Environmental Drivers of Densities of Key Rift Valley Fever Virus Vectors and the Role of Host-Vector Interaction in Virus Maintenance in Epidemic Regions

Joel Lumbasi Lutomiah

A thesis submitted in partial fulfillment for the degree of Doctor of Philosophy in Medical Parasitology and Entomology in the Jomo Kenyatta University of Agriculture and Technology

2015

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

Signature_____ Date_____

Joel Lumbasi Lutomiah

This thesis has been presented for examination with our approval as the university supervisors.

Signature_____ Date_____

Rosemary Sang, PhD
KEMRI, Kenya

Signature_____ Date_____

Juliette R. Ongus, PhD
JKUAT, Kenya

Signature_____ Date_____

Kenneth J. Linthicum, PhD
USDA/ARS, USA

DEDICATION

TO MY PARENTS

This work is dedicated to my father Mr Reuben Lutomiah Kweyu, posthumously, who believed in everything right, first among them education for his children. For believing in me from the time he bent my left hand over my head to reach the right ear in order to determine if I had attained the right age to join school, and for providing me with the most needed guidance and support when I took my first small steps towards becoming a pupilage at Makunga Primary School. And to my mother, Sarah Ondieri w'Okoti, for her prayers and encouraging words while I was a candidate at Namulungu Boys Secondary School, and I quote: "I have never heard of anyone dying of working hard to excel, but if you will be the first, so be it". These words lit the fire in my belly that led me to excel beyond my own expectations.

ACKNOWLEDGEMENTS

I am greatly indebted to my supervisors and mentors, Dr. Rosemary Sang, Dr. Juliette Ongus and Dr. Kenneth J. Linthicum for their exceptional support and guidance at each and every step of my research work and thesis writing that gave form and shape to this document. I also thank Benedict Orindi for analysing the data and Dr. Lillian Musila for critically reviewing my thesis.

My sincere and utmost gratitude goes to members of the Arbovirology/ VHF laboratory and the entire Center for Virus Research (CVR) fraternity for the technical support that they provided, individually or collectively, during my study. Specifically Collins Mutai (Bloodmeal analysis), James Mutisya, Dunstone Beti, Reuben Lugalia, John Gachoya, Francis Mulwa, Betty Chelangat and Gilbert Rotich (mosquito collection and identification). The significant contributions they made will forever remain a part of this document.

Finally, in a special way, I wish to thank my wife, Betty Violet Lutomiah, whose welcoming smile I always looked forward to at the end of every single day that I worked my fingers to the bone as I walked the journey. Your support and encouragement provided the strength I needed. Indeed you were the wind beneath my wings. And for my children, I appreciate your patience and support for the duration I was deeply involved in this work.

MAY GOD BLESS YOU ALL

This study was funded by the USDA/ARS.

TABLE OF CONTENTS

LIST OF APPENDICES

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
APHIS	Animal and Plant Health Inspection Service
BABV	Babanki virus
BLAST	Basic Local Alignment Search Tool
BOLD	Barcode of Life Data systems
Bs	*Bacillus sphaericus*
Bti	*Bacillus thuringiensis israeliensis*
BUNV	Bunyamwera virus
CHIKV	Chikungunya virus
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
CO1	Cytochrome c oxidase subunit I
CO_2	Carbon dioxide
CPE	Cytopathic effect
cDNA	Complementary deoxyribonucleic acid
Cytb	Cytochrome b
DENV	Dengue virus
DEET	N,N-Diethyl-meta-toluamide
DI	Disseminated infection
DIR	Disseminated infection rate
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DPF	Day post flooding
DTT	DL-Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EEEV	Eastern equine encephalitis virus
EFSA	European Food Safety Authority

EIP	Extrinsic incubation period
ELISA	Enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
FAO	Food and Agriculture Organization
FBS	Fetal bovine serum
FCCMC	Florida Coordinating Council on Mosquito Control
FDA	Food and Drug Administration
GPS	Global positioning system
IEP	Inter-epizootic period
ILRI	International Livestock Research Institute
IgG	Immunoglobulin G antibodies
IGR	Insect growth regulators
IJR	Ijara
IPM	Integrated pest management
ITN	Insecticide treated net
KRV	Kamiti river virus
KEMRI	Kenya Medical Research Institute
LAC	La Crosse virus
LN_2	Liquid nitrogen
MA	Massachusetts
MEGA	Molecular Evolutionary Genetic Analysis
MEM	Minimum Essential Media Eagles
mtDNA	Mitochondrial Deoxyribonucleic acid
MI	Mid gut infection
MIR	Mid gut infection rate
MPL	Maximum Permissible Limits
$NaHCO_3$	Sodium bicarbonate
NCBI	National Center for Biotechnology Information
ND	Number of dips

