et al.,* 2009). In Uganda, new species of flavivirus have been isolated from *Mansonia africana nigerrima* and *Culex quinquefasciatus* (Cook *et al.,* 2009). New viruses have also been isolated from different mosquito species in Kenya. Ochieng *et al*., (2013), for example, reported the presence of previously unknown viruses in the L. Baringo basin of Kenya such as Ndumu virus (NDUV) from *Culex rubinotus* and *Mansonia* species. The study also isolated the same virus from *Aedes mcintoshi*, *A. ochraceous*, and *A. tricholabis* trapped from Garissa. Similarly. Sindbis, Babanki and Usutu viruses were also isolated from species of *Culex* trapped in Naivasha, Kisumu and Budalangi with the main vector being *Culex pipens.*

These studies therefore show that, the range of species for mosquitoes that could be held culpable for transmission of the arboviruses is expanding. The sporadic outbreaks can no longer be associated with the previous known strains of viruses alone, for there is evidence of new mutants (Hall *et al*., 2012). This demonstrates the need to intensify research in this field of science to avoid looming disaster in future, either in isolated habitat or a wide ecological area.

In most of the previous studies on mosquitoes, the sampling is bi-annually and limited to the wet seasons when the population of vector is usually high. It is also limited to sampling within the homestead at night. The methodology of sampling within the homesteads only and at night leave communal grazing fields and other rich ecologies within the habitats un-sampled. This study therefore sought to sample within the homesteads at night, and also in communal grazing field during the day and night. The communal grazing areas were set aside by the community where livestock from the village graze during the day before being herded into their owners' homesteads at night. In these grazing areas livestock grazed alongside wildlife such as zebra, ostriches, some antelope species and monkeys among others. The study also targeted indigenous poultry residents within the homesteads for pathogen analysis for the first time.

2.5.4 Laboratory diagnostic tests for arboviruses

There are several diagnostic tests that are used for isolation and identification of arboviruses. They range from conventional to most advanced molecular techniques. Conventional approaches include; Plaque Reduction Neutralisation Tests (PRNT); virus neutralisation tests (VNT); Haemagglutination Inhibition Assay (HI);

Immunofluorescence Assay (IFA) and Enzyme Linked Immunosorbent Assay (ELISA) (Hall *et al.*, 2012). Recent tests are Rapid Microneutralisation Assays, Lateral Flow; Biosensors and Microfluidic Systems (Hall *et al*., 2012).

Nucleic acid is very important in molecular biology and the processes of extraction from the organism are very crucial. There are different protocols for extraction that are used in molecular studies, each depending on the available manufactures kit. Polymerase Chain Reaction (PCR), a recent innovation**,** is one of the technologies that have revolutionized science globally. The use of PCR technique in molecular biology to amplify thousands to millions of copies of DNA sequence with several protocols for optimization of genomic DNA is now in place (Shivani and Shabad, 2012). Real-time RT-PCR, Standard-PCR and Nested PCR methods have been developed for rapid detection of RVFV and other viruses (Hall *et al*., 2012). This molecular techniques have been made easier through development of multi-complex primers that are used in detection of DNA and RNA of pathogens samples to identify particular viruses in blood and mosquitoes. In a study carried out in Northern Europe by Johnson *et al.* (2012), PCR was used to identify vectors and arboviruses they transmit. In another study done during the outbreak of RVF in Kenya 2006/2007, a team of investigators led by Sang *et al.* (2010), used PCR to identify and analyze RVF virus from samples collected from Garissa and Baringo.

In furtherance to this, a post-PCR detection sequencing technology called Sanger Sequencing that is able to zero-in on the specific attributes of the pathogens has being developed (Munshi, 2012). Sanger sequencing technology was developed by Frederick Sanger and colleagues in 1977 (Bisht and Amrita Kumari Panda, 2013).

The technology is also known as the chain termination method and is a technique for DNA sequencing based upon the selective incorporation of chain-terminating dideoxynuleotides during in-vitro DNA replication (Munshi, 2012).

Continuance need to undertake sequencing that would lead to better understanding of metagenomics that is, identifying organisms present in a habitat, samples or any medium at a reduced cost and faster than Sanger sequencing has led to development of Next-Generation Sequencing (NGS) (Metzker, 2010). Such NGS technologies includes, Illumina (Solexa) sequencing, Roche 454 sequencing, Ion torrent- Proton / PGM sequencing, and SOLiD sequencing. This enables in-depth analysis of entire genomes at unprecedented levels, allowing researchers to explore questions and hypotheses that previously would have required years to answer by determining the precise order of nucleotides within DNA molecule (Thomas, 2001) and (Metzker, 2010) . Knowing which organisms are present in a particular environment is critical to research in ecology, epidemiology, microbiology and other fields (Metzker, 2010).

2.6 Transmission of arboviruses and associated risks factors

2.6.1 Environmental factors that favour emerging vectors

Environmental factors, availability of the host and interaction with the vector are some of main factors that determine the risk of contracting or getting infected with an arboviral disease. In addition, exposure to the multiple vector species lead to heighted risk of infection from the bites. Environmental and land use changes that trigger an outbreak of most of vector-borne diseases include flooding, drought, irrigation and damming.

The survival of primary vectors such as *Aedes* (subgenera *Neomelaniconium* and *Aedimorphus*) mosquitoes that emerge after flooding is therefore dependent on drought-resistant eggs that remain viable for long periods (Bird *et al*., 2009). It is reported that the first species to emerge are *Aedes* mosquitoes (*A. lineatopennis, A. cumminsii,* and *A. sudanensis*) followed a few days later by so called secondary amplifying vectors such as *Culex* and *Anopheles* species (Anyamba et al., 2001). Research also shows that measurements of vegetation conditions expressed in the form of the normalized difference vegetation index (NDVI) are strongly correlated with rainfall in semi-arid areas and can be used as a surrogate for rainfall predications (Anyamba *et al*., 2001). Heavy precipitations that follow up due to warm El Niño Southern Oscillation is said to be a factor in transmission of RVF (Bird *et al*., 2009).

Drought can also bring indigenous poultry hosts and vector mosquitoes into close contact during periods of scarce watering thus facilitating epizootic cycling and amplification of arboviruses within the populations (Pradier *et al*., 2012). Investigations conducted in Colorado showed that WNV infection rates among *Culex* species of mosquitoes declined with increasing wetland cover, suggesting that preservation of large wetland areas may represent a valuable ecosystem-based approach for controlling WNV outbreaks (Pradier *et al.*, 2012). Instances where poultry are reared in urban areas, clustered settlements and with their nests located in close proximity to human dwellings may therefore play a role in transmission of WNV.

2.6.2 Emerging and re-emerging disease vectors

Emerging and reemerging vectors are documented to result in outbreaks of new vector-borne diseases in a habitat or geographical location. In some instances, some vectors that are human and bird biters are increasingly becoming competent bridge vectors as evidenced by hybridization of *Culex pipens* in transmission of West Nile Fever virus (Fonseca *et al*., 2004). Viral isolation and vector competence studies have shown that *Aedes albopictus* mosquito is an efficient vector of more than 20 arboviruses (Ngoagouni *et al*., 2017). In central Africa, *Ae. albopictus* was first reported in 2000 in Cameroon and has since been found in almost all countries of the region. *Ae. albopictus* is often found with resident species of *Aedes aegypti* in the same city and larval breeding sites (Ngoagouni *et al*., 2017). Introduction of a new vector in an ecology results in changes of the disease transmission patterns (Weetman *et al*., 2018).

2.6.3 Evolutionary selective pressure on arboviruses

Arboviruses are prone to mutations with new strains occurring in a local ecology or transmitted to new ecologies. The ease of transmission and their virulence is dependent on several factors. Once a pathogen is established, ecological factors related to vector and host characteristics can shape the evolutionary selective pressure and result in increased use of hostas transmission hosts (Khan, 2015). The survival, establishment, and spread of animal diseases depend on climate, geographic factors, host species and their distribution (Bram *et al*., 2002). Mutants of local RVFV strains due to environmental or caused because of using attenuated vaccines is of significant concern globally. Phylogenetic analysis have shown the occurrence of reassortment of RVFV based on geographic regions: West Africa, Egypt and Central-East Africa

(Sall *et al*., 1999).. However, phylogenetic analysis does not categorize the arboviruses as pathogenic or not. The coding based pathogenicity is entirely based on clinical and pathology manifestation including economic loses of the disease.

2.7 Socio-economic status of Rift Valley Fever

A 2012 zoonosis report by ILRI indicated that zoonotic diseases mostly affect poor livestock keepers, causing about 2.3 billion human illnesses and 1.7 million human deaths per year (ILRI, 2012). With an estimated national ruminant livestock population of cattle being 17,467,774, sheep 17,129,606, goats 27,740,153 and camels 2,971,11, the contribution of ruminant livestock to Kenya is estimated to stand at Ksh 318.971 billion which is 43 % of Agricultural Gross Domestic Product and 12% of the total National GDP (Behnke and Muthami, *et al*., 2007). On a macroeconomic scale, losses from an outbreak of RVF in 2006-2007 is estimated to have been in range of Ksh 2.1 billion (US$32 million) on the Kenyan economy, based on its negative impacts on agriculture and other sectors like transport and services among others (Rich and Wanyoike, 2010) and (Karl and Wanyoike, 2010).

2.8 Research gaps identified in the study

It is therefore evident from the literature that, most of the studies in Lake Baringo basin have focused on mosquito population especially during the heavy rains, Elnino and when there is an outbreak of mosquito-borne diseases. This study was undertaken during times of an extraordinary unprecedented flooding along the Lake Baringo shoreline arising from the rise of the lake water and not from rain. So far only one study have attempted to understand the mosquito dynamics from different habitats found in Lake Baringo basin concurrently, shoreline, swamp marshy and dry

rangeland, as described in this study and over a several seasons (two (2) wet seasons and two (2) dry seasons). Similarly, only a few studies have collected data during the dry season. In an investigation by Ondiba (2017), mosquitoes were sampled from Baringo County in habitats he characterized into riverine, midland, lowland, and highland with Kapkuikui (swamp marshy area) and Salabani-Ngambo (lake shoreline) habitats characterized as lowlands and Kimelel (dry rangeland). The study was during the normal rain season. It is therefore noteworthy that there are no studies on changes in mosquito composition over a seasonal cycle and the influence of varied habitats that have focused on such rare phenomena as the flooding that took place in Lake Baringo, 2010-2014

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

3.1.1 Location

Lake Baringo basin is located in Baringo County at the floor of the central arm of the Great Rift Valley in Kenya and at an altitude of 950m above sea level. The basin covers 1,678 km^2 and is located at latitudes $00^0$28'N and 00°32'N and longitudes $36^0$58'60'E and $36^0$00'E (Figure 3.1). It consists of undulating hills, plains, and swamps. On the southern side of Lake Baringo is Lake Bogoria which is surrounded by Loboi plains. To the north of Lake Baringo are steep escarpments that separate the basin from the dry rangeland and Tugen hills. It is a fresh water lake with no outlet and is fed by three seasonal rivers, River Perkerra, Molo and Ol Arabel. Lake Bogoria, which is saline, is located at an altitude of 990 m above sea level, latitude 0°14'11.7"N and longitude 36°06'20.8"E. The field analysis of samples was carried out at Public Health Laboratories located in Marigat Sub-county at 0°28'29.3"N and 35°58'56.8"E, in Baringo County. International Livestock Research Institute and Directorate of Veterinary Services are located in Kabete, Nairobi, Kenya. Further studies were carried out at Macrogen Laboratories in Maryland, United State of America.

3.1.2 Climate and geomorphology

The Lake Baringo basin is located in semi-arid area, ecological zone VI (Jenny Johansson and Jakob Svensson, 2002). The basin experiences bimodal rainfall with the long rainy season starting from March–June and a short rainy season from October – November, averaging 1200 mm per year with a range of 300-700mm (Wetang'ula, Kubo, and Were, 2012). Temperatures vary from 15^0C to 35^0C with an average of 28^0C for the most part of the year (Wetang'ula *et al.*, 2012).

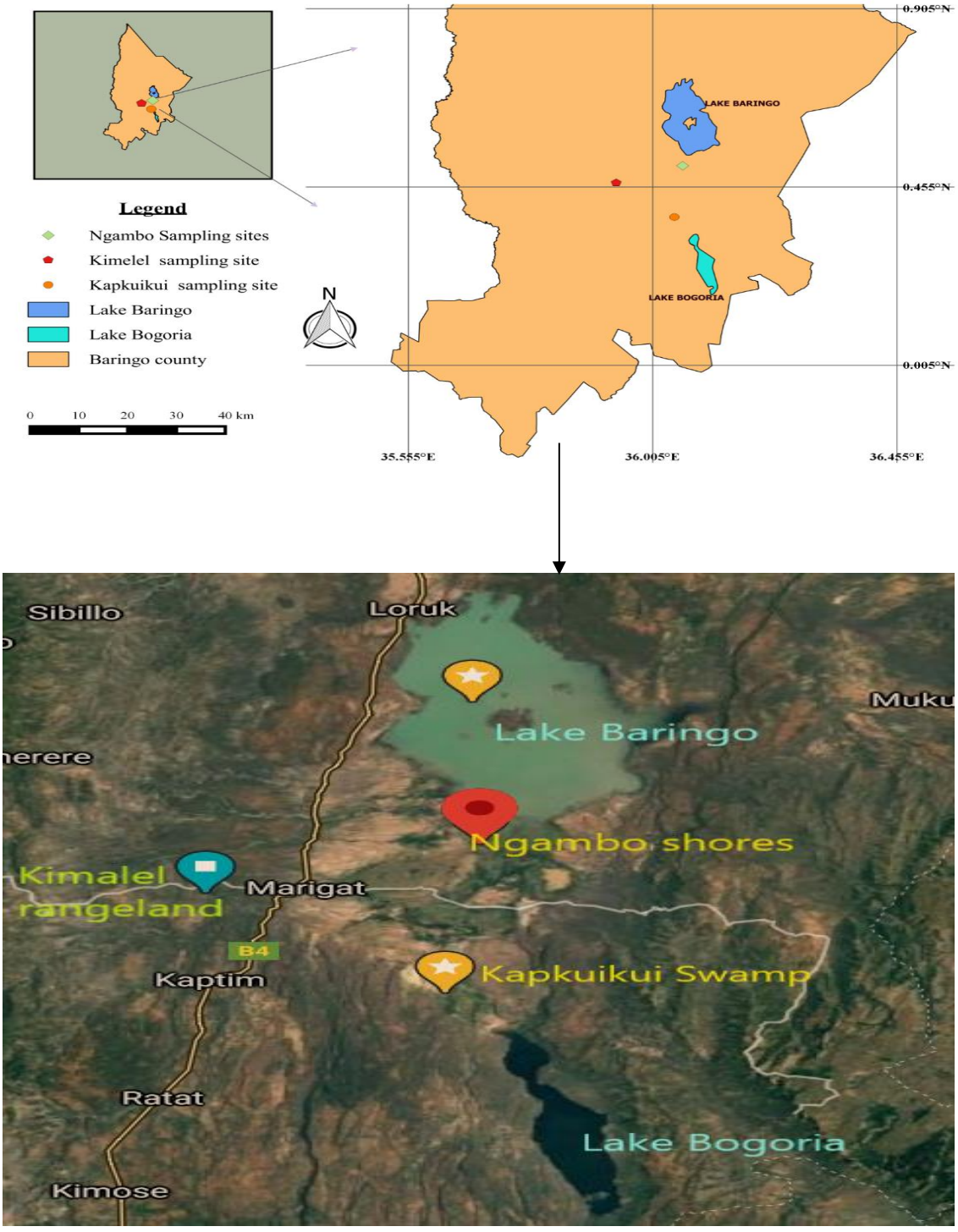

Figure.3.1: Location of study sites from Lake Baringo basin (flooding lake shoreline at Ngambo; swamp marshy at Kapkuikui and dry rangeland ecological habitats at Kimelel. Modified from Google Map)

3.1.3 Fauna and flora characteristics in the study site

The Lake Baringo basin has a diverse fauna and flora community, including diverse fish, birds and mammals. Because of its rich biodiversity, the lake was declared a Ramsar site in 2002, an indicator of its international importance as a waterfowl site (Omondi *et al*., 2016). Over 470 species of birds have been identified in the basin. Lake Baringo is particularly famous for rare raptors such as martial eagle, bat hawk, crown cranes, flamingos and storks such as marabou stocks (Bennun and Njoroge 2000; Omondi *et al*., 2013).

Being a fresh water lake, Lake Baringo harbour a number of fish species including *Aplocheilichthys spp, Barbus intermedius, B. lineomaculatus, Clarias gariepinus, Labeo cylindricus, Oreochromis niloticus* and *Protopterus aethiopicus* (Omondi *et al*., 2013). These fish species support a vibrant fishery where fish are marketed both locally and to urban centres such as Nairobi and Nakuru. Together with fish, other important animals include the hippopotamus (*Hippopoptamus amphibious*) and crocodiles (*Crocodylus niloticus*) which attracts a large number of tourists (Odada, Onyando, and Obudho, 2006). The lake basin harbour a number of swamps around it, which are mainly dominated by emergent water plants and macrophyte species such as *Cyperus papyrus*, *Phragmites* and other floating and rooted aquatic macrophytes (Terer *et al*., 2012). The basin is also covered by extensive invasive species, *Prosopis juliflora* which offer cool shades and moist soils (Lelenguyah *et al.*, 2016).