NE	Northeastern
NDUV	Ndumu virus
NRI	Ngari virus
NSP	Nonstructural protein
PBS	Phosphate buffered saline
PGAV	Pongola virus
PH	Potential of hydrogen
P.i.	Post infection
RNA	Ribonucleic acid
RNP	Ribonucleic protein
RR	Relative risk
RRV	Ross river virus
rpm	Revolutions per minute
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SFV	Semliki forest virus
SGB	Salivary gland barrier
SINV	Sindbis virus
TOT	Transovarial transmission
TSI-GSD	The Salk Institute–Government Service Division
USA	United States of America
USAMRIID	United States Army Medical Research Institute of Infectious Diseases
USDA	United States Department of Agriculture
VHF	Viral hemorrhagic fever
WHO	World Health Organization
WNV	West Nile virus
YFV	Yellow fever virus

ABSTRACT

Rift Valley fever virus (RVFV) is a mosquito-borne *Phlebovirus* that causes outbreaks such as witnessed in Kenya in 1997-1998 and 2006-2007. Vertebrate animals become infected through bites of infected mosquito vectors while exposure to tissues or body fluids of infected animals is the main mode of transmission to humans. Generally, there is limited knowledge on the transmission dynamics and vertebrate reservoirs of Rift Valley fever virus (RVFV) and other arboviruses. The effect of environmental variables of the breeding habitats on the type of mosquito species has been studied elsewhere but not in Kenya. Therefore this study was conducted in the Northeastern, Garissa County, to evaluate the association between environmental variables and larval composition and densities of key RVFV vectors, and the role of host-vector interaction in the maintenance of the virus in El-Humow, an RVF epidemic region. The results of this study showed a significant difference in species composition between Marey and Wakab-Harey ($p=0.005$). However there was no association between species composition and physical characteristics of *dambos* in both areas. There was also no significant association between the water temperature and species composition, (Marey: $p=0.9$; Wakab-Harey: $p=0.100$). On the other hand, there was a significant association between species composition and the levels of pH ($r=0.45$, $p=0.001$). Transovarial transmission (TOT) was demonstrated for Ndumu virus (NDUV) in *Culex pipiens* mosquitoes collected as larvae. Both *Aedes ochraceus* and *Aedes mcintoshi* obtained bloodmeals from the same vertebrate hosts in almost equal proportions with goats (*Capra hircus*), cattle (*Bos taurus*) and donkeys (*Equus asinus*) being the most common sources. The percentages of *Ae. ochraceus* and *Ae. mcintoshi* with human (*Homo sapiens*) blood were nearly identical at 6% and 7.3% respectively. Among domestic animals, camels (*Camelus dromedarius*) were the least fed upon. Midgut infection rates (MIR) of 9.5% in *Ae. ochraceus* were highest in specimens that had fed on sheep. While there was no RVFV isolate from bloodmeals from goats, 68.4% (13/19) of NDUV isolates were from mosquitoes with bloodmeals from goats of which 46.2% (6/13) had disseminated infection. This study demonstrated an association between physical and

environmental characteristics of *dambos* and the composition and densities of secondary vectors but not primary vectors of RVFV. It has also demonstrated that RVFV vectors fed randomly on available vertebrate hosts, although preferably on goats, during the 2006-2007 RVF outbreak. Some viruses such as NDUV are also maintained in nature by TOT. More studies on species succession during wet seasons should be conducted in diverse RVFV epizootic regions. Specifically, the effect of physical and environmental characteristics of breeding habitats on the diversity and density of mosquito spp. Studies on TOT and the prevalence of RVFV and other arboviruses in bloodfed field-collected mosquitoes during wet seasons should also be conducted further.

CHAPTER ONE
INTRODUCTION

1.0 Background Information

In Kenya, for example, RVF outbreaks, including the 2006-2007 outbreak (Sang *et al.*, 2010), have historically occurred repeatedly in two ecologically distinct regions; the Northeastern (NE) and Rift Valley, and are associated with anomalous heavy rainfall (Davies, Linthicum & James, 1985; Woods *et al*., 2002; King *et al*., 2010). Both regions are generally very hot with semiarid conditions, and are occupied by pastoral and agropastoral communities that rear large herds of livestock which are good amplifiers of the virus. Some of the hotspots of the 2006-2007 RVF outbreak were El-Humow in Garissa and Sangailu in Ijara, Garissa County NE Kenya; and in Marigat, Baringo County in the Rift Valley.