3.1.4 Land use systems in the study area

The L. Baringo basin is the home of Perkerra Irrigation Scheme, which is located 4-5 km west of Marigat town. Several water canals feed the irrigation farms. Other main irrigation land is at Kapkuikui located in Loboi Swamp where diverse crops such as maize, fruits and vegetables are grown in the area for local consumption and sale to the urban centres such as Nakuru.

Livestock farming is practiced within all homesteads with the main breeds of animals being indigenous cattle, goats, sheep and poultry. A few donkeys are kept for transportation of households and farm produce. The animals are left to free graze in the fields and in common grazing grounds and herded back to homesteads in the evenings. Beekeeping is also practiced with most of hives located in the forest and in areas with shrubs and acacia trees. Fishing is practiced by a few farmers who live along the lake.

3.1.5 Human and animal health

The common diseases in the basin are malaria, leishmaniasis, tuberculosis, and helminthiasis for humans. Livestock diseases in the area include Foot and Mouth Diseases, Peste des Petits Ruminants (PPR), East Coast Fever (ECF), Anaplamosis and trypanosomiasis. Both humans and livestock, however also suffer from zoonotic diseases such as RVF, WNF, and Chikungunya. These diseases are occasional occurring when environment is favourable for their vectors. Most of this zoonotic diseases are caused by viruses (Tigoi *et al*., 2015). According to the Public Health Department in Marigat, there are increased cases of persistent unexplained fever at

times manifesting itself like influenza virus infection, a typical cause of viral infections in humans and death of livestock.

A number of entomological surveys along the Lake basin area have shown circulation of arboviruses of significant public health interest (Ofula *et al.*, 2016). Most of this arboviral diseases are transmitted by mosquitoes whose composition, diversity and abundance are influenced by various factors including environmental and climatic changes. In 2006/2007, new cases of Rift Valley virus were reported in the L. Baringo basin affecting both humans and livestock for the first time after heavy Elnino rains (Nguku *et al*., 2010).

3.2 Selection of study sites

Three study sites were selected to represent three habitats occurring in the Lake Baringo basin; flooded lake shoreline, swamp marshy and dry rangeland habitats (Figure 3.1). The flooded lake shoreline included several villages with Ngambo (Site 1) selected to represent the rest because of its accessibility. It lies at 0°30'30.6"N and 36°03'36.7"E, and an altitude of 995 metres above sea level and is used for crop and livestock farming. The area was flooded in 2010-2013 with lake water rising from the Lake Baringo and overflowing over to farms used for intensive cultivation of farm crops and human settlements (Plate 3.1; 3.2)., Macrophytes, water vegetables and *Prosopis juliflora* were the dominant weeds covering large tracks of land and water surface (Plate 3.1). These are said to offer conducive habitat for mosquitoes (Mwangi and Swallow, 2005).

Plate 3.1: Flooded lake shoreline habitat of the Lake Baringo following extreme flooding. Houses previously stood on the flooded site.

Plate 3.2: Floating macrophytes following extreme flooding in the lake shoreline ecological habitat.

The swamp marshy wetland is located at Kapkuikui (Site 2) between Lake Baringo and Lake Bogoria and within Loboi wetland. It lies at an altitude of 1004 metres above sea level, 0°22'44.6"N and 36°02'42.1"E. The swamp forms an extensive soggy ground that is fed by springs and River Loboi. The main features of the swamp are extensive growth of emergent macrophytes dominated by *Cyperus papyrus* and *Typha domingenis* (Terer *et al*., 2012). It is a fragile ecosystem which is easily destabilized by both natural and anthropogenic activities (Odada *et al*., 2006). The area is extensively used as a communal grazing area for livestock (Plate 3.3a, b). In this habitat, livestock graze alongside wildlife such as Zebras, Ostriches, and different species of antelopes.

Plate 3.3a, b: Different **t**rapping sites from **s**wamp marshy habitat (Kapkuikui) from Lake Baringo basin located within Loboi wetland.

The dry rangeland habitat is located in Kimelel (Site 3) at latitude 0°27'57.2"N, longitude 35°56'15.7"E and altitude 1172 meters above sea level. There is a manmade dam for watering livestock during the rainy season but dries up during the dry season. The habitat is characterized by scattered acacia species, grass and sparse shrubs (Plate

34 a, b). Unlike the other habitats around Lake Baringo, only a few *P. juliflora* grow in the area. This habitat is separated from the other two by a sharp escarpment.

Plate 3.4 a, b: Dry rangeland habitat of Lake Baringo basin with scattered shrubs and water pan where mosquitoes breed and serve as a watering point for livestock.

3.3 Research design

This was a longitudinal, descriptive and quantitative analysis research covering 12 months from October 2012 - October 2013. Mosquitoes data on population and composition in terms of genera was collected from three stratified habitats; flooded lake shoreline, swamp marshy area and dry rangeland. The habitats were further stratified into homesteads and communal open grazing areas microhabitats. Livestock were randomly identified for the study with those showing signs of illness purposively selected for arboviruses screening. Arboviruses were screened from livestock blood samples. Blood samples were collected from cattle, sheep, goats and poultry after every two months coming to a total of six (6) sampling months from October 2012 – October 2013.

3.4 Sampling of mosquitoes for abundance, composition and diversity

3.4.1 Sampling of mosquitoes from habitats

Sampling of mosquitoes was carried out at both night and daytime. Mosquitoes were trapped using 18 CDC light traps in three ecological zones; swamp marshy, flooded lake shoreline and dry rangeland habitats. Two (2) CDC light traps were hanged at a vantage point on a tree in the field at daytime, two (2) at night and two (2) more within homesteads in the night. The trapping sites were separated from each other by a distance of approximately 300 to 500 metres. The GPS coordinates were then taken. Trapping in daytime was from 3pm to 6am, with CDC traps baited with iced carbon dioxide in an insulated container inorder to enhance attraction of mosquitoes in the field at night (Plate 3.5). The iced CO_2 was allowed to diffuse out and dispense into the air slowly inorder to attract the mosquitoes. At night, trapping was done from 6pm to 6am and dry iced CO_2 and illuminating light from CDC traps used to attract mosquitoes. After the trapping period, the mosquito cages were labelled with a unique identification barcode before transporting to the field laboratory for chilling and sorting into different genera and sex.

Plate 3.5: CDC light trap accompanied with iced CO_2 to imitate CO_2 from livestock intended to enhance attraction of mosquitoes in the field at night.

3.4.2 Sampling of mosquitoes around homesteads

Two homesteads were randomly selected from each habitat and sampled at night from 6pm to 6am. Traps were laid at the animal sheds and poultry nests where livestock were herded together and enclosed for a night. The CDC mosquito traps, each with a barcoded cage, were placed on a vantage point at approximately one (1) metre above the ground with bulb illuminating throughout the night. The barcoded cages were removed in the morning with the trapped insects chilled in a minus 20 degree centigrade (-20^0C) freezer for thirty minutes before identification.

3.4.3 Sorting and identification of mosquitoes in the field

The laboratory benches were disinfected with absolute alcohol before commencement of the bench work with samples handled using powder free nitrile gloves. All chilled insect samples in the cage were placed on a white background surface where mosquitoes were separated from non-mosquitoes (Plate 3.6a). The mosquitoes were then sorted into different sexes and genus, based on morphological identification features as outlined by Edwards, (1941), Rueda (2004) and Walter Reed Biosystematics Unit, (*http://www.wrbu.org/aors/africom_Keys.html*). Females of the same genus were counted and put together into pools of 50 or less. All sorted mosquitoes were stored in nitrogen cylinders or in carriers with iced CO_2 awaiting extraction of the nucleic acid components. The data of genus, sexes and GPS coordinates was entered into a Microsoft Excel® spreadsheet (Plate 3.6b).

Plate 3.6a, b: Sorting, identification and pooling of mosquitoes trapped from a site in the field into sexes and genus. The pooled mosquitoes were later barcoded before stored for further analysis in laboratory.

3.4.4 Extraction of total nucleic acid from mosquitoes

Total nucleic acid (DNA and RNA) was extracted from the pooled mosquitoes using a MagNA Isolation kit and protocol (Roche Diagnostics GmbH, Mannheim Germany). During the extraction process, previously sorted mosquitoes stored in nitrogen cylinders in the laboratory were placed in an Eppendorf tube and crashed into a paste before transferring into another 1.5 ml Eppendorf tube containing silica magnetic beads. A volume of 500*u*l MagNA Lysis buffer was added to bind nucleic acid and inactivate the enzymes. The sample was then centrifuged at a speed of 7000 revolutions per minute for 60 seconds. After centrifuging, the lysate samples were placed in a sample cartridge and purified using automated MagNA LC Instrument (Roche Diagnostics GmbH, Mannheim Germany) resulting in a purified nucleic total RNA. The extracted purified nucleic acid was quantified using a Nanodrop® (Thermo Fisher Scientific, USA) to obtain its optical density and then stored in a freezer at minus -80^{0}C for subsequent processing of mRNA and cDNA.

3.4.5 Synthesis of copies of arboviruses DNA (cDNA)

RNA from arboviruses degrades at room temperature. The RNA was therefore required to be converted into a stable product which involved several processes for the synthesis of First Strand and Second Strand cDNA using Klenow polymerase enzymatic reaction. The first step was to convert stored frozen total nucleic acid to total mRNA. This was then purified using oligo dT beads and transcribed into cDNA using the protocol prescribed in the TruSeq® RNA Preparation Guide v2 (Illumina).

Total RNA was diluted with RNA nuclease-free water to 50 *u*l. It was then incubated in a preheated thermo cycler heat block at 65^{0}C for 5 minutes. The heated product was

then purified using protocol for purification of poly A+ mRNA with the resultant end product been purified mRNA. The First Strand cDNA was synthesized using Superscript™ Kit II (Invitrogen Technologies). A volume of 11.1 *u*l mRNA end product was mixed with one (1) μl of Oligo (dt) primer using Superscript™ II Kit (Invitrogen Life Technologies, California). A volume of one (1) *u*l Superscript™ II was added to the sample to catalyse the synthesis processes. It was then incubated in a thermal cycler at 25°C for 10 minutes; 42°C for 50 minutes; 70°C for 15 minutes and left to cool at 4°C, resulting in synthesized First Stand cDNA that was then stored in a freezer at -20^0C.

The Second Strand cDNA was synthesized from stored First Strand cDNA using AMPure XP beads as described in QIAquick PCR Purification™ Kit (Qiagen, Hilden, Germany). This process uses unbound strand of mRNA from First Strand cDNA and random primers provided in the PCR purification kit to synthesize a replacement strand to generate a double-stranded cDNA (Illumina®, 2014) (Plate 3.7).

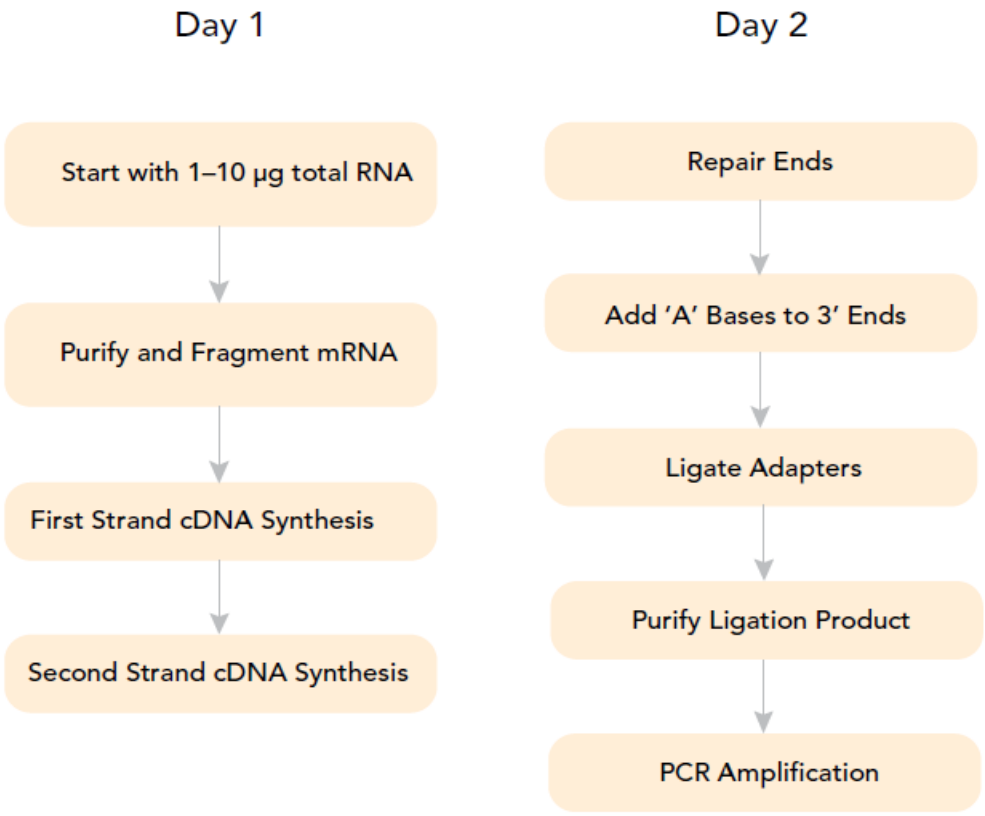

Plate 3.7: Workflow for preparation of DNA libraries for sequencing using illumina MiSeq sequencers system to enable comparison of DNA (Source Illumina TruSeq® RNA Guide v2, 2014)

3.4.6 Amplification and quantification of ds-Strand DNA

Amplification of synthesized ds-strand was carried out using MinElute PCR Purification Kit (QIAGEN, Germany) and enriched using PCR. It was incubated for 30 seconds at 98°C; followed by 15 cycles each of 10 seconds at 98°C, 30 seconds at 65°C, 30 seconds at 72°C; followed by another incubation for 5 minutes at 72°C; and cooling at 4°C. A PCR Primer Cocktail (PPC) supplied in TruSeq RNA Sample Prep v2 kit (Illumina), was used for annealing the ends of adaptors in accordance to manufacturers protocol. The end product, enriched DNA libraries to be used in next generation sequencing, was validated using Qubit 2.0 Fluorescent and Assay Kit (Life

Technologies, Thermo Fisher Scientific Inc. USA) and stored at minus -20^{0}C in readiness for sequencing.

3.4.7 Sequencing of quantified DNA

The DNA libraries stored at -20^{0}C were thawed and prepared in readiness for Next Generation Sequencing (NGS). The thawed DNA libraries were loaded into Illumina ® MiSeq® system instrument operating on a MiSeq Reporter software® (MiSeq Sequencing System Guide, 2017). The automation process was left to run for 12 hours. The system was able to sequence DNA libraries into thousands of mosquito and pathogen DNA/RNA sequence reads. The software then classified mosquito DNA and pathogens DNA/RNA sequence reads into several taxonomic levels: kingdom, phylum, class, order, family, genus, and species based on the Greengenes database (http://greengenes.lbl.gov/). Many studies have used Next Generation sequencing, a recent advanced molecular tool, to analyse the pathogens isolated from animals with one such a study by Michuki (2016) following mass deaths of Laughing Doves (*Streptopelia senegalensis*) in different localities throughout Kenya. In another study, Lwande *et al.,* 2013 used next generation sequencing to identify arboviruses from tick and mosquito from samples collected from livestock and wild animals in Ijara District in Kenya.

The fragment (reads) were further aligned with the nucleotide sequences and subjected to Basic Local Alignment Search Tool (BLAST) that compared data against National Centre for Biotechnology Information (NCBI) and Vectorbase of Bioinformatics Resource for Invertebrate Vectors of Human Pathogens, both for the

significance matches and evolutionary relationships (*https://www.ncbi.nlm.nih.gov/; https://www.vectorbase.org/*).

3.5 Identification and characterisation of arboviruses

3.5.1 Livestock sample size

A combined sample size of the cattle, sheep and goats that was screened for the diseases and for epidemiological data collection was calculated using the formula:

Sample Size n = (Z-score) ² x p (1-p) ÷ (margin of error 'q')² (Thrusfield, 2005)

where: n = sample size

Z= mean score, 1.96, at 95% Confidence Level

Anticipated prevalence from presumed target population, p= 0.187 (Bird *et al*., 2009).

Margin of error, q= 10% or 0.1

To calculate for sample size

n= $(1.96)^2$ * 0.187(1-0.187) ÷ $(0.1)^2$ = 58 total number of all species of livestock

The presumed prevalence (0.187), adopted in this study was from the findings by Bird *et al.,* 2009, which was the most recent prevalence of RVF infections for Lake Baringo basin during the Elnino rains that caused floods and outbreak of RVF in 2006/2007. In order to accommodate migrating livestock and those being translocated to other areas because of the floods, a 10% margin of error was used. Further, the selection of livestock and poultry used in the research was purposive. It targeted those which were suspected to be unhealthy or ill after a visual assessment.

3.5.2 Sampling of blood from livestock

Laboratory investigations of the arboviruses from cattle, sheep, goats and chicken was carried out on the whole blood that was drawn from the ear vein. In case of indigenous poultry, blood was drawn from wing vein. Vacutainer tubes coated with EDTA were used in both cases. The exercise was carried out once after every two months coming to a total of six (6) months. The ETDA coated tubes with blood were bar-coded, placed in a cool box with ice and transported to the field laboratory where it was aliquoted into 2ml cryovials. The numbered aliquots were carried to laboratory in a container packed with iced CO_2 where they were stored and preserved in biorepository nitrogen liquid tanks.

3.5.3 Extraction of RNA from blood samples of livestock

The blood samples were screened for arboviruses at International Livestock Research Institute (ILRI) in Nairobi, Kenya. Blood samples were thawed and kept inside iced container. In an RNase-free tube, a volume of 200 μl sample whole blood was added to 60 ul of red blood cell lysis buffer. The contents were incubated for 5 minutes at room temperature and centrifuged for one (1) minute to obtain serum for laboratory testing using QIAamp™ viral RNA extraction kit (Qiagen, Inc., Valencia, California, USA). The products were pre-filtered, isolated, purified and concentrated into a total RNA end product. The extracted RNA was then eluted with $\geq$6 μl of RNase-free water.

3.5.4 Synthesis of cDNA from livestock using standard RT-PCR assay

The First Strand DNA was synthesized using Superscript II Kit from Life Technologies, California and in accordance to instructions from the manufacturer.

Briefly, one (1) *u*l of Oligo (dT) 12-18 (500μg/mL) primer was added to 4*u*l of sample into a nuclease-free microcentrifuge tube. An additional one (1) *u*l of Deoxynucleotide Triphosphate (dNTP) was added to the mix in the tube and sealed with aluminium foil before incubating at 65^0C for 5 minutes. A new tube with water was used as a negative control. From the Superscript™ II kit, 4 μl First-Strand buffer, 2 μl of 0.1 M Dithiothreitol (DTT) and 1μl RNase were thawed, mixed and added into the total nucleic acid mixture. The resultant mixture was centrifuged gently before incubating at 25^0C for 2 minutes. A 1μl of Superscript™ II Reverse Transcript (RT) enzyme was added to the resultant mixture and mixed by pipetting gently, inactivated by heating at 70^0C for 15 min to form cDNA that was then stored at -20^0C for further use.

3.5.5 Preparation of nested PCR products

A nested PCR amplification process (first and second PCR), was used in this study inorder to increase sensitivity of target sequence detection (Ondiba *et al*., 2019). Preparation of the PCR product involved use of gene specific primers for RVF, WNF, and FLAVI adopted from (OIE, 2016). The forward and reverse primers used were, WNF Forward primer ‘5GGG-CCT-TCT-GGT-CGT-GTT-C 3’ and Reverse ‘5GAT-CTT-GGC-YGT-CCA-CCT-C3’; FLAVI sequence Forward primer and Reverse, 5'AAGCCGGAATAACGTGTGAC3' and 5'AAGTTTGAACCCACCGTCAGG3'. The forward and reverse RVF primers were NS3a (5’-ATG-CTG-GGA-AGT-GAT-GAG-CG-3’) and NS2g (5’-GAT-TTG-CAG-AGT-GGT-CGT-C-3’) respectively (OIE, 2016)

Table 3.1: Sequences of primers used for nested PCR sourced from Inqaba Biotech South Africa

Virus target	PCR stage	Primer
Rift Valley Fever virus	First stage	Forward NSca (5-'CCT-TAA-CCT-CTA-ATC-AAC-3')
		Reverse: NSng (5'-TA-TCATGG-ATT-ACT-TTC-C-3')
	Second stage	Forward: NS3a (5'-ATG-CTG-GGA-AGT-GAT-GAG-CG-3')
		Reverse: NS2g (5'-GAT-TTG-CAG-AGT-GGT-CGT-C-3')
West Nile Fever virus	First stage	Forward 1401: 5'-ACC-AAC-TAC-TGT-GGA-GTC-3'
		Reverse 1845R: 5'-TTC-CAT-CTT-CAC-TCT-ACA-CT-3'
	Second stage	Forward 1485F: 5'-GCC-TTC-ATA-CAC-ACT-AAA-G-3'
		Reverse 1732R: 5'-CCA-ATG-CTA-TCA-CAG-ACT-3'
Flavivirus	First stage	Forward: 5' GAG GCT GGG GAA ATG GCT G 3'
		Reverse: 5' CCT CCA ACT GAT CCA AAG TCC CA 3'
	Second stage	Forward: 5'AAG-CCG-GAA-TAA-CGT-GTG-AC3'
		Reverse: 5'AAGTTTGAACCCACCGTCAGG3'

The first-round PCR was performed according to instructions provided in the Superscript^{R} III kit from manufacturer. Briefly, to prepare 50 *u*l of PCR elute, a volume of 5 *u*l of 10x PCR enzyme buffer, 5 *u*l 25 mM $MgCl_2$, 5 *u*l 10 mM dNTP, 2 *u*l of each primer pair of the gene specific (inner and outer RVF, WNF, FLAVI), 5 *u*l Taq DNA polymerase were pippeted separately into a single PCR tube containing 23 *u*l of sterile water. A volume of 5 *u*l of cDNA (DNA template) was added then amplified at 50^0C for 30mins; followed by pre-denaturation at 95^0C for 1 min; annealing at 56^0C for 30 seconds each and lastly with an extension at 72^0C for 10 mins. The first PCR product was then stored under -20^0C until further use (Lorenz, 2012).

To prepare second PCR product, a volume of 1*u*l each of primers for RVF, Flavivirus, WNV; 1*u*l of dNTP at concentration 10 mM; 7.3 *u*l of 10X PCR buffer; 8 *u*l $MgCl_2$ at 25mM; and 1.0 *u*l of Taq DNA polymerase with the 26.3 *u*l of RNAse were added into 2*u*l of first PCR product. The mixer was then incubated in the thermocycler at 95^0C for 2 minutes, then subjected to 30 cycles at 95^0C for 1 minute, 55^0C for 30 seconds and 72^0C for 45 seconds with the final thermal incubation at 72^0C for 10 minutes. The final product, nested PCR products, were coded and stored in -20^0C freezer. The PCR products were later packed and shipped to Macrogen Clinical Laboratories Inc., USA, for DNA sequencing. The sample sequence and visual representation of chromatogram using Applied Biosystems ABI PRISM 7700 Sequence Detection System® (Applied Biosystems, Inc), to produce AB1 file format, a binary files format for storing genetic data, was generated.

3.5.6 Analysis to identify arboviruses and mutant genes

The edited gene sequences were aligned with referenced sequences using CLUSTALW©, a DNA multiple sequence alignment programme, in order to identify the arboviruses and mutations. First the Basic Local Alignment Search Tool (BLAST) program was used to search for nucleotide with similarity sequence. The generated files were saved as Fast All alignment file format (multi-FASTA file format) and translated into proteins base sequences using Molecular Evolutionary Genetics Analysis software 6 (MEGA) before subjecting them to genomic trimming (NCBI, *https://www.ncbi.nlm.nih.gov/*). A start and stop codons were identified in order to make sure that no stop codons were introduced during trimming. Second, a protein sequences were then subjected to protein BLAST to identify the nature of the protein that would lead to organism identification and phylogenetic analysis. The taxonomic

information of the species was retrieved from Uniprot KB Database of UniProt Consortium©, 2017 (UniProt, *https://www.uniprot.org/*). The database provide a central hub of functional information on proteins, with accurate, consistent and rich annotation mainly the amino acid sequence, protein name or description, taxonomic data and citation information. Subsequently, the data on disease pathogenesis was evaluated using protein-protein interactions from programme (Virus Pathogen Database and Analysis Resource (ViPR), *www.viprbrc.org).* Screenshots of the variant gene sequence and position against the consensus sequence was taken.

3.5.7 Phylogenetic evolutionary analysis of the arboviruses

The procedure and protocol used in drawing and estimating the phylogeny tree was as detailed by Hall (2013). First, the homologous DNA sequences were downloaded into a MEGA 5 software and used the appropriate computer instructions. Second, the alignment of sequences in a FASTA file format was done by aligning the DNA codons using CLUSTAW, with the method to give an estimate of the positions of historical insertions and deletions. Using the saved mega file, the CLUSTAW programme was used to compute and draw phylogeny tree. The Maximum Likelihood [ML]) statistical method of analysis was used to estimate the tree. The reliability of a phylogenetic tree was computed using the **bootstrap** method (Hall, 2013) (Tamura *et al.*, 2011). The nodes whose bootstrap value was >70% were considered to be more reliable.

3.7 Data Analysis

3.7.1 Statistical analysis of mosquitos' abundance, composition, diversity

The data analysis involved use of various statistical formulas and software. The descriptive statistics was used to express an overall morphological characteristics of

mosquitoes and population of different genus that were trapped. A one way ANOVA was used to compare the means of the mosquitoes catches from the three habitats where H_0: $u_1=u_2=u_3$ (Null hypothesis: there was no difference in means of the mosquitoes catches from the three habitats), while the alternate hypothesis was H_0: $u_1 \# u_k$ (u_1, u_2, and u_3 were means of the three habitats) with *F* statistic test (an assessment for homogeneity of variance) performed with an assumption of homogeneity of equal variance to the study habitats (Singh, 2018). The mosquitos' data was transformed using log10(x+1).

The dominance and diversity of the mosquitos' genera was calculated using Simpsons and Shannon Weaner Indices of Diversity. This was calculated using Simpson's Index of Diversity for mosquito communities in the flooding lake shoreline, swamp marshy and dry rangeland ecological habitats where:

Simpson Diversity Index (D) = 1 - ∑n (n-1) ÷ N (N-1) (Simpson, 1949; Oguoma

and Ikpeze 2008)

∑ = sum (total)

n = the number of individuals of each different species

Shannon Weaner Index of Diversity was calculated using (H'= − p ∑ i ln pi) and expressed as Shannon Equitability (*H'/lnN*).

The calculated Simpson Diversity Indices were then used for pairwise comparison of habitats using Tukey HSD at P=0.05. The use of Simpson's index in diversity analysis was because of its robustness to take into account the number of species richness in a

habitat, and the relative abundance, that is, evenness where species evenness ranges from zero to one, with zero signifying no evenness and one, a complete evenness.

3.7.2 Arboviruses prevalence and variations

The percentage or the number of sample cases that were infected with arboviruses of medical interest in a herd was expressed as a period prevalence measure to show the potential risk (n/N). The significance differences in arbovirus infection for livestock resident in three ecological habitats was calculated using ANOVA and paired comparison between two habitats at a time using t-test with rejection level set at $P<0.05$. Statistical analysis to determine significance differences of infections in each habitat was calculated using ANOVA.

The resultant nucleotide sequences were aligned against those in the National Center for Biotechnology Information (NBCI) sequence databases (U.S. National Library of Medicine 8600 8600 Rockville Pike, Bethesda MD, 20894, USA) in order to compare the regions of similarity. Phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA 6) software (Tamura *et al.,* 2011; Kumar, Tamura, and Nei 1994; Tamura *et al*., 2011) with a Poisson correction model (Tamura *et al.,* 2011) was used to Estimates of evolutionary divergence between the sequences.

CHAPTER 4: RESULTS

4.1 Spatial-temporal changes in mosquito dynamics and diversity

4.1.1 Spatial-temporal variation in mosquito abundance and composition

During the 12 months sampling period, from November 2012 –November 2013, a total of 386,624 individual mosquitoes were captured all belonging to two families, Anopheline and Culicinae. Anopheline comprised of only one genus, *Anopheles* while Culicinae comprised of 10 genera: *Mansonia, Coquillettidia, Culex, Ficalbia, Aedeomyia, Aedes, Theobaldia, Uranotaenia, Orthopodomyia* and *Hodgesia* making a total of 11 genera occurring in the area.

Of the 11 mosquitoes genera, four (4), *Anopheles, Aedes, Culex* and *Mansonia,* occurred in all three habitats but in different proportions. However five (5) genera occurred only in the flooded shoreline and swamp marshy areas, probably due to the habitats wetted characteristics. *Orthopodomyia* and *Hodgesia* which were very rare genera were only captured in the flooded shoreline and swamp marshy area respectively and in very few numbers (Table 4.1).

Table 4.1: Occurrence of mosquitoes in different habitats of Lake Baringo basin, Kenya during a period of unprecedented extreme flooding, 2012-2013

Family	Genera	Flooded shoreline	Swamp marshy	Dry rangeland habitat
Anopheline	*Anopheles*	✓	✓	✓
Culicinae	*Aedes*	✓	✓	✓
	Mansonia	✓	✓	✓
	Culex	✓	✓	✓
	Coquillettidia	✓	✓	
	Uranotaenia	✓	✓	
	Aedeomyia	✓	✓	
	Ficalbia	✓	✓	
	Theobaldia	✓	✓	
	Orthopodomyia	✓		
	Hodgesia		✓	

From the 10 genera of family Culicinae, eight (8) species were positively identified morphologically while 14 were unidentified (Table 4.2). The genus *Aedes* had the highest number of species with three (3) confirmed species and four (4) unidentified species distributed across the three habitats. *Culex* was similarly diverse with three (3) confirmed species and four (3) unidentified species. *Mansonia* had only three (3) species while the other genera were each represented by one species.

Table 4.2: List of mosquito species identified found in Lake Baringo basin, Kenya, during periods of heavy flooding, November 2012- November 2013.

Family	**Genera**	**Species**
Anopheline	Anopheles	*Anopheles gambiae s.s.* complex
		Anopheles arabiensis
Culicinae	Aedes	*Aedes aegypti*
		Aedes lineatopennis
		Aedes albopictus
		*Aedes spp (*unidentified (4)
	Culex	*Culex pipens quinquefasciatus*
		Culex pipiens pipiens
		Culex tritaeniorhynchus
		Culex spp (unidentified (3*)*
	Ficalbia	*Ficalbia* spp (unidentified 1)
	Aedeomyia	*Aedeomyia* spp (unidentified 1)
	Mansonia	*Mansonia uniformis*
		Mansonia africana
	Coquillettidia	*Coquillettidia* spp (unidentified 1)
	Theobaldia	*Theobaldia* spp (unidentified 1)
	Uranotaenia	*Uranotaenia* spp (unidentified 1)
	Orthopodomyia	*Orthodomyia* spp (unidentified 1)
	Hodgesia	*Hodgesia* spp (unidentified 1)

The catches fluctuated over time with high catches registered during the wet season, November to December and March to July. Low mosquito catches occurred during the dry season in month of October to November and August to September. The mean catches of mosquitoes in the three habitats found in Lake Baringo basin was significantly different at $F_{(2,\ 27)} = 3.35$; P=0.04). Similarly the catches were

significantly different between homesteads and communal grazing areas at ($F_{(8, 9)}$ = 3.94; P=0.00065). The flooded lake shoreline had the highest number of individual mosquitoes captured 343,730 (88.9 %) throughout the sampling period. The swamp marshy area harboured 41,734 (10.8 %) of the mosquito population while the dry rangelands harboured only 1,161 (0.3%) (Table 4.3).

The monthly catches of mosquitoes from Oct 2012-Oct 2013 significantly varied at $F_{(11, 12)}$ =2.87; P=0.04). Catches from the microhabitats showed that 192,333 (50%) of the total individual mosquitoes caught were from the homesteads. Of this, 182,375 (47%) were caught at night in the grazing fields and 11,917 (3%) daytime. Further, the total catches of mosquitoes caught in homesteads and grazing area microhabitats were proportionately different to each other. From the flooded lake shoreline the proportion of mosquitoes from homesteads to grazing areas was 1:1. There were more catches in homesteads than from the grazing fields in swamp marshy area at the ratio of 3:2 and as well in dry rangeland ecological habitats at the ratio of 7:3 respectively (Table 4.3).

Table 4.3: Comparison of mosquito species abundance in microhabitats in the three ecological study sites (flooded shoreline, swamp marshy and dry rangeland habitats) of Lake Baringo basin, Kenya, Oct 2012- Oct 2013

Microhabitat	**Flooded shoreline**	**Swamp marshy**	**Dry rangeland**	**Total for microhabitat**
Homestead	167,564	24,010	759	192,333 (50%)
Grazing field night-time	165,816	16,268	291	182,375 (47%)
Grazing field daytime	10,350	1,456	111	11,917 (3%)
Total for habitats	343,730 (88.9 %)	41,734 (10.8 %)	1,161(0.3%)	

4.1.2 Spatial-temporal analysis of individual mosquito genera

The mosquito community of the Lake Baringo basin during the period of unprecedented flooding was dominated by the genus *Mansonia* (family Culicinae), constituting 84.9% of the total number of individuals captured (Table 4.4). *Culex* was the second most abundant genera constituting about 10.6% of the total number of individuals captured while *Anopheles* constituted a small proportion of about 3.0%. Other genera were rare, constituting only 1.6% of the total number of individuals captured.

Table 4.4: Overall percentage composition of mosquito genera in Lake Baringo basin, Kenya, during a period of heavy flooding in November 2012-November 2013

GENERA	No. of individuals	%
Mansonia	328090	84.9
Culex	40911	10.6
Anopheles	11656	3.0
Ficalbia	3761	1.0
Aedeomyia	1351	0.4
Aedes	330	0.1
Coquillettidia	313	0.1
Theobaldia	97	0.0
Uranotaenia	55	0.0
Orthopodomyia	55	0.0
Hodgesia	6	0.0
Total number	**386625**	**100.00**

The flooded shoreline was dominated by *Mansonia* 321,899 (97.8%). *Culex* dominated the swamp marshy mosquito community constituting 26,150 (64.1%) of the total mosquito population and 14,420 (35.4%) in flooded shoreline area. In the dry rangelands, the proportion of all genera was very low, each genera constituting less than 2%. Of the total catches of *Anopheles* mosquitoes, 8,066 (66%) were from swamp marshy habitat, 34,492 (8.2%) from the flooded lake shoreline, 704 (5.8%) from dry rangeland. *Aedes*, which is the primary vector of arboviruses, mainly occurred in swamp marshy habitat constituting 96.9% (Table 4.5). This percentage is critical as this area is one of the common communal areas for grazing livestock hence increasing the transmission chances of arboviruses. Similarly, the highest catches of *Aedeomyia* 1,236 (99.8%) were from the flooded shoreline habitat.