These two regions are dominated by the key mosquito vectors of Rift Valley fever virus (RVFV) such as *Ae. mcintoshi* and *Ae. ochraceus* in the NE Kenya and *Mansonia uniformis* and *Mn. africana* in the Rift Valley (Lutomiah *et al*., 2013). It is not clear what factors influence the difference in mosquito species predominance in the respective areas and how RVFV and other viruses are maintained in nature between epidemics. However occurrence of numerous *dambos* (seasonally waterlogged areas) in the NE Kenya, the primary breeding habitats of floodwater *Aedes* mosquitoes (primary vectors of RVFV), and the swampy areas in Marigat (preferred by *Mansonia* spp.) could be responsible for the differences in species distribution and dominance in the respective areas. Therefore the species that initiate virus transmission vary from region to region and not necessarily the floodwater *Aedes* spp. However the secondary vectors (*Culex* spp.), which disseminate the virus to perpetuate the outbreaks cut across the two regions. Understanding the habitats of floodwater *Aedes* mosquitoes is fundamental in the efforts to manage RVF through vector control.

Although the change in population structure of mosquito species over time (species succession) has been studied extensively, the findings are usually not representative of geographically distinct regions. This is because of the aforementioned

geographical variation in species composition. For instance, in a previous study in a forest in Kenya, it was found that floodwater *Aedes* mosquitoes: *Ae.* (*Aedimorphus*) *cumminsii, Ae.* (*Aed.*) *dentatus, Ae.* (*Mucidus*) *sudanensis, Ae.* (*Neomelaniconion*) *mcintoshi, Ae.* (*Neo.*) *circumluteolus*, etc, were the first to emerge from flooded *dambos,* followed by *Culex* and *Anopheles* spp. (Linthicum *et al.,* 1983). In another study conducted in a permanent marsh habitat, *Cx. pipiens* were the predominant species observed (Logan *et al*., 1991b). It was not clear how the species structure in these two studies would compare with one in a different ecological setting that is prone to RVF outbreaks. Therefore species succession in Sangailu, NE Kenya and how it would influence the epidemiology of RVF is not well understood.

Transovarial transmission (TOT) of RVFV has been demonstrated on two occasions only (Linthicum *et al*., 1985b; Mohamed *et al*., 2013). Efforts to demonstrate TOT in the laboratory have been less successful. For instance Romoser and his colleagues (2011) demonstrated limited TOT by detection of the virus in individual developing eggs without infection of the entire ovary. As such there is limited evidence on TOT of RVFV as well as other arboviruses. Therefore continued field studies, especially in RVF epidemic areas, may provide further evidence of the occurrence of this phenomenon in nature. This is important to understand the epidemiology of diseases. While targeting to demonstrate TOT of RVFV in field collected mosquitoes, it is possible that other arboviruses are also found to be maintained in nature through this process. This is because several arboviruses have been found circulating concurrently during outbreaks and the inter-epidemic period (IEP) (Bowen *et al.,* 2001; Turell *et al*., 2002; Gerrard *et al*., 2004; Crabtree *et al*., 2009; LaBeaud *et al*., 2011a).

Although RVFV has been associated with many mosquito spp., there is limited laboratory evidence to support the vector competence of some of them. This is because vector competence studies require appropriate containment facilities not available in many laboratories. Besides, it is extremely difficult or thus far impossible to rear some of these species in the laboratory. Therefore, one of the ways of determining if a mosquito sp. is a vector is by analysing the infection and dissemination status of

bloodfed/ engorged mosquitoes collected during an outbreak. This analysis can also provide insights into which species may have initiated transmission and those that possibly perpetuated the epidemic especially if the collection dates are put into consideration. Therefore knowledge of the vectorial capacity of a vector and its host-feeding preference in field studies can help to define the role of vertebrates in pathogen amplification and maintenance in nature (Townzen *et al*., 2008; Kent, 2009) and the epidemiology of arboviruses.

1.1 Statementof the Problem

RVF epidemics occur irregularly every 5-10 years in the Northeastern part of Kenya resulting in huge economic losses due to deaths of, and abortions in domestic animals (Kaschula, 1957; Easterday, 1965; Munyua et al., 2010) and trade embargoes (Little *et al*., 2001). Human infection causes significant disease in 1-2% of the cases (McIntosh *et al*., 1980; Woods *et al*., 2002). A case fatality rate of 10–20% has also been documented (Madani *et al*., 2003; Swanepoel & Coetzer, 2004; Rich & Wanyoike, 2010).

Despite the Northeastern part of Kenya being a RVF hotspot, few studies relating to RVFV have been conducted there. The primary vectors of RVFV breed in *dambos* which become active during the rain season. Livestock and wildlife become exposed to transovarially-infected mosquitoes when they come to these *dambos* in search of drinking water. However TOT of RVFV has not been studied in RVF hotspots. *Dambo* characterization has also not been conducted; and breeding habitat preference not evaluated. Similarly the host-feeding patterns of key vectors of RVFV during an outbreak have not been determined. All these factors are important in designing targeted and effective control measures in the event of increased risk of virus transmission.

1.2 Justification

Mosquito spp. succession has been studied only to a limited extent in Kenya (Linthicum *et al.,* 1983) in areas that have not experienced explosive RVF. There is need to understand the biology and ecology of RVFV vectors, especially given the recent

occurrence of RVF outbreaks in non-outbreak areas. These include the relationship between physical and environmental factors of breeding habitats and their productivity for key vectors of RVFV. This is important for making informed decisions regarding the type of habitats that should be targeted for larviciding to avert an outbreak when it is anticipated. Current evidence for TOT of RVFV is lacking as there is limited evidence of this phenomen in mosquitoes (Linthicum *et al.,* 1985a; Mohamed *et al.,* 2013). There is also no data on vector-host interaction during RVF outbreaks. The host-feeding preferences by key vectors of RVFV during the 2006-2007 outbreak in the NE is unknown as are the hosts that were most significant in the amplification of RVFV. Hence blood-meal analysis and virus detection assays will provide a better understanding of the role of vectors and vertebrate hosts in the transmission and amplification of RVFV in the RVF hotspots. Knowledge on the host-feeding pattern of these vectors is important to inform scientists involved in studies on RVFV about ways in which the virus is maintained and the host animals that are involved.

1.3 Research Questions

1) How does the population structure of mosquito species in *dambos* change over time during and after flooding?
2) Is there an association between species composition and densities and the physical and environmental characteristics of the *dambos*?
3) What is the role of transovarial transmission in the maintenance of Rift Valley fever virus and other arboviruses in nature?
4) What were the host feeding patterns of key Rift Valley fever virus vectors during the 2006-2007 Rift Valley fever outbreak?
5) What was the prevalence of Rift Valley fever virus and other arboviruses in bloodfed mosquitoes collected during the 2006-2007 Rift Valley fever outbreak and inter-epizootic period?

1.4 Hypotheses

1.4.1 Null hypotheses:

1. The population structure of mosquito species does not change during and after flooding
2. There is no association between physical and environmental variables of *dambos* and the composition and densities of key RVFV vectors
3. Arboviruses are not maintained in nature by TOT
4. Bloodfed RVFV vectors collected during the 2006-2007 outbreak did not feed on specific preferred vertebrate hosts
5. Bloodfed field-collected mosquitoes are not infected with arboviruses

1.5 Objectives

1.5.1 General Objective

To evaluate the association between environmental characteristics and larval densities of key RVFV vectors and the role of host-vector interaction in the maintenance of the virus in an RVF epizootic/ epidemic region

1.5.2 Specific Objectives

1. To evaluate the trend in population structure of mosquito species (species succession) in Sangailu, NE Kenya, during and after flooding
2. To evaluate the relationship between *dambo* surface area, depth, water pH, temperature and the larval composition and densities of RVFV vectors in Sangailu
3. To determine TOT of RVFV and other arboviruses in mosquitoes in nature
4. To determine the host-feeding patterns of key vectors of RVFV collected during the 2006-2007 outbreak
5. To determine the prevalences of RVFV and other arboviruses in bloodfed field-collected mosquitoes

1.6 Study Limitations

This current study had a few limitations. For example the species succession data presented is based on one-time sampling during short rains. This may not represent the succession trend that would be expected during prolonged rains. This is because the short rains are usuaaly insufficient to support complete development and proliferation of many mosquito spp. due to the quick drying of breeding habitats. Also due to the rough terrain in the Northeastern and the unpredictability of the rainfall pattern, there were challenges in reaching the study site in good time to commense on sampling.