Table 4.5: Mosquito species abundance in three ecological habitats (flooded shoreline, swamp marshy and dry rangeland) of Lake Baringo basin, Kenya, during periods of unprecedented flooding, Oct 2012-Oct 2013

	Mosquito habitat					
Genus	Flooded shoreline	% catches in habitat	Swamp marshy area	% catches in habitat	Dry rangelands	% catches in habitat
Mansonia	321899	97.8%	6473	2.0%	200	0.1%
Culex	14420	35.4%	26150	64.1%	211	0.5%
Ficalbia	3558	94.9%	190	5.1%	0	0.0%
Anopheles	3449	28.2%	8066	66.0%	704	5.8%
Aedeomyia	1236	99.8%	1	0.1%	2	0.2%
Coquillettidia	147	47%	166	53%	0	0
Theobalbia	97	100.0%	0	0.0%	0	0.0%
Uranotaenia	28	36.4%	21	27.3%	28	36.4%
Aedes	25	1.9%	1289	96.9%	16	1.2%
Orthodomyia	2	50.0%	2	50.0%	0	0.0%
Hodgesia	0	0.0%	6	100.0%	0	0.0%

Of the 5 dominant mosquito genera, *Culex*, *Anopheles*, *Mansonia*, *Aedes* and *Aedeomyia*, the highest individual catches from homestead microhabitat were of genus *Culex* at 16,774 (67.9%) and *Anopheles* at 4403 (61.1 %). The highest catch of mosquitoes of genus *Aedes* (416; 92.6%) was in grazing fields' microhabitat while *Hodgesia* were 74 constituting 98.7 %. Highest catch of genus *Mansonia* (5,772) was from the communal grazing fields constituting 71.1% while homestead catch was 2,347 constituting 28.9% (Table 4.6).

Table 4.6: Comparison of mosquito species **i**nfestation (%) in different microhabitats homestead and grazing areas of Lake Baringo basin, Oct 2012-Oct 2013

Genera	Microhabitat			
	Homesteads		Communal grazing fields	
	Individual	% catch	Individual	% catch
Aedes	37	7.4%	461	92.6%
Culex	16774	67.9%	7934	32.1%
Aedeomyia	13	76.5%	4	23.5%
Anopheles	4403	61.1%	2798	38.9%
Mansonia	2347	28.9%	5772	71.1%
Ficalbia	176	92.6%	14	7.4%
Hodgesia	1	1.3%	74	98.7%
Uranotaenia	21	91.3%	2	8.7%
Orthodomyia	1	100.0%	0	0.0%

The population of the genus *Anopheles* fluctuated from month to month and from one habitat to another, suggesting that other factors other than just floods, influenced its abundance, probably the presence of diverse breeding sites and microhabitats (Figure 4.1). In dry rangeland habitat, high catches coincided with wet season and filling up of the water dam within the area in months of March to April and wet cold season in June to July. The infestation by this genus was significantly different in all three habitats during the study period ($F_{(12, 13)}$ =2.60; P=0.038). Further Tukey HSD tests showed that the catches of this genus, *Anopheles*, was significantly different between swamp marshy and dry rangeland habitats but insignificant between wetted two habitats, flooded lake shore and swamp marshy ecological habitats.

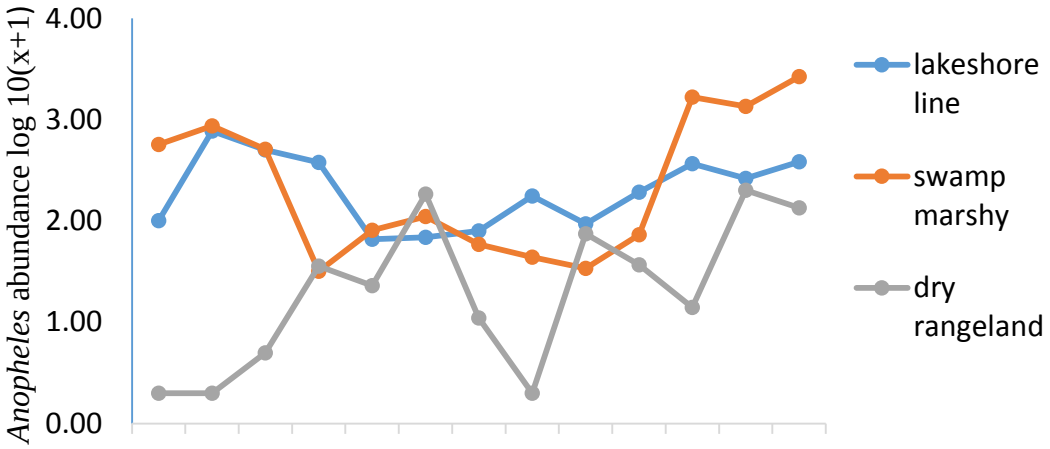

Figure 4.1: Monthly abundance of mosquitoes of genus *Anopheles* in the three habiatats, 2012-2013.

The abundance of the genus *Mansonia* differed significantly between the three sites ($F_{(2, 39)}$ =3.23; P=0.005). The genus inhabited both flooded shoreline and swamp marshy ecological habitats throughout the twelve months study period with the flooded lake shoreline being more infested. In the dry rangeland, the infestation was low with no catches during the dry months of January to March and September to October (Figure 4.2).

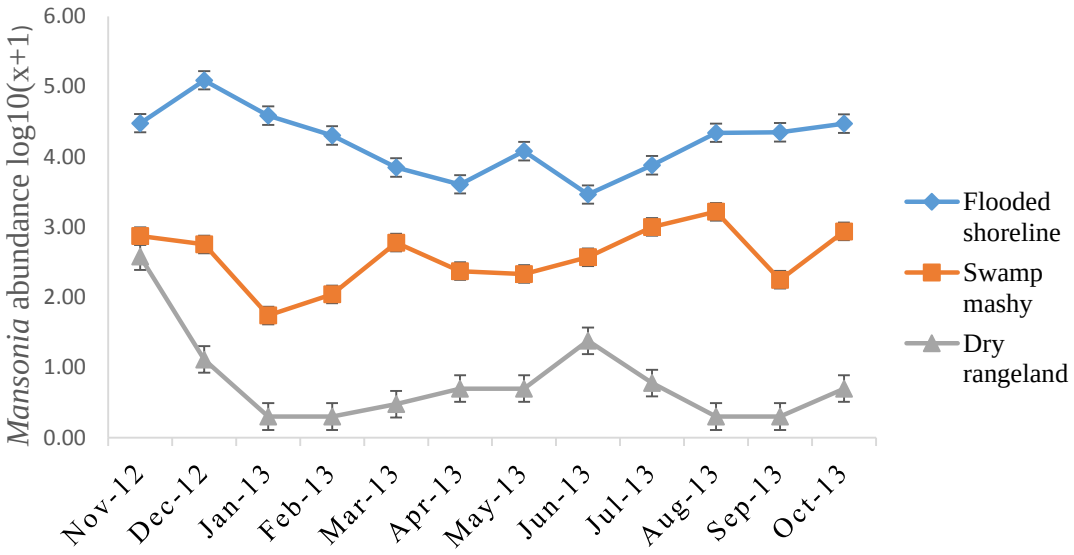

Figure 4.2: Spatial-temporal changes for mosquitoes of genus *Mansonia* from the three ecological habitats of Lake Baringo basin, Kenya Nov 2012-Oct 2013

The genus *Aedes* was absent from flooded shoreline and dry rangeland sites during the dry season from December to March despite the lake shoreline been flooded during that period. In the swamp marshy habitat, the mosquitoes of genus *Aedes* were consistently caught throughout the 12 months study period due to the existing fresh water pools and springs, occasional precipitation and short rains experienced from June to October. This characteristic was unique to swamp marshy area and not to the other habitats (Figure 4.3).There were very few catches of this genus in dry rangeland almost in entire study period apart from the wet rain month of May. The infestation differed significantly between the three differing habitats ($F_{(2, 36)}$ =3.25; P=0.028). Further post hoc Tukey HSD test revealed that the catches of *Aedes* was significantly different between catches from swamp marshy and dry rangeland habitats.

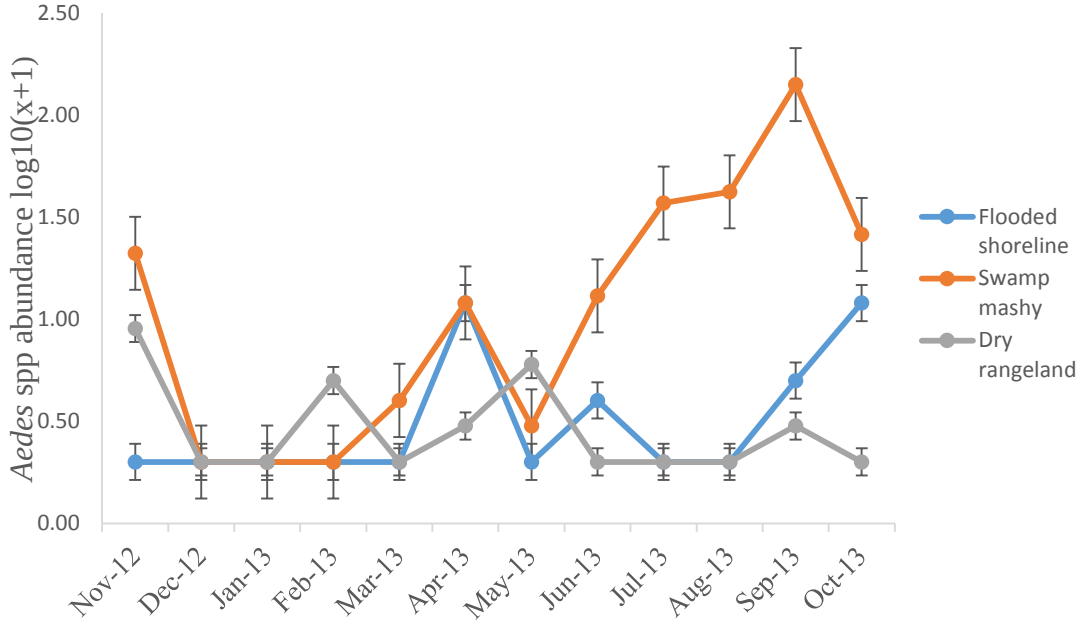

Figure 4.3: Infestation of mosquitoes of genus *Aedes* in the three ecological habitats in Lake Baringo basin, Kenya, Nov 2012-Oct 2013.

The population of the genus *Culex* differed significatly between the three ecological habitats ($F_{(2,36)}$ = 3.25; P=0.028), with the swamp marshy habitat harbouring the highest numbers and the dry rangelands the least. The post Tukey HSD test showed that the catches from these two habitats differed significatly from each other. However the monthly catches during the study period were statistically insignificant. From the two habitats, genus *Culex* was trapped in almost equal propotions throughout the study period, with populations peaking from June to October (Figure 4.4). Except for a small occasional peaks, populations of *Culex* remained relatively low in the dry rangeland habitat.

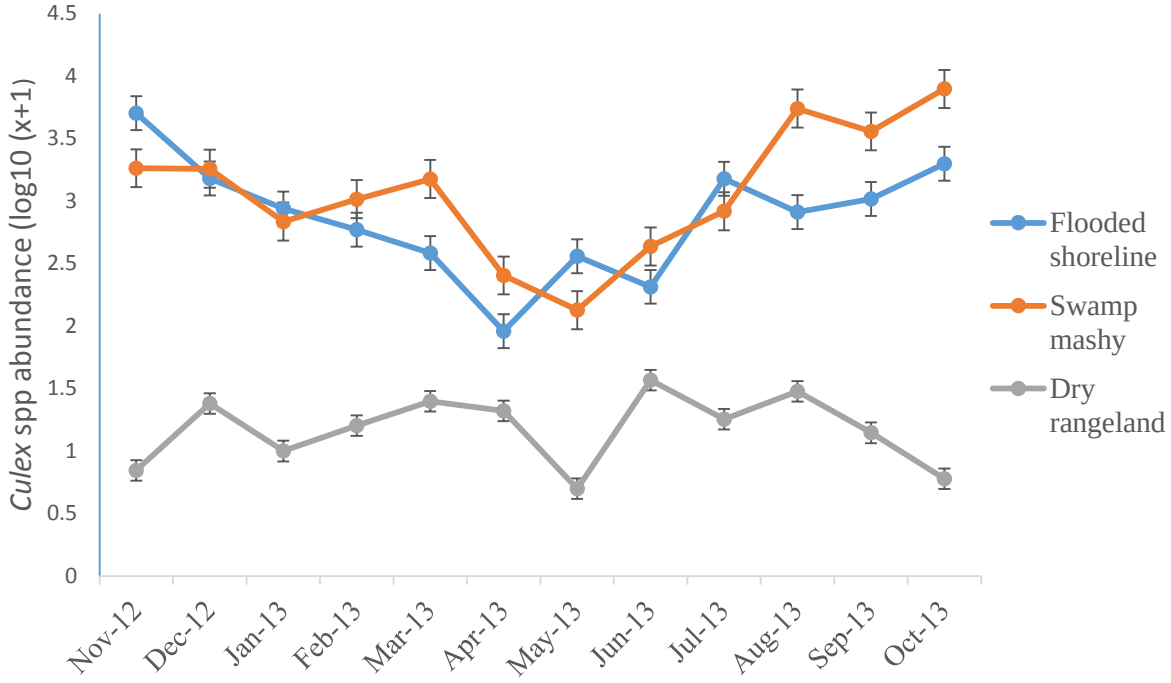

Figure 4.4: Infestation of mosquitoes of genus *Culex* in the three ecological habitats of Lake Baringo basin, Kenya, Nov 2012 Oct 2013

4.1.3 Spatial-temporal diversity of mosquitoes from Lake Baringo basin

Lake Baringo basin has a wide range of habitats exhibiting rich to low diversity of mosquitoes. The diversity for mosquito genera composition was calculated using Simpson Index of Diversity (1-D) and Shannon Equitability Index in the habitats and not species since a number of species were unidentifiable. The Simpson Diversity indices for swamp marshy was 0.56 (Shannon equitability index= 44) and 0.57 (Shannon equitability index=0.45) for dry rangelands indicating evenness of the genera in the two habitats. The flooded shoreline had the lowest Simpson's Index 0.13 (Shannon equitability index =0.13), an indication of an unevenness and dominance by a single genus, in this instance, genus *Mansonia*. However the genera richness for swamp marshy habitat was 10. Similarly the genera richness for the flooded shoreline ecological habitat was equally 10 while for dry rangeland was six (6).

4.2 Arboviruses isolated from mosquitoes and livestock

4.2.1 Arboviruses isolated from the mosquitoes in Lake Baringo basin

A total of 12 arbovirus families were isolated from the mosquitos' caught in the Lake Baringo basin during the period of unprecedented flooding (Table 4.7). The families were composed of 18 species with Flaviviridae and Poxviridae contributing four and three species respectively. Polydnaviridae and Alloherpesviridae contributed two species each while the others contributed one species each. The family of interest to the study was Flaviviridae with the species *Aedes flavivirus* and *Culex flavivirus*. No virus of family Bunyaviridae was isolated from mosquitoes during this period of unprecedented extreme flooding.

Table 4.7: Family and species of viruses found harboured in midgut of mosquitoes from Lake Baringo basin

Arbovirus family	Species
Flaviviridae	*Culex flavivirus, Aedes flavivirus, Hepatitis C virus, Bovine viral diarrhoea virus 1*
Polydnaviridae	*Glypta fumiferanae ichnovirus, Hyposoter fugitivus ichnovirus*
Poxviridae	*Taterapox virus, Cowpox virus, Rabbit fibroma virus*
Baculoviridae	*Culex nigripalpus nucleopolyhedrovirus*
Alloherpesviridae	*Ictalurid herpesvirus 1, Tupaiid herpesvirus*
Microviridae	*Microvirus*
Picornaviridae	*Encephalomyocarditis virus*
Retroviridae	*Human immunodeficiency virus 1*
Totiviridae	*Saccharomyces cerevisiae killer virus M1*
Papillomaviridae	*Alphapapillomavirus*

4.2.2 Arboviruses isolated from livestock blood from Lake Baringo basin

Blood was drawn from 77 animals. Of all animals, 16 (20.8%) tested positive while 61 (79.2%) tested negative to arboviruses suggesting high risk of infections in that an infection in a single animal with a vector-borne disease puts the whole herd at risk. Of the positive livestock species, eight (8) were infected with *Aedes flavivirus* species while another eight (8) had Rift Valley Fever virus in their blood (Table 4.8). The study revealed the presence of *Aedes flavivirus* in livestock for the first time in the region.

Table 4.8: Number of livestock infected in the three ecological habitats of Lake Baringo basin, Kenya, during unprecedented flooding, Nov 2012 - Oct 2013

Species of livestock	**No. tested**	**No. +positive**	**No. -negative**
Caprine	17	4	13
Ovine	16	4	12
Indigenous poultry	22	5	17
Bovine	22	3	19
TOTAL	**77**	**16 (20.8 %)**	**61 (79.2 %)**

4.2.3 Prevalence of arboviruses in livestock

The probability of the target livestock been infected with either *Aedes flavivirus* arbovirus was 10.4%. Similarly the probability of livestock been infected with Rift Valley Fever virus was 10.4%. The probability of getting *Aedes flavivirus* from livestock resident in Lake Baringo basin was highest for indigenous poultry (18%) followed by Caprine (12%). On the other hand, the probability of an infection with

Rift Valley Fever virus was highest in Ovine (23%) followed by Caprine (12%) and Bovine (9%) each (Table 4.9).