CHAPTER TWO

LITERATURE REVIEW

2.0 Arboviruses

Arboviruses are a large group of single-stranded RNA viruses which are mostly transmitted by blood-sucking arthropods. Approximately 134 of them are known to cause diseases in humans (Karabatsos, 1985). These viruses generally have a global distribution, with the majority occurring in tropical areas where climatic conditions permit year-round transmission (Gubler, 1996). The presence of arboviruses in an area depends on the availability of specific arthropod vectors and animal hosts. Favourable environmental conditions are also prerequisites to successful virus amplification and transmission (Randolph & Rogers, 2010). Human infections (epidemics) result from mosquito bites which become infected by feeding on viraemic vertebrates.

Arboviruses are usually restricted to specific habitats and are seasonal with transmission corresponding to the abundance of competent vector species (Day & Shaman, 2011, Ochieng *et al.*, 2013). The geographic and seasonal distributions of many infectious diseases, including Rift Valley fever (RVF), are linked to climate.

2.1 Rift Valley fever virus (RVFV)

RVF is a vector-borne zoonotic disease of domestic ruminants that causes epizootics and associated human epidemics in Africa and the Middle East (Peters & Linthicum, 1994; CDC, 2000a). It was first described by Daubney, Hudson & Graham, (1931) during an outbreak in sheep in Naivasha in the Rift Valley, Kenya. However, periodic RVF epizootics were first observed in Kenya as early as 1912 in the then Rift Valley Province (Montgomery & Stordy, 1913; Stordy, 1913). Although the virus has been associated with a wide variety of animals, the disease seems to be limited to domestic ruminants and humans (Meegan & Bailey, 1988).

RVFV is a tri-segmented negative-sense single-stranded RNA virus comprising large (L), medium (M) and small (S) genome segments (Schmaljohn *et al.*, 1996). RVFV belongs to the genus *Phlebovirus* and family *Bunyaviridae* (Murphy *et al.*, 1995; Torres-Velez & Brown, 2004), a group of enveloped RNA-viruses (Flick & Bouloy,

2005). The L, M and S segments are packaged together into virions by ribonucleoproteins (RNP), with the 3' and 5' terminal sequences complementary to each other, forming panhandle structures, and giving the RNP its circular shape (Elliott, 1996). Also in the family *Bunyaviridae* are other viruses of public health importance such as Hantavirus and the Crimean Congo hemorrhagic fever virus. RVFV causes high mortality in newborn ruminants, especially sheep and goats, abortion in pregnant animals and human disease (Davies, Linthicum & James, 1985; Peters & Linthicum, 1994) (Fig 2.1).