Table 4.9: Prevalence of arboviruses in livestock during the period of extreme flooding of Lake Baringo, Nov 2013 - Oct 2014

Pathogen	**Caprine** n=17	**Ovine** n=16	**Avian** n=22	**Bovine** n=22	**Overall** N=77
Aedes flavivirus	2 (R=0.12)	1(R= 0.06)	4 (R= 0.18)	1 (R=0.05)	8 (R=10.4%)
Rift Valley Fever virus	2(R=0.12)	4 (R=0.25)	1 (R=0.05)	2 (R=0.09)	8 (R=10.4%)

R= Risk of infection computed as number infected as a ratio of number of animals sampled.

Livestock grazing in the swamp marshy area had the highest risk of infection with arboviruses followed by flooded shoreline habitats (Figure 4.5). This was corroborated by statistical analysis where the risk of infection with arboviruses was significantly lower in the dry rangeland habitat than in flooded shoreline ($t_{(6)} = 1.94$; P=0.024).

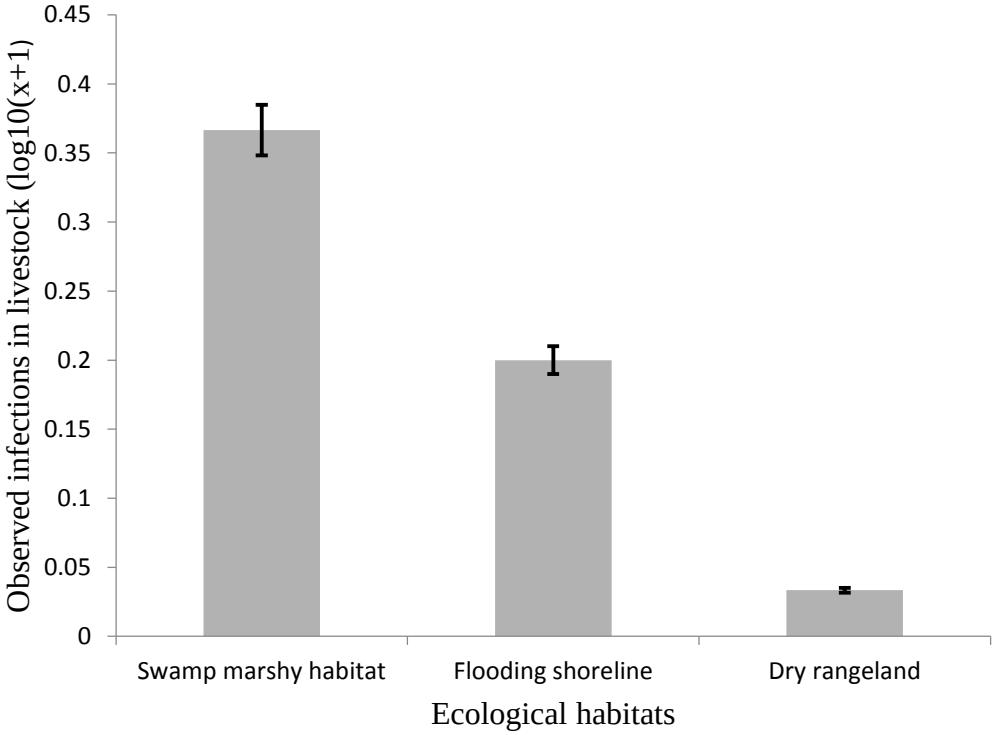

Figure 4.5: Observed infections in livestock with arboviruses from three habitats from Lake Baringo basin during an extreme flooding, Oct 2012-Oct 2013

Livestock from swamp marshy ecological habitat and flooded shoreline had an equal chance of having *Aedes flavivirus* (14%) in their blood while livestock resident in dry rangeland habitats faced just a 4% risk. Avian (indigenous poultry) had a higher chances of been found with nucleic acid of *Aedes flavivirus* (18%) in their blood than other species (Table 4.10).

Table 4.10: Risk of livestock to infections with *Aedes flaviviruses* in three ecological habitats of Lake Baringo basin, Kenya.

***Aedes flavivirus* prevalence**

Livestock species	Swamp marshy		Flooded shoreline		Dry rangelands		Total N
	n	**+ve**	**n**	**+ve**	**n**	**+ve**	**+ve**
Caprine	5	1 (20%)	5	0 (0%)	7	1 (14%)	2 (12%)
Ovine	8	0 (0%)	3	1 (33%)	5	0 (0%)	1 (6%)
Avian	8	2 (25%)	8	2 (25%)	6	0 (0%)	4 (18%)
Bovine	8	1 (13%)	6	0 (0%)	8	0 (0%)	1 (5%)
TOTAL	**29**	**4 (14%)**	**22**	**3 (14%)**	**26**	**1 (4%)**	**n= 8 N=77 (10.4 %)**

The test for RVFV revealed eight (8) positive samples of livestock blood. Six (6) of positive samples for RVFV, representing 75%, were from livestock resident in swamp marshy habitat while the remaining two (2), that is 25%, were from livestock resident in flooded shoreline habitat (Table 4.11). None of the blood samples collected from livestock resident in dry rangeland habitat was positive for Rift Valley Fever virus. The risk of infection was much higher in swamp marshy habitat (21%) as compared to flooded (9.1%) and dry rangeland habitats (4%). On the other hand, Ovine was at higher risk of infection with Rift Valley Fever virus (19%). No livestock was infected with Rift Valley Fever virus in dry rangeland. From the swamp marshy habitat, caprine was more at risk (40%) compared to other species (Table 4.12).

Table 4.11: Livestock species positive for RVF viruses resident in different habitats from Lake Baringo basin

	RVF POSITIVE SAMPLES		
Species	Swamp marshy ecological habitat (Kapkuikui)	Flooded shoreline ecological habitat (Ngambo)	Dry rangeland ecological habitat (Kimelel)
Bovine	RVF52898	RVF53008	-
Caprine	RVF53206	-	-
Caprine	RVF53155	-	-
Ovine	RVF51631	RVF53177	-
Ovine	RVF52890	-	-
Poultry	RVF53082	-	-

Table 4.12: Risk of livestock to infection with Rift Valley Fever virus from three ecological habitats of Lake Baringo basin, Kenya

	RVF prevalence						
	Swamp marshy		Flooded shoreline		Dry rangelands		Total N
Livestock species	n	+ve	n	+ve	n	+ve	+ve
Caprine	5	2 (40%)	n=5	0 (0%)	n=7	0 (0%)	2 (12%)
Ovine	8	2 (25%)	n=3	1 (33%)	n=5	0 (0%)	3 (19%)
Avian	8	1 (13%)	n=8	0 (0%)	n=6	0 (0%)	1 (4.5%)
Bovine	8	1 (13%)	n=6	1 (17%)	n=8	0 (0%)	2 (9%)
							n=8 N=77
	29	**6 (21%)**	**22**	**2 (9.1%)**	**26**	**0(0%)**	**(10.4%)**

4.3 Characterisation of arboviruses in livestock

4.3.1 Characterisation of *Aedes flavivirus*

The BLASTN results to characterize the fragments of *Aedes flavivirus* isolated from livestock blood samples revealed similarity of the flavivirus polyprotein in the NS3-h region of the gene. It further revealed that all eight (8) *Aedes flavivirus* isolates from Lake Baringo basin (A51674, A52801, A52805, A52833, A52871, A52945, A52977 and A53170) were related. They had 98-100% similarly to NCBI nucleotide accession number AB488411 (*Aedes flavivirus*, strain: Narita-24.) (Table 4.13).

Table: 4.13: Analysis of *Aedes flavivirus* subspecies pathogen isolated from blood of livestock referenced to sequence accession ID AB488411.1, NC012932.1, AB488423.1

Code	Species	Habitat	strain	Variation/ mutations of nucleotide bases to referenced NCBI sequence
A51674	Caprine	Swamp marshy	Narita 24	polyprotein (NS3-h region) 99% similarity, 1% dissimilarity
A52801	Ovine	Flooded lake shores	Narita 24	Sample sequence: 100% similarity, 0% dissimilarity
A52805	Indigenous poultry	Flooded lake shores	Narita 24	Sample sequence: 98% similarity, 2% dissimilarity
A52833	Bovine	Swampy-marshy	Narita 24	Sample sequence: 99% similarity, 1% dissimilarity
A52871	Indigenous poultry	Swampy-marshy	Narita 24	Sample sequence: 100% similarity, 0% dissimilarity
A52945	Caprine	Dry rangeland	Narita 24	Sample sequence: 100% similarity
A52977	Indigenous poultry	Swampy-marshy	Narita 24/21	Sample sequence: 99% similarity, 1% dissimilarity
A53170	Indigenous poultry	Flooded shoreline	Narita 24	Sample sequence: 99% similarity, 1% dissimilarity

Further BLASTN and alignment of the *Aedes flavivirus* sequences from blood samples of livestock showed that all eight (8) isolates from samples blasted were evolutionary 100% related to each other (Table 4.14).

Table 4.14: Distance matrix for nucleotide sequences between paired sequences of *Aedes flavivirus* from blood samples of livestock species resident in Lake Baringo basin

	A51674	A52801	A52805	A52833	A52871	A52945	A52977	A53170
A51674								
A52801	0.000							
A52805	0.000	0.000						
A52833	0.000	0.000	0.000					
A52871	0.000	0.000	0.000	0.000				
A52945	0.000	0.000	0.000	0.000	0.000			
A52977	0.000	0.000	0.000	0.000	0.000	0.000		
A53170	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

Further, when blasted against the sequences in the NCBI database, the results yielded a number of the mutant strains circulating in the study area. Three (3) sequences of virus from bovine species (A52801) resident in flooded lake shores area, indigenous poultry (A52871) resident in swamp marshy habitat, and caprine from dry rangeland had 100% nucleotide similarity to the referenced sequences accession (AB488411) of *Aedes flavivirus* in NCBI database. Another five (5) virus sequences were observed to have different nucleotide bases mutations after alignment with NCBI referenced *Aedes flavivirus* strains accession number AB88411.1, NC0129321.1, AB488423.1. Some of the variations and mutations included new insertion of the bases, deletion

(gaps), and or exhibiting single nuclear polymorphisms (SNPs) (Plate 4.1, 4.2, 4.3, 4.4, 4.5, and 4.6).

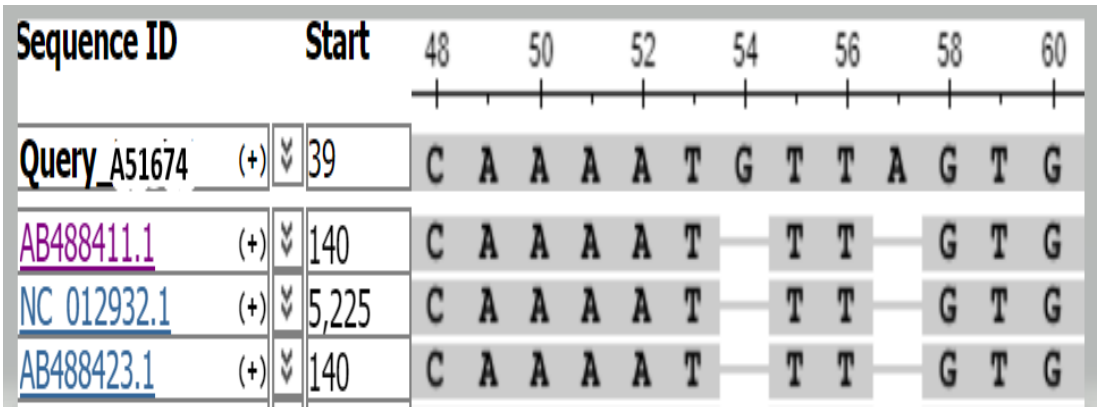

Plate 4.1: A section of *Aedes flavivirus* sequence obtained from caprin**e** blood resident in swamp marshy habitat (A51674). When referenced to NCBI sequence accession number AB48811, it returned a similarity value of 99% and 1% dissimilarity. The 1% dissimilarity constituted 2 gaps (marked white) that corresponded to position 54 and 57 of the consensus sequence.

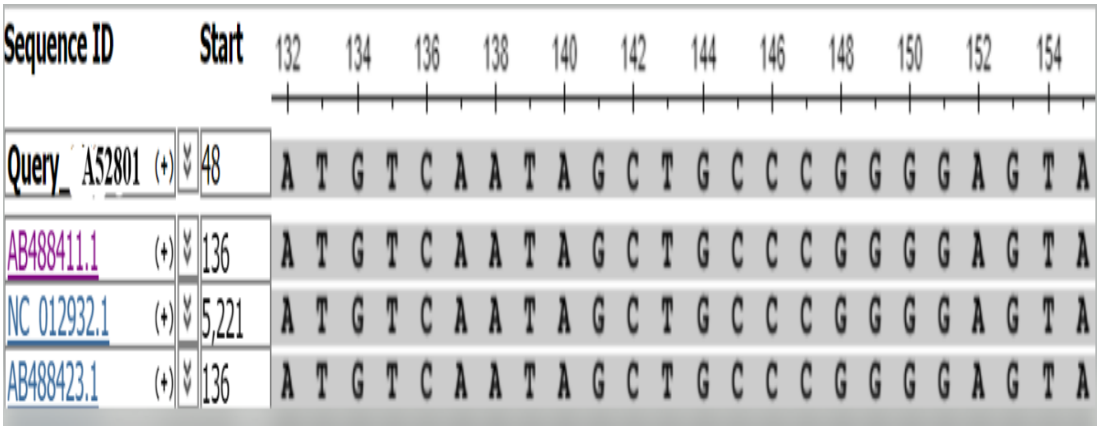

Plate 4.2: A section of *Aedes flavivirus* sequence obtained from ovine blood (A52801) from flooded shoreline habitat referenced to NCBI sequence showing 100% similarity and 0% dissimilarity to referenced ID AB44811. No mutation was observed.

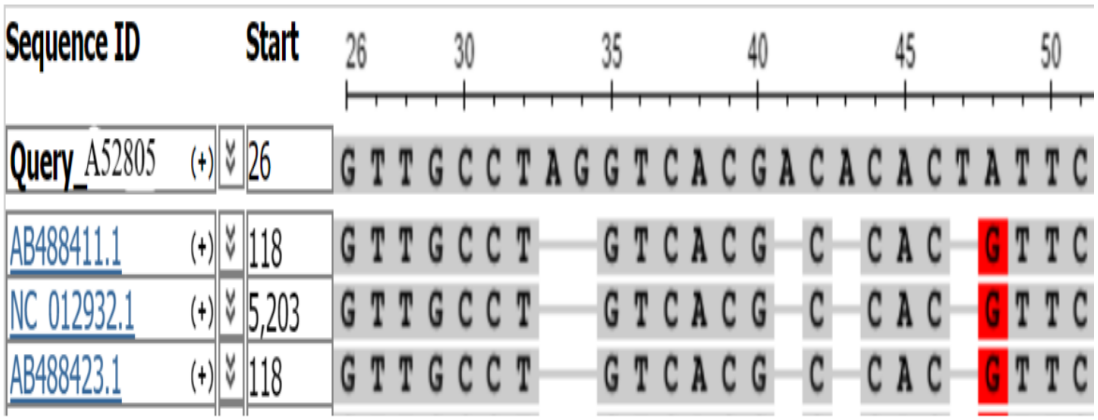

Plate 4.3: A section of *Aedes flavivirus* sequence obtained from blood of indigenous poultry (A52805) from flooded shoreline area referenced to NCBI sequence accession ID AB48811, NC012932.1, AB488423 showing 98% similarity, 2% dissimilarity and 5gaps. The 2 % dissimilarity was based on alignment sequence observed at nucleotide bases (marked clear) for the associated gaps and translation of purine A/G at referenced marked red.

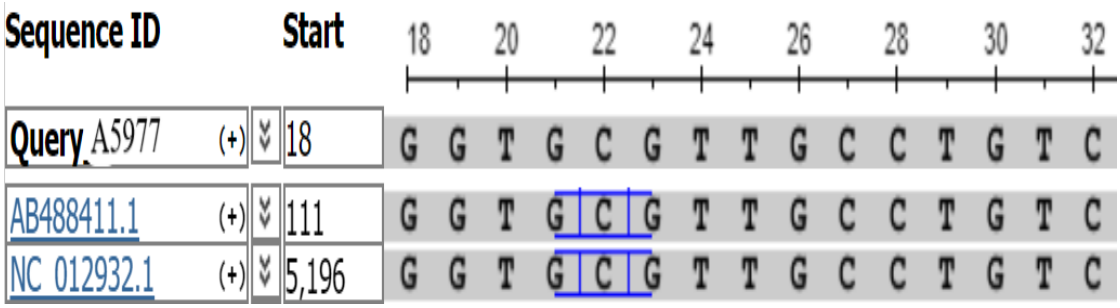

Plate 4.4: A section of *Aedes flavivirus* sequence obtained from blood of indigenous poultry (A52977) from flooded shoreline area referenced to NCBI sequence accession ID AB48811.1 and NC012932.1 showing 99% similarity and 1% dissimilarity. The 1% dissimilarity was based on alignment sequence from 2 insertions that corresponded to alignment sequence position marked with blue colour.

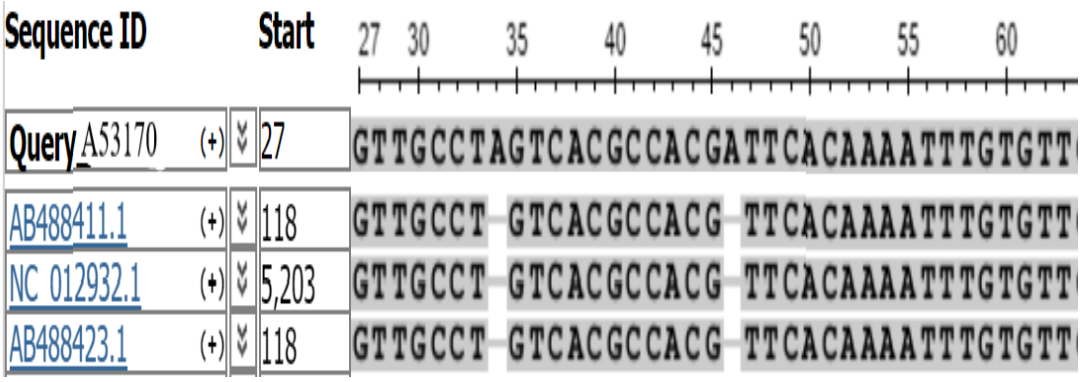

Plate 4.5: A section of *Aedes flavivirus* sequence obtained from blood of indigenous poultry (A53170) resident in swamp marshy area referenced to NCBI sequence accession ID AB48811.1 and NC012932.1 showing 99% similarity and 1% dissimilarity. The 1% dissimilarity was based on alignment sequence from 2 insertions that corresponded to alignment sequence position marked white

4.3.2 Characterization of Rift Valley Fever virus

The BLASTN of RVFV sequence showed similarities with several others isolated in various geographical regions globally. Three (3) isolate sequences from bovine (RVF 52898), ovine (RVF51631) and ovine (RVF52890), all resident from swamp marshy habitat (Kapkuikui) were 100% identical to each other (Plate 4.6). However, they all had 99% similarity, 1% dissimilarity and 0% gap in nucleotide base arrangement to the referenced NCBI sequences accession: KX944844.1, KX944836.1, KX944832.1, KX944831.1, and KM210509.1.(NCBI, *https://www.ncbi.nlm.nih.gov/*). The 1% mismatch was at nucleotide bases corresponding to aligned consensus base positions 6, 10 and76 (Plate 4.7).

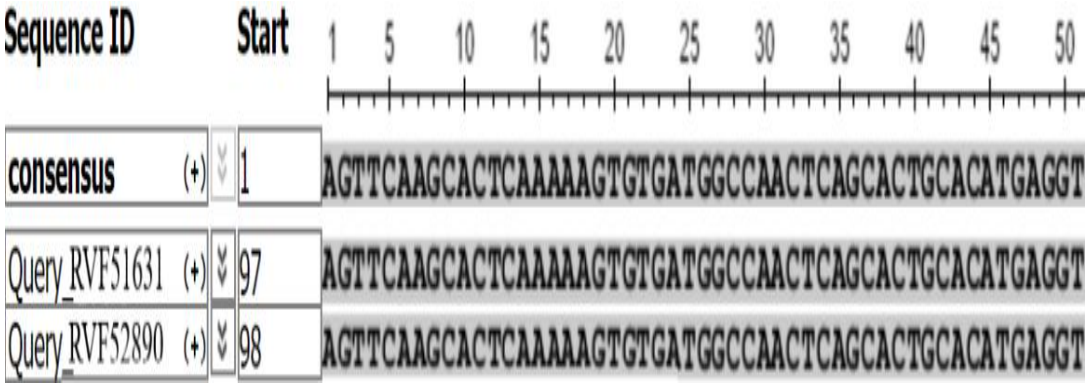

Plate 4.6: Section of the sequence of the strain of RVF virus from ovine species (RVF51631 and an ovine (RVF 52890), both resident in lake shore habitat, including bovine (RVF 52898), from swamp marshy ecological habitat. The sequences were 100% identical to each other.

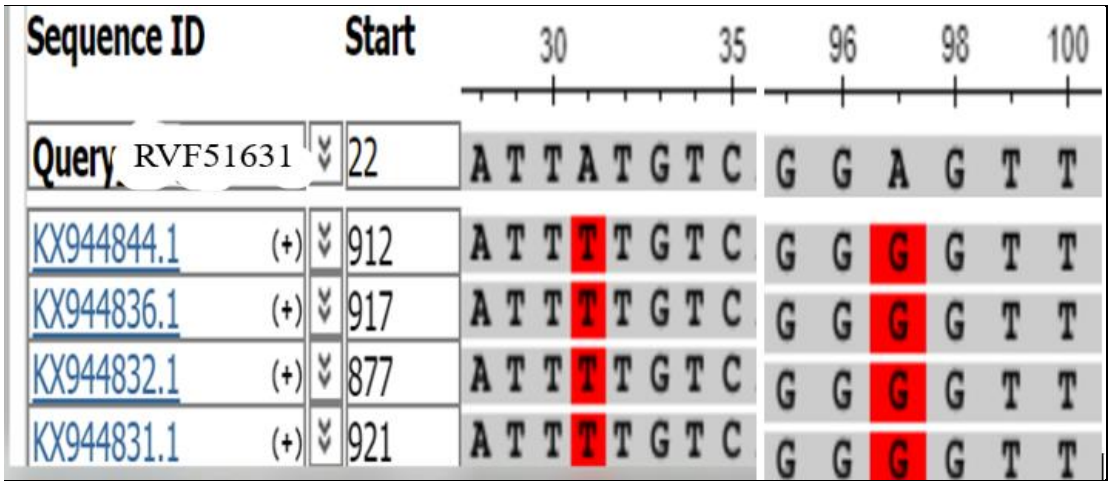

Plate 4.7: Screenshot of sequences of bovine (RVF5298), ovine species (RVF51631, RVF52890) from swamp-marshy ecological habitat showing positions of the nucleotide mutations position marked red.

Two (2) sequences of caprine (RVF53206; RVF53155), both from swamp marshy habitat, had their sequences differently aligned to each other. The RVF virus sequence from caprine (RVF53206) when subjected to NCBI blast returned results of 98% similarity and 1% dissimilarity to NCBI reference sequence accession number

KY3663321.1 and KY366332.1. The nucleotide sequence of RVF virus of second caprine species (RVF53155), also from swamp marshy habitat showed a 6% mismatch in nucleotide to NCBI reference. When the two (2) caprine sequences were aligned to each other, the blast results showed 8% dissimilarity and only 92% similarity (Plate 4.8, 4.9, 4.10, 4.11).

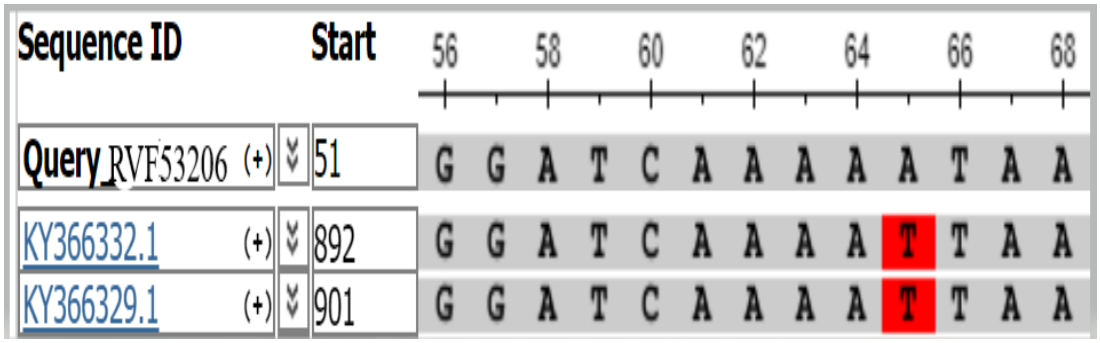

Plate 4.8: Section of RVF virus sequence from caprine (RVF53206) resident in swamp marshy habitat with mutation observed at alignment base positions marked red

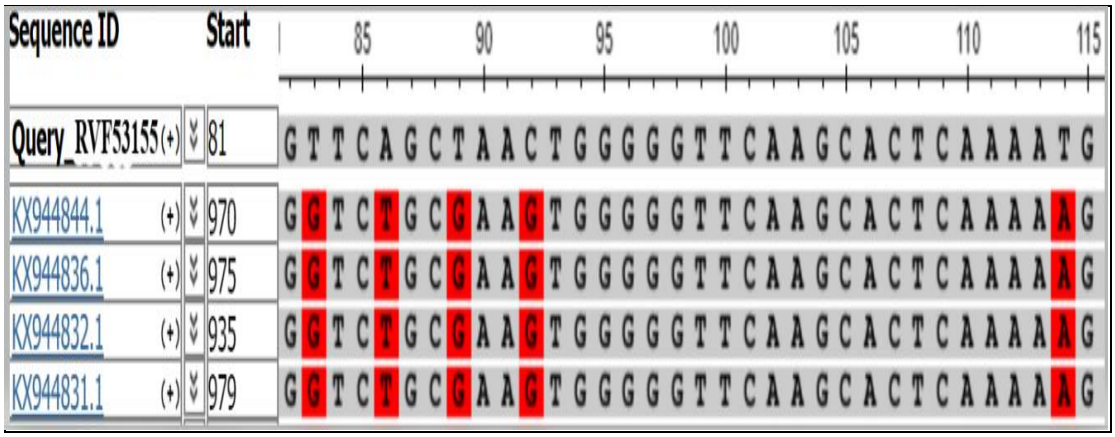

Plate 4.9: Section of RVF virus sequence from a caprine (RVF53155) resident in swamp marshy habitat. The sequence mismatch to NCBI reference accession KX944844.1 was 6% at alignment base position marked red

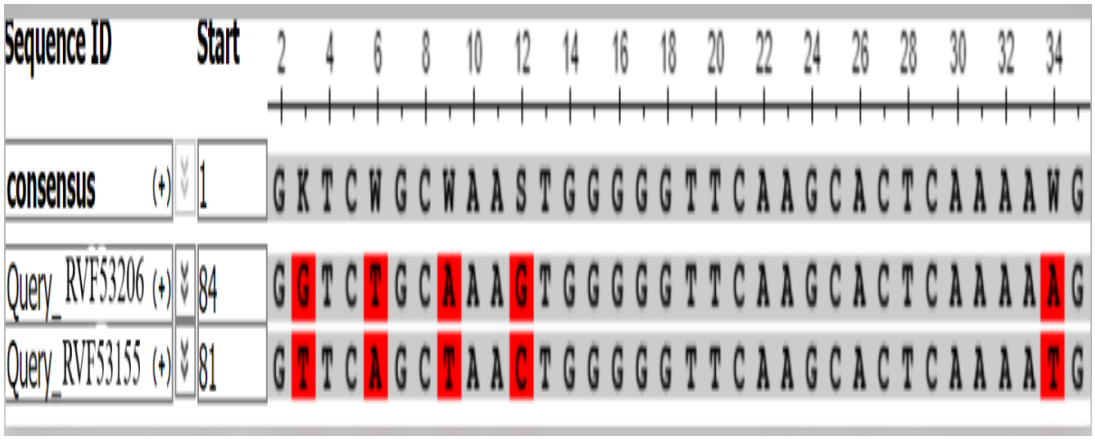

Plate 4.10: Sections of RVF virus sequence from caprine (RVF53206; RVF53155), aligned to each other. The sequences were 8% dissimilar to each other with mutations observed at consensus sequence base position marked red.

From indigenous poultry (RVF53082), the isolate sequence of RVF virus returned an extraordinary high dissimilarity value of 14% after alignment with NCBI database sequences. The isolate sequences had mutations that numbered more than 10, and included base translations, insertions and gaps at various nucleotides base positions (Plate 4.11)

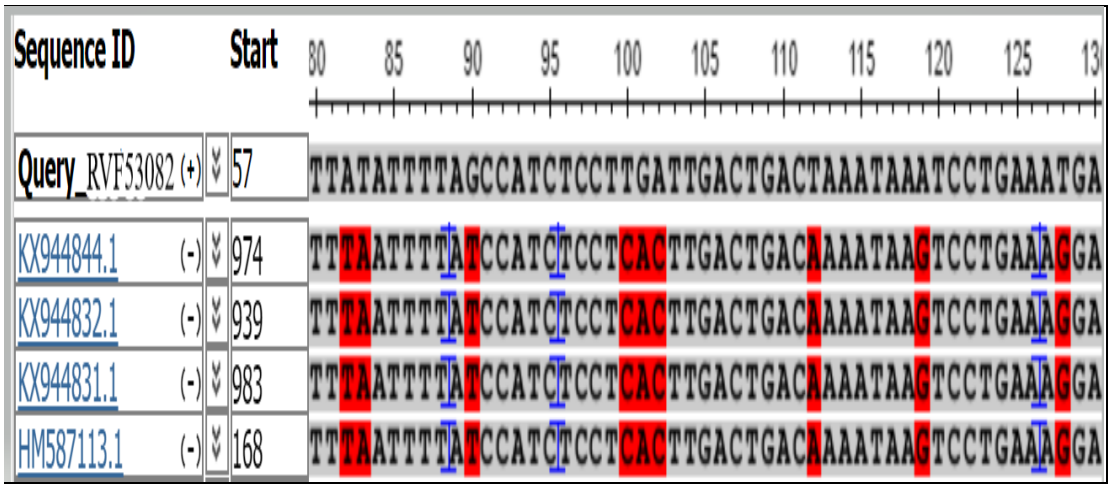

Plate 4.11: Screenshot of sequences of RVF virus isolate from indigenous poultry resident in lake shoreline habitat (RVF53082) aligned to sequences from NCBI database displaying a 14% dissimilarity (marked red and insertions positions marked blue) and 86% similarity.

Two (2) RVF virus isolates from bovine (RVF52898) resident in swamp marshy area was different from the strain isolated from a bovine resident in lake shore habitat (RVF53008). When these two isolates of RVF virus from bovine but resident in different habitats were aligned together, they exhibited the highest dissimilarity value of 24% to each other and a low similarity value of 86% (Plate 4.12). The sequence of RVF from bovine (RVF52898) resident in swamp marshy was 99% identical to NBCI reference sequence KX944841, KX944836, KX944832 and KX94431. The sequence of the RVF virus isolate (RVF53008) from blood of a bovine resident in lake shoreline ecological habitat, was different with a variation of 4% and 96% similarity to the NCBI reference sequence. The variation was observed at nucleotide base alignment positions 8, 17 and 65 (Plate 4.13).

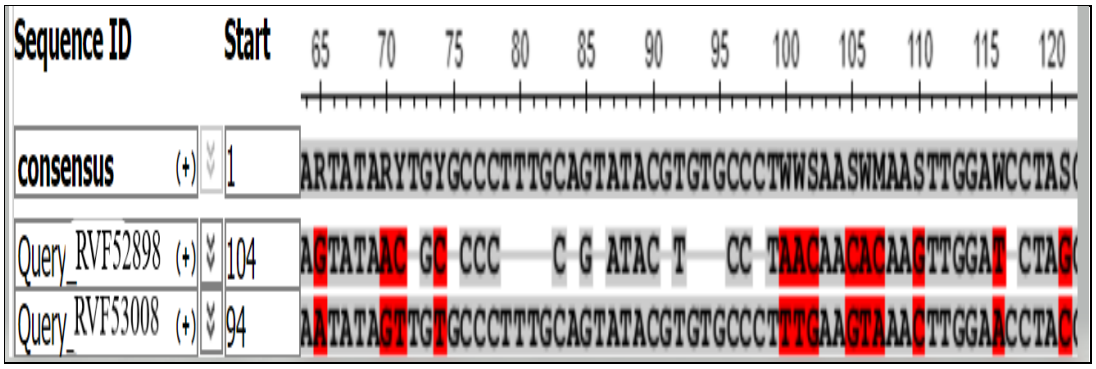

Plate 4.12: Section of variations of RVF virus sequence base positions (marked red) for bovine species resident in swamp marshy habitat (RVF52898) and (RVF53008) resident in lake shoreline. The two isolates, had a 24% dissimilarity value at positions marked red and white gaps

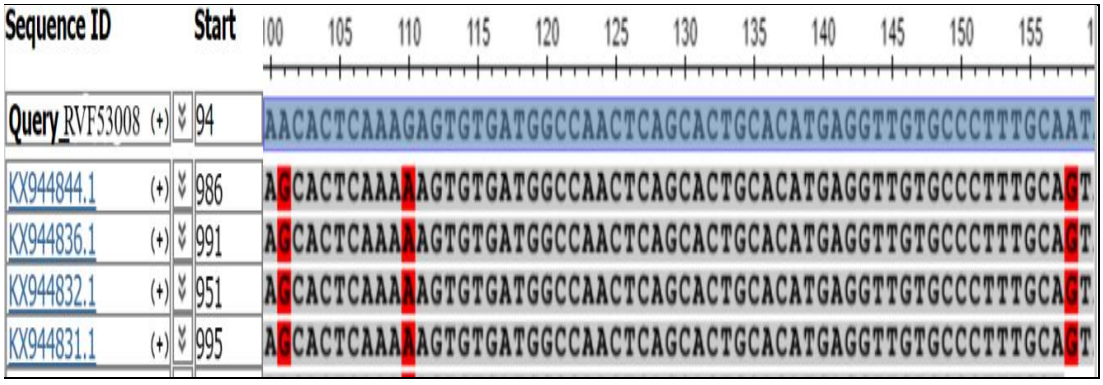

Plate 4.13: Screenshot of sequence of RVF strain from bovine resident in lake shoreline habitat (RVF53008). The isolate sequence was 96% identical to NCBI isolates with a 4% mismatch marked red.

Two (2) ovine species from the same area, swamp marshy habitat, that tested positive had RVF sequences aligned100% identical to each other (Plate 4.14). The two sequences showed 10% mismatch and 90% identity to NCBI sequence of isolates KX944844, KX944832, KX944831, HM587114, HM587113, HM587110, JF784387, DQ380189 and DQ380188. When the same sequence (ovine species resident in swamp marshy habitat) were aligned to RVF virus sequence from ovine species resident in lake shoreline (RVF53177) habitat, a 10% mismatch of the nucleotide bases was observed at alignment positions, 7, 9, 13, and 21.