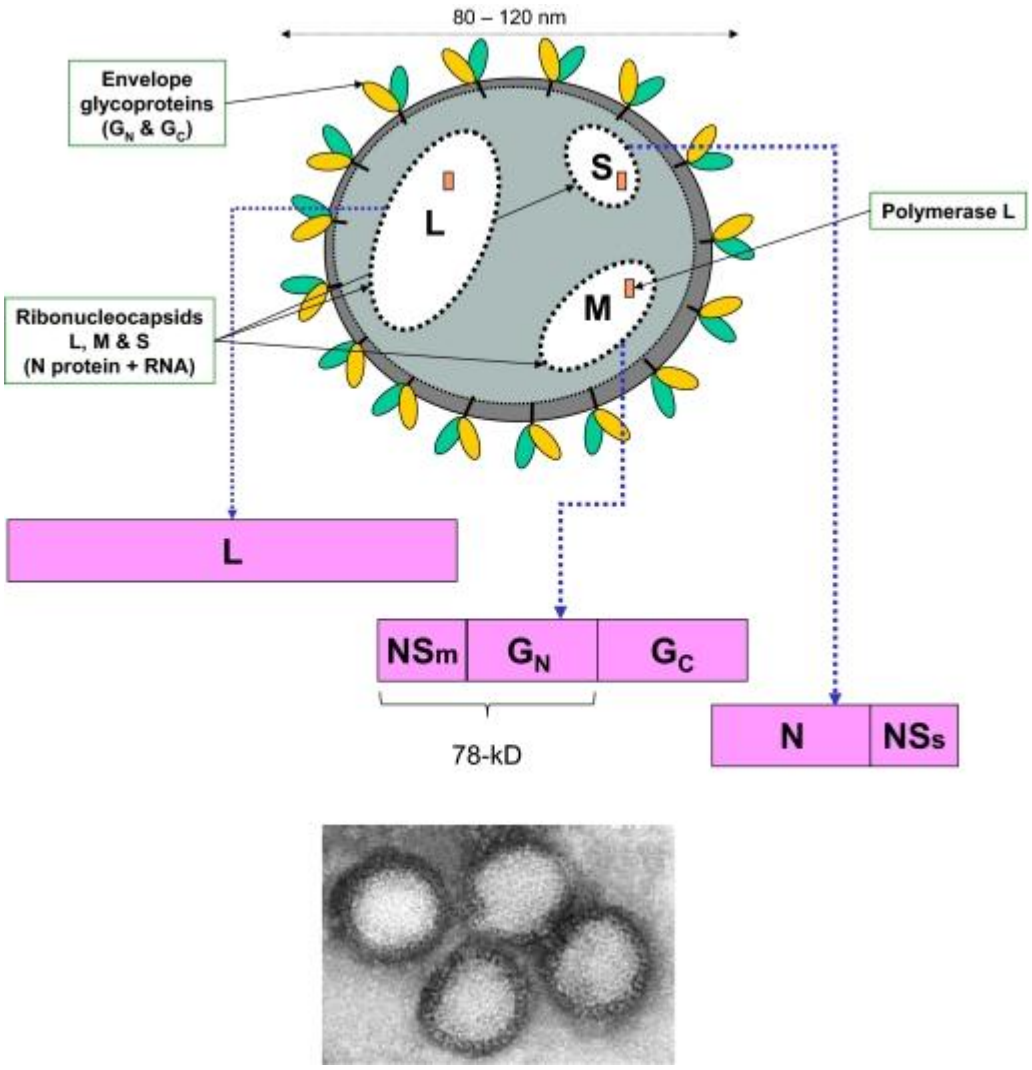


Figure 2.1: Schematic structure of RVFV (electron micrograph from Linda Stannard Theiler M). The structure shows the three segments comprising of large (L), medium (M) and small (S) genom segments. Adopted from Tolou H., Plumet S., Leparc-Goffart I., Couissinier-Paris P., (2009). Le virus de la fie`vre de la valle´e du Rift: e´volution en cours, Me´d. Trop. 69:215–220

2.2 Epidemiology of RVFV

2.2.1 Virus transmission and associated risk factors

The bite of infected mosquitoes constitutes the main means of transmission of RVFV in animals (Bouloy *et al*., 2008; Bird *et al*., 2009) and dissemination between herds or flocks over short distances (Chevalier *et al*., 2004). On the other hand, direct contact of animals and humans with infected tissues such as aborted foetuses is the predominant infection route during the amplification stage of the epizootic (Swanepoel & Coetzer, 1994; Morrill & McClain, 1996; Swanepoel & Coetzer, 2004). Aborted foetal material and placental membranes contain large numbers of virus particles which can either contaminate the local environment or infect animals that come in direct contact (Pe´pin *et al*., 2010). This infection route is significant because the virus can persist for long in the environment (Andrewes & Horstmann, 1949; Theiler, 1957; Craig *et al*., 1967). Human infections also occur through exposure to blood and secretions/ excretions of infected animals, as well as through ingestion of raw milk from infected animals and inhalation of aerosols especially in laboratory personnel (Swanpoel & Coetzer, 1994; WHO, 2010).

Exposure to tissues of infected ruminants was the main cause of human infection among veterinarians during the 2006-2007 and the 2008 epidemics in Kenya and South Africa respectively (Archer *et al*., 2011). This is also the main reason that shepherds and wildlife rangers are at high risk of infection (Abu-Elyazeed *et al.*, 1996). While mechanical transmission to laboratory animals by mosquitoes and biting flies has also been demonstrated (Hoch *et al*., 1985), human-to-human transmission is uncommon and has only been documented in one fatal case (Arishi, Aqeel & Al Hazmi, 2006). The virus is presumed to gain entry through abraded skin, wounds, mucous membranes and aerosols (Swanpoel & Coetzer, 1994).