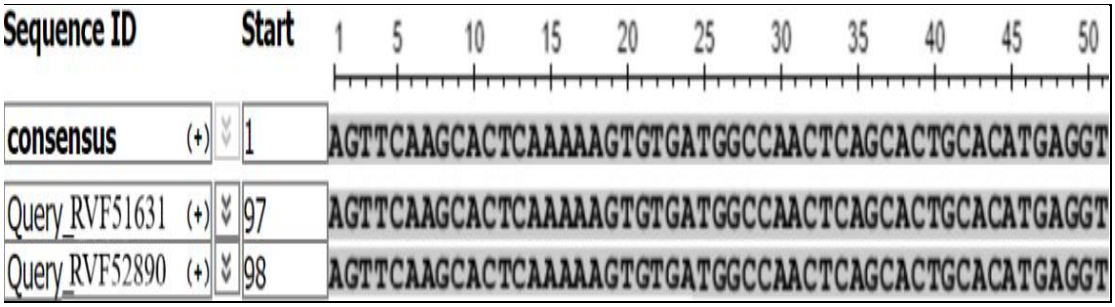

Plate 4.14: Sequences of RVF from ovine species resident in lake shore and swamp marshy habitat. They had 100% march to each other with nucleotide base positions and 0% gaps.

4.3.3 Phylogenetic analysis of *Aedes flavivirus*

The phylogenetic mapping of the *Aedes flavivirus* sequences used the Zika virus NS3 protein (GB accession: KJ461621.1, Tahiti strain) as the rooting outgroup. The choice of Zika outgroup was informed in that it was distantly related to flaviviruses of flaviviridae family isolated from mosquitoes in the study habitats. The MEGA 6 software was used to align the sequences, remove gaps, select suitable models, draw the tree, and perform bootstrapping (Tamura *et al.*, 2011). The evolutionary relationships was inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). The bootstrap consensus tree inferred from 1500 replicates was taken to represent the evolutionary history of the taxa for flavial virus (Hall 2013; Felsenstein 1993) (Figure 4.6).

From this study, the eight (8) *Aedes flavivirus* strains isolated from blood of livestock species resident in Lake Baringo basin were 100% evolutionary related. The eight (8) strains were also evolutionary related to the strain of *Aedes flavivirus* reported in Nagasaki and Toyama in Japan (GB accession AB488423.1, AB488415.1,

AB488411.1). Further, from the phylogenetic tree, it can be deduced that the strains of Aedes flavivirus from Lake Baringo basin are still evolving (Figure 4.6).

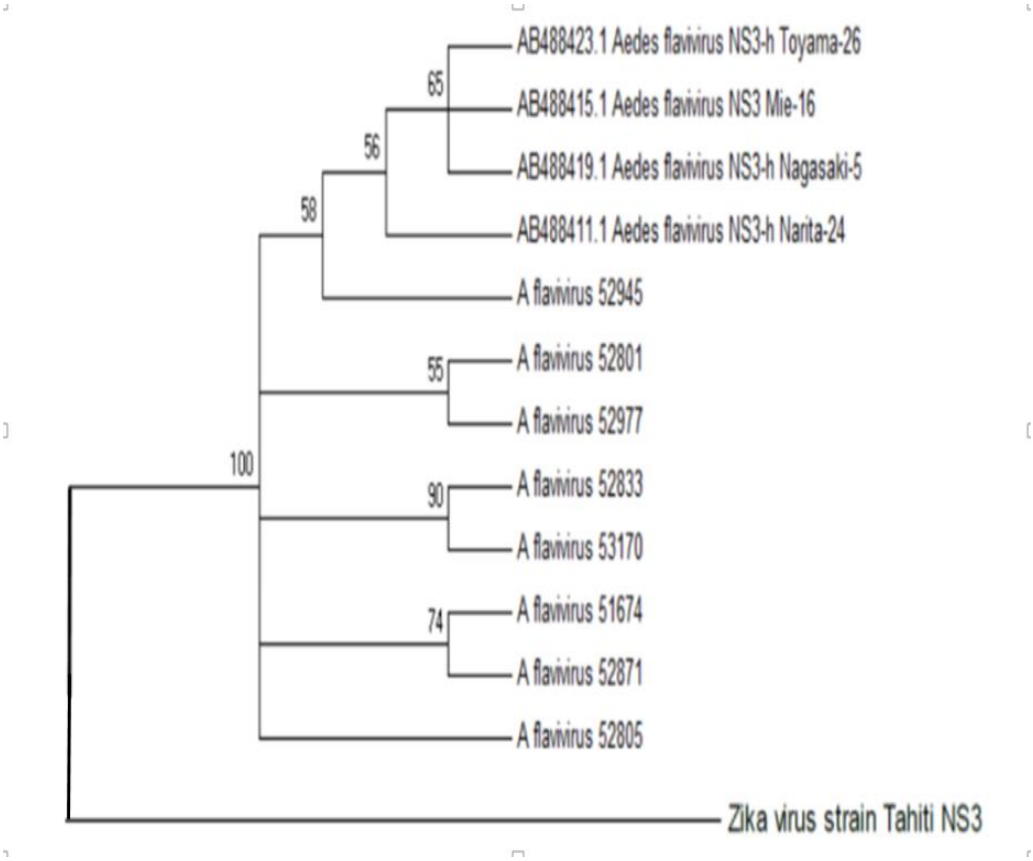

Figure 4.6: Phylogenetic tree for *Aedes flavivirus* isolated from livestock species resident in Lake Baringo basin. Molecular Phylogenetic analysis for evolutionary history was inferred by using Maximum Likelihood method rooted on Zika virus based on the Kimura 2-parameter and Tamura-Nei model (Kimura, 1980**;** Tamura *et al.*, 2011).

4.3.4 Phylogenetic analysis of RVF virus

The phylogenetic mapping of the Rift Valley Fever virus sequences used the Hantavirus HTN/Far (GB Accession: AF172442.1) as the rooting outgroup. This is because it is a member of the Bunyaviridae family that is not closely related to the RVF virus. A phylogenetic consensus tree was drawn to reveal the evolutionary divergence of the RVF virus sequences isolated from livestock in the three habitats.

The RVF virus sequences comprised three (3) clades. The first clade was from ovine (swamp), caprine (swamp), ovine (flooded) and poultry resident in swampy marshy habitat and clustered together (RVF51631, RVF53206, RVF53177, RVF53082). The second clade was from bovine (RVF53008) and caprine (RVF53155) resident in swamp marshy habitat. A third clade formed a distinct separate group of strain that was found in bovine (RVF52898) (Figure 4.7).

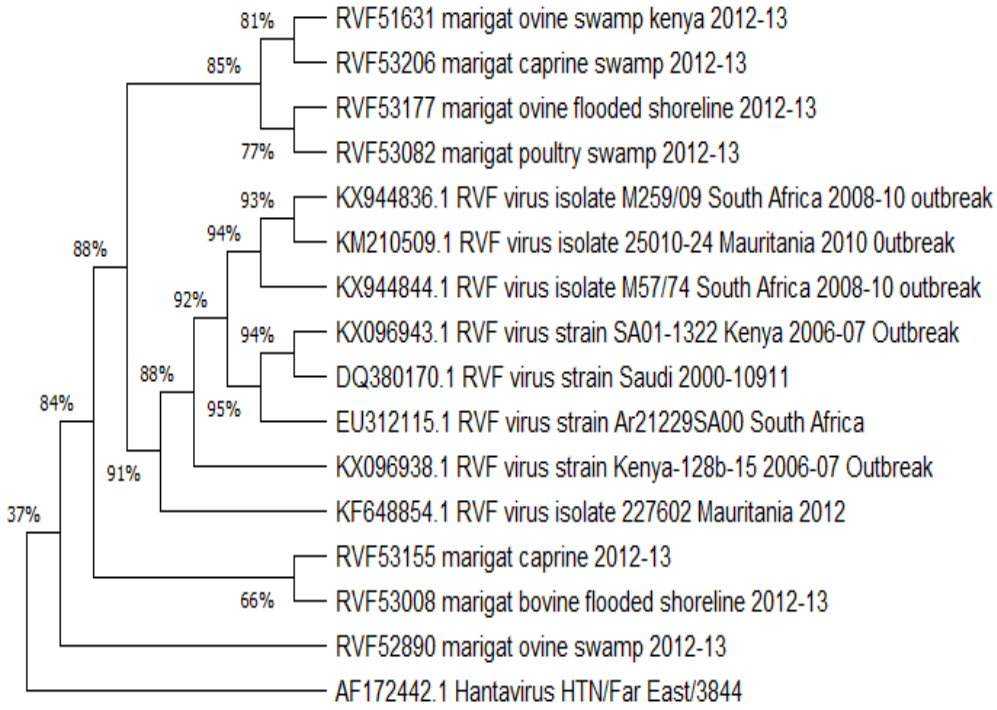

Figure 4.7: Phylogenetic tree for RVF virus isolates from blood of livestock resident from Lake Baringo basin ecological habitats rooted on Hantavirus. Note: The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model [1]. The tree with the highest log likelihood (-8007.47) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with superior log likelihood value. Evolutionary analyses were conducted in MEGA X [2]. The numbers at the branches are confidence values based on Felsenstein's bootstrap method.

CHAPTER 5: DISCUSSIONS, CONCLUSIONS AND RECOMMEDATIONS

5.1 Discussions

Exogenous drivers such as amount of rainfall, state of water, floods and other abiotic factors influence the survival of insects and their habitation in an ecology. This study aimed to understand the spatial and temporal changes of mosquito abundance, composition and diversity in flooded lake shoreline ecology alongside other habitats of Lake Baringo during the unprecedented extreme flooding in 2011-2014.

In this study, with an overall catches of 386,625 individual mosquitoes and an abundance averaging monthly 32,218 mosquito catches, it leaves no doubts that this was among the few studies that have reported such remarkable high catches. The catches were recorded in the entire period of study, during the wet and dry season and was considered as a high infestation when compared with other reported catches. For instance, a study by Ondiba *et al.* (2017) had a low total catches of 9,729 in the lowland area of the lake basin averaging monthly catches of 811 individual mosquitoes while trapping by Sang *et al.* (2010) during floods from Elnino rains of 2006/2007 averaged 5,269 individual mosquitoes catches.

There are variety of microhabitats where various genus and species of mosquitoes optimally thrive in Lake Baringo basin. A total of 11 mosquito genera from all habitats as recorded is probably the highest number of genera caught in a single study conducted in the Lake Baringo basin. Sang *et al.* (2010), in a study carried out during the Elnino floods of 2006-2007 had nine (9) genera reported, while Ondiba *et al.* (2017) found a lesser number of eight (8) genera. The high number of genera reported

in this study is as a direct consequence of existing wetted conditions in lake shoreline habitat whereas in swamp marshy and dryland, the presence of high diverse number of genus was as a result of intermittent precipitation that supported mosquitoes breeding sites and niches.

Not all mosquitoes could be identified morphologically due to the high number that were caught. Advanced techniques had to be used to identify more species correctly. In this study, the Next Generation Sequencing (NGS) was able to amplify from pooled mosquito DNA libraries new sequences that were identical to those of *Aedes albopictus* species. This discovery suggested presence of this new species which had not been reported in the study area and Kenya prior to this study. The finding of this species confirms the richness of the ecology of Lake Baringo basin in terms of mosquito composition from the area. The species is generally found to occur in Asian forests and America (Parker *et al.*2019). It is an emerging vector in Africa and recently was identified in Gabon, West Africa (Ngoagouni *et al.*2017). The species is reported to transmit other lethal viruses such as Japanese Encephalitis, Zika, Dengue, and Chikungunya among other viral diseases (Parker *et al.*2019). Its occurrence in Kenya should be a major concern for health.

The present study found out distribution of mosquitos' genera in the basin is dependent on various environmental factors. Genus *Mansonia* is the most abundant in areas with water and dense macrophytes which provides adequate shade and resting places (Pratiwi *et al.* 2019). From the results of the study, the high abundance of genus *Mansonia* (84.9%) was due to wetted conditions in the flooded lake shoreline and swamp marshy habitats which provided favourable environmental conditions

consisting of submerged substrates, water, macrophytes and emergent vegetation ideal for the breeding and proliferation of this species. Sang *et al*. (2010), reported 87.0% catch of *Mansonia* during the Elnino in 2006, while Ajamma *et al.* (*2*016) reported 57.9%. A study by Paula *et al.*(2012) reported that artificial flooding from hydroelectric water dams result in increased mosquitoes of genus *Mansonia* concluding that this species benefits from the flooding relative to others. The high abundance of genus *Mansonia* should be of interest because the species are incriminated in transmission of arboviruses such as RVF as reported by Tantely *et al.* (2015). The high infestation from Lake Baringo basin is therefore a public health concern. This because humans and livestock from flooded lake shoreline habitat face the risk of infection with zoonotic arboviruses than those residents in dry rangeland where *Mansonia* were caught at very low densities.

Mosquitoes of *Aedes* species usually appear in a habitat after precipitation that form pools of clear water on ground, containers, tree-holes and vegetation (Kweka *et al.* 2018). The distribution and abundance of the mosquitoes of this genus is often affected by the quality of water. In flooded lake shoreline habitat, the lake water flood caused changes in quality of water and oviposition sites. In this habitat, the flooded animal waste continued to degrade and release dissolved nutrients into water further polluting the ecology. This made the habitat less favourable for most of *Aedes* mosquito species to oviposit. These affects the spatial and temporal abundance, diversity and richness of mosquitoes. Further to this, the high catches of the genus *Aedes* (79.0%) in the swamp marshy grazing field microhabitat demonstrated the important role the microhabitats play in harbouring some of primary vectors. It is most

likely that initial infections are picked from the grazing fields as animals graze and amplified by mosquitoes in homesteads.

The eggs of *Culex* females are laid on permanent or temporary water surfaces, with larval habitats being ponds, flooded ground, irrigated crops, river banks and tree holes, clear water, The swamp marshy habitat supported *Culex* abundance at 64.1%. The presence of several species of *Culex* (*Culex pipens pipens, Culex pipens quinquefasciatus, Culex tritaeniorhynchus,* and another unidentified 3 *Culex* spp) in the Lake Baringo basin underpins the importance of this genus in a complex transmission cycle and as primary and secondary vectors of arboviruses. In another study by Cornet *at el.,* (2019), *Culex pipens* was implicated in transmision of avain malaria protozoa including arboviruses. The presence of these *Culex* species therefore increases the risk of infection with RVF, avian malaria and other arboviruses in lake Baringo basin.

Mosquitoes of genus *Theobaldia* are regarded as flood mosquitoes. They are rare and prefer to feed on birds which are non-viremic (Harbach *et al.,* 2007). The genus appear in habitats that are flooded and in this study, they were only caught from flooded lake shoreline habitat. This study therefore associates the appearance of mosquitoes of genus *Theobaldia* to the unprecedented flooding that created conducive environment for the emergence of the genus. These means that, in this study, they could have equally facilitated the transmission of RVFV from non-viremic birds to mammals.

The prolonged unprecedented floods resulted in low mosquito richness as well as diversity in the flooded lake shoreline area. It affected the richness and evenness

distribution and composition of mosquitos' genus. The flood waters resulted in a poor diversity while the swamp marshy and dry rangeland habitats supported rich and even mosquitos' community. In a study by Duchet *et al.* (2017) it was found out that flash floods disturb and negatively affect phytoplankton and zooplankton which influence selection of mosquito oviposition habitat because of the loss of food resources in ephemeral pools.

Arboviruses are associated with variety of diseases in humans and livestock. This study isolated two arboviruses from mosquitoes of Lake Baringo basin, *Aedes flavivirus* and *Culex flavivirus* both of which are Insect Specific Flaviviruses (ISF). Ochieng *et al*. (2013) reported presence of insect specific viruses, Ndumu virus and Kamiti River viruses from Lake Baringo basin. When viewed in addition to *Aedes flavivirus* and *Culex flavivirus* then the basin can be regarded as a rich habitat for Insect Specific Flaviviruses.

Arboviruses are associated with morbidity and mortality in humans and livestock. However, majority of arboviruses are non-pathogenic. This is the first ever study to report presence of *Aedes flavivirus* isolate from livestock blood. This contrary to according to Nasar *et al*.(2015) thast *Aedes flavivirus*, is an insect specific virus, non-pathogenic and do not replicate in vertebrate cells (Roiz *et al.*2012). Further, Nasar *et al.* (2015) states that the *Ae. flavivirus* is transmitted vertically in mosquitoes and that their transmission horizontally through feeding process is not yet well established. The probable explanation for *Aedes flavivirus* reported from this study in livestock is that mosquitoes, while feeding, inject fragments of nucleic acid of *Aedes flavivirus* that are detectable by PCR in livestock blood but which do not replicate.

The strains of *Aedes flavivirus* from Lake Baringo basin and those from NCBI when cross-referenced with those from Japan were found to be evolutionary related. The phylogenetic tree indicated that *Aedes flavivirus* from Lake Baringo were grouped into five (5) novel clusters. This study therefore construes that the *Aedes flavivirus* viruses from Lake Baringo basin have the future potential to evolve into a distinct lineage consisting of several different antigenic groups. This is corroborated by the finding from Vázquez *et al.* (2012) that the degree of evolutionary divergence can be indicative of a likelihood to evolve into a new antigenic group of viruses.