2.2.2 Clinical manifestations and diagnosis of RVF

A sudden increase in the rates of abortions in ruminant livestock, especially in sheep and goats, should always raise the suspission of RVF as a possible etiological cause (Kapoor, 2008). Clinical symptoms of RVF vary among patients, and human

infection can either be asymptomatic or associated with an acute syndrome of fevers, weakness, myalgias, dizziness, anorexia, weight loss, and headaches (Madani *et al.*, 2003). The fever is biphasic in that temperature rises for 2 days, decreases, and then rises again (Kapoor, 2008). RVF is also characterized by nausea, vomiting and diarrhoea, and the patient may also present with conjunctivitis and photophobia in 5% of the haemorrhagic cases, which occasionally leads to death (Swanpoel & Coetzer, 1994). Haemorrhagic cases occur in less than 1% of patients and may appear as early as two days after the onset of symptoms (Swanepoel, Manning & Watt, 1979). Kenya has RVFV seroprevalence of up to 32% in high-risk regions (Woods *et al.*, 2002). During IEP the seroprevalence rates are between 1 and 19% (urban and rural settings respectively) with an overall inter-epidemic RVFV seropositivity rate of 13% (LaBeaud *et al.*, 2007; LaBeaud *et al.*, 2008). Although the lethality for humans infected with RVFV is below 2%, case fatality rate of 23% was reported in the 2006-2007 outbreak in Kenya (LaBeaud *et al.*, 2008).

The disease is clinically difficult to diagnose in the absence of haemorrhagic fever, which occurs in less than 1% of patients (Swanepoel, Manning & Watt, 1979), or specific organ manifestations. Definitive diagnosis depends mainly on reliable laboratory tests (Pepin *et al*., 2010). However, RVF may be suspected when there is concurrence of a sudden outbreak of febrile illness with headache and myalgia in humans, and abortions in domestic ruminants and deaths of young animals (Pepin *et al*., 2010).

Laboratory diagnosis of RVF is achieved using various techniques, including virus isolation (Anderson *et al*., 1989) from serum or whole blood, antigen detection (Niklasson *et al*., 1983, Meegan *et al*., 1989), PCR techniques (Drosten *et al*., 2002; Garcia *et al*., 2001) and detection of RVFV-specific antibodies (Swanepoel, Struthers, & Erasmus, 1986). Some of these techniques have disadvantages in that they require a long time to perform, hence are not suited for public health institutions faced with an RVF epidemic that requires immediate intervention (Pepin *et al*., 2010). In the recent past, more advanced and sensitive techniques have been developed for the rapid detection and

identification of RVFV. These include real time PCR (RT-PCR) TaqMan probe (Ibrahim *et al.,* 1997; Garcia *et al*., 2001, Sall *et al.,* 2002; Bird *et al.,* 2007; Wanja *et al.,* 2011)) and real-time reverse-transcription loop-mediated isothermal amplification assays (RT-LAMP) (Peyrefitte *et al*., 2008, Le Roux *et al*., 2009).

2.2.3 Treatment and management of RVF infection in humans

There are no therapeutic drugs for RVF hence treatment is entirely supportive (CDC, 1995). However, medications that are metabolized by the liver are not recommended (Kapoor, 2008).

2.3 Vectors of RVFV

Prior to 1987, transmission of RVFV was associated with a limited number of mosquito species (Pepin *et al*., 2010). These included members of the subgenera *Neomelaniconion* and *Culex*, which are responsible for maintenance and amplification of RVFV (McIntoshi *et al.,* 1983; Jupp & Cornel, 1988). RVFV vectors are classified into primary and secondary vectors. The primary vectors, also referred to as ‘‘reservoir/ maintenance’’ vectors, are members of the sub-genera *Neomelaniconion* and *Aedimorphus.* In sub-Saharan Africa, *Ae.* (*Neomelaniconion*) *mcintoshi*, *Ae.* (*Neo.*) *circumluteolus* and *Ae. ochraceus* are the most important primary vectors (Gargan *et al*., 1988a). On the other hand, secondary or ‘‘epidemic/ amplifying’’ vectors belong to a wide variety of species in the genera *Culex* and *Aedes* (Linthicum *et al.,* 1985a). The virus has been isolated from a large number of species during and between epidemics (Linthicum *et al.,* 1985a; Traore-Laminzana *et al.,* 2001; Jupp *et al.,* 2002; Miller *et al.,* 2002; Diallo *et al.,* 2005; Sang *et al*., 2010). Laboratory studies have also shown that there is a worldwide distribution of competent vectors for RVFV, including North America, Saudi Arabia and Africa (Turell & Bailey, 1987; Gargan *et al.,* 1988b; Jupp & Cornel, 1988; Turell, Bailey & Beaman, 1988; Jupp *et al.,* 2002).