Rift Valley Fever have been reported in several parts of Africa and Asia, particularly Kenya. The characterization of RVFV isolates from livestock species in this study reveals new mutations as evidenced from genomic sequences showing several new variations, but it is not clear whether the mutations will result in virulence or not in future. In this study, it was observed that the detected variations of RVF virus sequences were local and share close lineage with isolate Kenya 128-15 which caused outbreak of RVF in 2006-2007. The variations and mutations could have been induced by larger external environmental factors prevailing in Lake Baringo basin over time. Further, the isolated RVF virus sequence shows relation to isolates from South Africa, South Sudan and Mauritania that caused outbreaks in 2008-2010. According to Freire *et al.*(2015) and Bird *et al.*(2007), the RVF virus from different parts of world are still evolving and purifying, and this could hold true to RVF virus from Lake Baringo basin.

The results of this study suggest that over time, repeated prolonged floods will hasten the process of mutations. According to Maluleke *et al.* (2009) the mutation takes years

and pathogenic mutants could be from the same habitat contributing to evolutionary dynamics. In this study, the divergence observed relative to other isolates can be explained by geographical separations over time scale factor since the last outbreak that occurred in 2006/2007. There is still potential for the isolates to be widely spread in other geographical areas where it may result in genetic reassortment posing the threat to humans and livestock.

Phylogenetic analysis of viruses from this study shows no host related grouping. In some instances, the phylogenetic analysis show that isolates from ovine, poultry and caprine belong to the same descendant. Sall *et al.* (1999) explains that, where there is no evolutionary grouping of viruses based on host, this is because of frequent exchange of viruses between different hosts. It was observed that all livestock species from the study area graze together side to side with wildlife. This behavior would facilitate exchange of viruses from domestic to wildlife host and vice versa as mosquitoes bite and take bloodmeal which has the potential of resulting in reassortment of the viruses and eventually causing an outbreak. Longdon *et al.* (2014) concluded that most of zoonotic outbreaks, epidemic or pandemic are as a result of pathogen jumping from its original host into a novel species, a behavior referred to as host shift. In this study, the encountered mutations are due to environmental changes and gene exchange as livestock graze alongside wildlife over time and space.

This research has already resulted in three outputs, two publications in peer reviewed journal (Appendix III and IV) and a presentation in a conference (Appendix V).

5.2 Conclusions

From these study, it is concluded that:-

i. Lake Baringo basin was infested by diverse mosquito species totalling to 11 species, and the unprecedented flooding of Lake Baringo basin affected mostly mosquito abundance, composition and diversity from flooded lake shoreline habitat and not in the other two habitats resulting in the highest number of individual mosquitos' catches, with 97.3% been of genus *Mansonia*.

ii. There were three species of arboviruses isolates, *Aedes flavivirus*, *Culex flavivirus* and Rift Valley Fever virus. RVF virus was more prevalent in livestock resident in the swamp marshy and least from dry rangeland.

iii. Characterization of the genome sequences of isolated arboviruses revealed mutations from the Lake Baringo basin that are continuous, environmental dependent and still evolving with *Aedes flavivirus* showing a common original homology, as well as for Rift Valley Fever virus.

5.3 Recommendations

This study recommends that:-

i. The community be sensitized on protection against infection from mosquitos' bites and disease prevention.

ii. With the new understanding of risk of livestock to RVFV informed from this study, it is recommended that both the relevant departments and agencies for livestock and human health develop a strategic approach for the control and mitigation against RVFV in wetlands and floods prone areas.

iii. A further spatial mapping of the mosquito species inhabiting Lake Baringo basin be carried out to identify the emerging vectors species.

5.4 Recommendations for further studies

Based on this study, the following areas would require further investigations:-

i. Environmental factors that cause low circulation of viruses as opposed to those which triggers large outbreaks.

ii. The role of viremic birds and poultry in sustenance of RVFV in livestock within the homestead.

iii. Studies on the internal physiological factors that act as a barrier inside the vertebrate cells and that stops the replication of ISF viruses such as *Aedes flavivirus*.

iv. More studies and advanced analysis of the ISF be carried out inorder to establish the role they play in suppression of viral disease infections and amplifications in consideration as candidate for biological control.

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APPENDIXES

Appendix I: Letter of engagement collaborative research

MINISTRY OF AGRICULTURE, LIVESTOCK AND FISHERIES

Telegrams: "VETLAB", Kabete
Telephone: 020 – 631390/4/5/7, 631287 and 631291
Fax: 254-20- 631273

When replying, please quote:
REF: GEN TSE/KAB/VOL1/144
All correspondences should be addressed to:
The Director of Veterinary Services
Parcel by rail: Nairobi Station

DEPARTMENT OF VETERINARY SERVICES,
VETERINARY RESEARCH LABORATORIES,
PRIVATE BAG,
00625 KANGEMI

14TH August, 2013

The Director General
International Livestock Research Institute
NAIROBI

LETTER OF ENGAGEMENT: COLLABORATIVE RESEARCH ACTIVITIES "*MOSQUITO-BORNE ARBOVIRUSES ISOLATION RESEARCH PROJECT (MAIRP)*"

Reference is made to the signed Memorandum of Understanding (MoU) between Department of Veterinary Services and International Livestock Research Institute for collaborative research work titled **'Mosquito-borne Arboviruses Isolation Research Project-MAIRP'.** Mr Samuel Kabochi Kamau who is the Chief Principal Zoologist in the Department of Veterinary Services, Division of Zoological Services is hereby appointed the Principal Investigator on the foregoing research work. He is therefore cleared and authorized to undertake field surveillance, collection of samples, and engagement of the community at the research sites. He is also to be involved in all laboratory analysis of the samples that will be stored in ILRI and in accordance to the MoU and communicate the progress and findings on regular basis to this office which will be used towards facilitating on crafting of appropriate control strategies of the vectors and vector-borne diseases and also inform on related Government policy.

DR PETER ITHONDEKA (PhD, MBS)
DIRECTOR OF VETERINARY SERVICES

Appendix II: Approval of research proposal

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke

Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 810901 Ext. 57530

Internal Memo

FROM: Dean, Graduate School

DATE: 25th June, 2014

TO: Kamau Samuel Kabochi
C/o Zoological Sciences Dept.

REF: I84/22567/12

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

This is to inform you that Graduate School Board, at its meeting of 11th June, 2014, approved your Research Proposal for the Ph.D Degree **Entitled, "Seasonal Variation of Mosquitoes Species (Culicidae), Abundance and Potential Risks to Arboviruses in Three Ecological Systems of Marigat District, Baringo County."**

You may now proceed with your Data Collection, subject to clearance with the permanent Secretary, Ministry of Higher Education, Science and Technology.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking Forms per semester. The form has been developed to replace the Progress Report Forms. The Supervision Tracking Forms are available at the University's Website under Graduate School webpage downloads.

By copy of this letter, the registrar (Academic) is hereby requested to grant you substantive registration for your Ph.D studies.

Thank you.

DAVID NJOROGE
FOR: DEAN, GRADUATE SCHOOL

c.c. Chairman, Department of Zoological Sciences

Supervisors:

1. Prof. John M. Mueke
   C/o Department of Zoological Sciences
   Kenyatta University
2. Dr. Michael M. Gicheru
   C/o Department of Zoological Sciences
   Kenyatta University
3. Dr. Benson M. Mwangi
   C/o Department of Zoological Sciences
   Kenyatta University
4. Dr. George N. Michuki
   Molecular Pathogen Discovery Laboratory
   International Livestock Research Institute (ILRI)
   C/o Department of Zoological Sciences
   Kenyatta University

Appendix III: Output 1: Scanned page of a publication in a Journal

http://www.dipterajournal.com/pdf/2020/vol7issue6/PartA/7-5-6

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Mosquitoes composition, abundance and distribution in swampy and flooded shoreline habitats of Lake Baringo, Kenya, during a period of extreme flooding (2012-2013)

Samuel K Kabochi, Benson M Mwangi, Michael M Gicheru, George N Michuki and Irene A Onyango

Abstract
Mosquito ecology is influenced by wetness in the environment. In 2011-2014, a rise in waters of Lake Baringo resulted in unprecedented flooding that inundated over 88km² of the shoreline. A longitudinal study carried out from October 2012-October 2013 assessed mosquito abundance and diversity in two habitats. A total of 386,624 mosquitoes were captured, 89% from flooded shoreline and 11% from swampy habitat. Family Culicinae constituted 10 genera. *Manzonia* dominated the catches with 98% from flooded shoreline and swampy habitat 2%. Genetic sequences of *Aedes albopictus* species was identified and reported for the first time in the basin. Diversity index was higher in swampy habitat (Simpson Diversity Index=0.56), compared to flooded shoreline (Simpson diversity index =0.13). Future recurring floods will result in drastic changes of the ecology and could lead to emergence and re-emergence of more species.

Keywords: extreme flooding, flooded shoreline, swamps, mosquito, diversity, Lake Baringo

1. Introduction

Mosquitoes are well known vectors of human pathogens, including arboviruses and are considered to be the main vectors of killer diseases such as malaria, Rift Valley Fever, Dengue haemorrhagic fever, Yellow Fever, West Nile Fever and Chikungunya Fever [1]. According to a report by WHO [2], malaria transmitted by mosquitoes is currently responsible for 1.4% of global disease burden, with the vast majority of burden from among children in sub-Saharan Africa. Flooding from either heavy rains, Elnino, or artificially induced floods mostly result in creation of several ecotope layers in a habitat that promote the proliferation of mosquitoes including distribution and breeding [3]. Lake Baringo basin has diversity of habitats whose composition of fauna and flora is influenced by human activities, environmental changes and that are affected differently by floods. The basin has severally experienced outbreaks of diseases such as Rift Valley Fever (RVF), mostly attributed to Elnino/Southern Oscillation phenomenon (ENSO) rains causing flooding [4]. The notable Elnino floods in Lake Baringo occurred in 1997-1998 [5] and 2006-2007 [6] resulting in livestock and human death. However, other than floods resulting from heavy rains, an extreme and unprecedented flooding occurred in 2011-2014 in Lake Baringo basin and was associated with changes in earth geological factors that contributed into rise of lake waters and submerged farms along the lake shores [7]. This extreme flooding phenomenon is rare and is said to be a 50 year cycle with previous recorded occurrence from the Lake Baringo drainage basin having occurred in 1901 and 1963 [5, 8]. The phenomenon affected the mosquito dynamics in the region and from different habitats. In a study by Lutomiah [9] carried out in Lake Baringo basin, it was reported that mosquitoes of the genus *Manzonia* dominates the swampy areas. According to Paula [3], flooding at initial stages results in a decrease of mosquitoes of genus *Manzonia* from a habitat, but after a few weeks, the population goes up due to emergence of macrophytes. The prolonged floods can also result in loss of diversity with excess nutrients causing eutrophication effects [10]. This study, which commenced in October 2012 and ended in October 2013, therefore sought to understand probable changes that take place during periods

Appendix IV: Output 2: Scanned page of a publication in a Journal.

https://eajsti.org/index.php/EAJSTI/article/view/296

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Epidemiological patterns of Rift Valley Fever from diverse habitats during an extreme unprecedented flooding of Lake Baringo basin, Kenya, 2012-2013

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Abstract

Mosquitoes' ecology and associated arboviruses are heavily influenced by precipitation and retention of water in the environment. In 2011 and 2014, unprecedented floods occurred in Lake Baringo basin inundating approximate 88 km2 of the shoreline land. This caused abrupt environmental changes raising fears of an outbreak of Rift Valley Fever (RVF) disease. This study was carried out to determine the situation of RVF disease in livestock from diverse habitats during the extreme unprecedented flooding phenomenon that occurred in Lake Baringo basin, in 2012-2013. Blood was drawn from ear vein of livestock selected randomly from the three study areas (lakeshore land, swamp marshy and dry rangeland habitats). Mosquitoes were trapped using CDC light traps and identified morphologically. From a total of 77 blood samples, eight were positive for RVF virus (RVFV) representing an overall infection of 12%. RVF prevalence from livestock resident in flooded lakeshore land habitat was 2.6% (N=77) compared to the swamp marshy habitat at 7.8% (N=77). No infections were recorded from dry rangeland (0%). Mosquitoes of genus Mansonia dominated the catches in flooded lakeshore (98%). Highest individual catches of mosquitoes of genus Aedes was from swamp marshy area whose abundance was 96.8% and below 2% in other habitats. The Simpson's Diversity Index for mosquitoes from swamp marshy habitat was 0.56, dry rangeland 0.57 and lakeshore land 0.13. The flooded lakeshore land was the most affected by the unprecedented floods resulting in uneven mosquito diversity and subsequently low prevalence of RVF in this habitat. This could be attributed to prolonged disruption of biotic and abiotic factors creating unfavourable breeding sites of multiple species of primary vectors of RVF in flooded lakeshore land unlike in other habitats.

Keywords: *Lake Baringo; unprecedented floods; RVF prevalence; livestock; habitat*

Cite as: *Kamau et al., (2021).* Epidemiological patterns of Rift Valley Fever from diverse habitats during an extreme unprecedented flooding of Lake Baringo basin, Kenya, 2012-2013. *East African Journal of Science, Technology and Innovation 2(3).*

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Introduction

Stratification of an ecosystem into several diverse habitats is important in understanding environmental connection with the hosts, pathogens and vectors (Restrepo *et al.*, 2016). These variations help in giving an insight into spatial epidemiological patterns of a disease that could be a precursor to an outbreak (Wangara *et al.*, 2019). Lake Baringo basin harbours diversity of habitats whose composition of fauna and flora is influenced by human activities and environmental changes (Odada *et al.*, 2006). The basin has

Appendix V: Output 3: Abstract of presentation during the Kenyatta University Biennial Research and Conference 23rd – 25th October 2019.

KUBRIC 2019

COMPOSITION OF MOSQUITOES IN ECOLOGICAL HABITATS OF LAKE BARINGO BASIN DURING UNPRECEDENTED FLOODING

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Geological disturbances were previously reported to cause unprecedented flooding of Lake Baringo 50 years ago. Recently, this phenomenon reoccurred between the years 2011 to 2014. A total of 88 Km^2 (61.3%) of shoreline land was submerged. Because of the importance of the basin as habitat to mosquitoes that transmit arboviral diseases, a study on their composition and distribution was carried out during the flooding phenomenon in three ecological habitats. The habitats were swamp marshy, flooding shoreline and dry rangeland habitats. Eighteen (18) CDC light traps were used to trap mosquitoes around homesteads and grazing fields located in the ecological habitats for a period of 12 months. A total of 386,624 mosquito individuals were captured. There were 10 genera of mosquitoes identified with genus Mansonia constituting 84.9%, Culex 10% and Anopheles 3%. Other genera were rare, constituting only 1.5%. The composition of mosquito genera also differed significantly in ecological habitats. In conclusion, the unprecedented flooding did influence the composition and distribution of mosquitoes in the basin which could affect the risk to arboviral diseases.

Key words: Mosquitoes species, flooding; composition

Appendix VI: Additional photographs during the extreme flooding 2012-2013

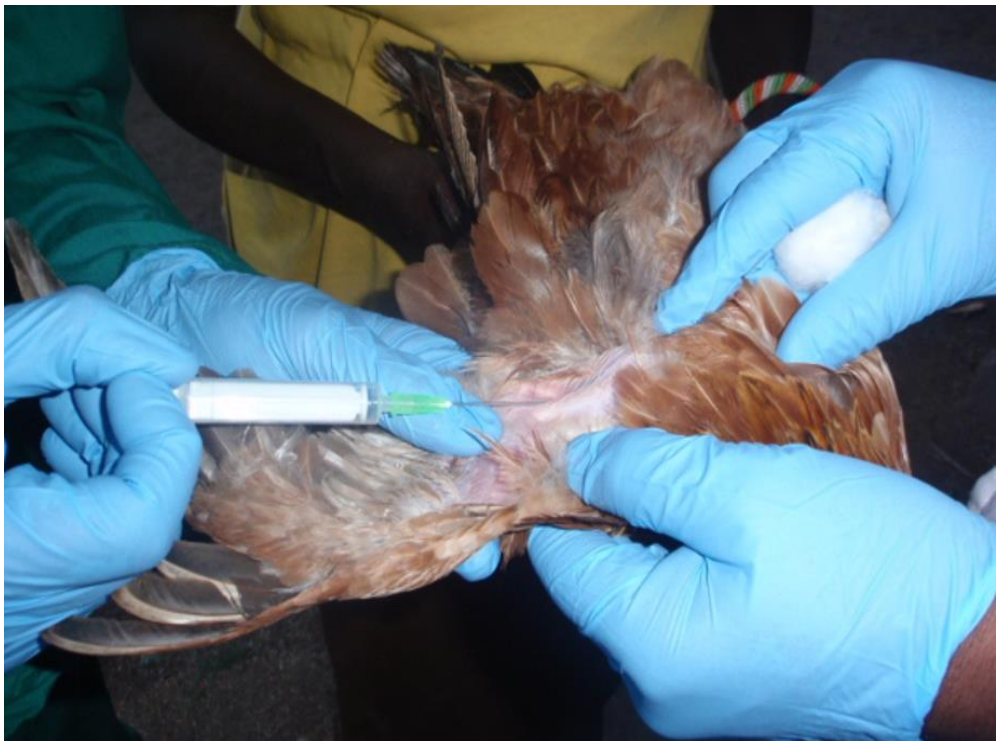
Homesteads before flooding of lake shoreline habitat in Lake Baringo basin

Submerged homestead after flooding of Lake Baringo in 2012

Submerged tourist hotel from the shores of Lake Baringo

Flooded and abandoned schools and offices following flooding of Lake Baringo in 2012

Livestock grazing alongside wildlife in swamp marshy habitat



Screening of poultry for arboviruses from a homestead in Lake Baringo basin