In Kenya, RVFV has been detected in many species, including *Ae.* (*Aedimorphus*) *cumminsii, Ae. mcintoshi, Anopheles christyi, An. pharoensis, Cx. antennatus, Cx. vansomereni, Cx. zombaensis*, and *Cx. rubinotus* (Linthicum *et al.,*

1985a). Logan *et al.*, (1991b) also isolated the virus from *Cx. pipiens, Cx. univittatus, Cx. ventrilloni, Coquillettidia pseudoconopas, Cq. versicolor, Cq. aurites, Mansonia africana, Ae. quasiunivittatus, Ae. dentatus, Ae. circumluteolus* and *An. squamosus*. During the 2006-2007 outbreak, RVFV was also isolated from *Ma. uniformis*, *Cx. bitaeniorhynchus, Cx. pocilipes* and *Ae. pembaensis* among other species (Sang *et al*., 2010).

However, other than virus isolation from most field collected mosquitoes, there is limited laboratory evidence to support the epidemiological role of many of these mosquitoes for RVFV transmission. This is because many laboratories lack appropriate containment levels to handle RVFV and its potential vectors coupled with the rearing challenges associated with these mosquitoes to allow for appropriate vector competence studies to be successfully conducted. Mosquito populations from different geographic areas also differ in their vectorial capacity for arboviruses (Gubler, 2001; Goddard *et al.*, 2002; Herrera *et al*., 2006) hence what is known for a species from one region may not apply for the same species in a different region.

2.3.1 Transmission cycle of RVFV

The transmission cycle of RVFV involves two processes: TOT and horizontal transmission.

2.3.1.1 Transovarial transmission (TOT)

TOT, also known as vertical transmission, is the transmission of a virus from a parent host vector to the offspring through the female ova (Hogenhout *et al.*, 2008). It involves virus entry into a fully formed egg during oviposition (Tesh & Cornet 1981), or venereally, though rarely, when an infected male mosquito passes over the virus to the offspring *via* the sperm during copulation with uninfected female (Simmons *et al*., 1931). During a RVF outbreak, parous floodwater aedines, including *Ae. mcintoshi* and *Ae. circumluteolus*, oviposit on the soils surrounding the standing water of the *dambos* (Mellor & Leak, 2000) and temporary water bodies (riverines) along large rivers and lakes (Knight & Baker, 1962; Cassani & Bland, 1978). When the heavy rains subside

and the *dambos* dry up, the already laid eggs in the soil, some infected with the virus, undergo an obligatory period of embryonation and dehydration in readiness to hatch during subsequent flooding (Mellor & Leak, 2000, Pepin *et al*., 2010). It is therefore thought that the virus is maintained in the drought-resistant eggs over long periods of time (Linthicum *et al*., 1985b; Gargan, Jupp & Novak, 1988a; Becker, 1989) especially during prolonged drought (Swanpoel & Coetzer, 1994). Current evidence for TOT is based on the isolation of RVFV from mosquitoes collected as larvae and pupae (Linthicum *et al.,* 1985b), as well as male mosquitoes collected as adults (Mohamed *et al*., 2013).

2.3.1.2 Horizontal transmission

Horizontal transmission is the transmission of a virus between a vector and a vertebrate host. During times of abnormally heavy rains when the *dambos* are flooded, and environmental factors such as water temperatures and dissolved oxygen are conducive (Rydzanicz, Kącki & Jawień, 2011), an explosion in the number of floodwater mosquitoes occurs (Linthicum *et al*., 1985c). Some of the larvae that hatch from eggs are already infected with the virus. When these develop into adults, they transmit the virus to susceptible livestock and wildlife, which may be attracted to the watering points. It has been argued that in East Africa RVFV is maintained over several years at animal watering sites by horizontal transmission between ruminants and mosquitoes during the rainy season and by TOT in *Aedes* mosquitoes during the dry season (Chevalier, Thiongane, & Lancelot, 2009). These animals serve to amplify the virus to high titres and, if the *dambos* remain flooded for 2 to 3 weeks, the floodwater *Aedes* spp. are then succeeded by *Culex* spp., which oviposit egg-rafts on the water surface, leading to a population explosion of *Culex* sp. mosquitoes, which become infected upon feeding on viraemic vertebrate hosts (Pepin *et al*., 2010). Whereas the floodwater *Aedes* spp. tend to remain in the immediate vicinity of the larval habitats and only feed at dusk and dawn, the more nocturnal *Culex* spp., disperse to find vertebrate hosts to feed on, leading to extensive dissemination of virus and occurrance of outbreaks (Pepin *et al*., 2010), (Fig. 2.2).

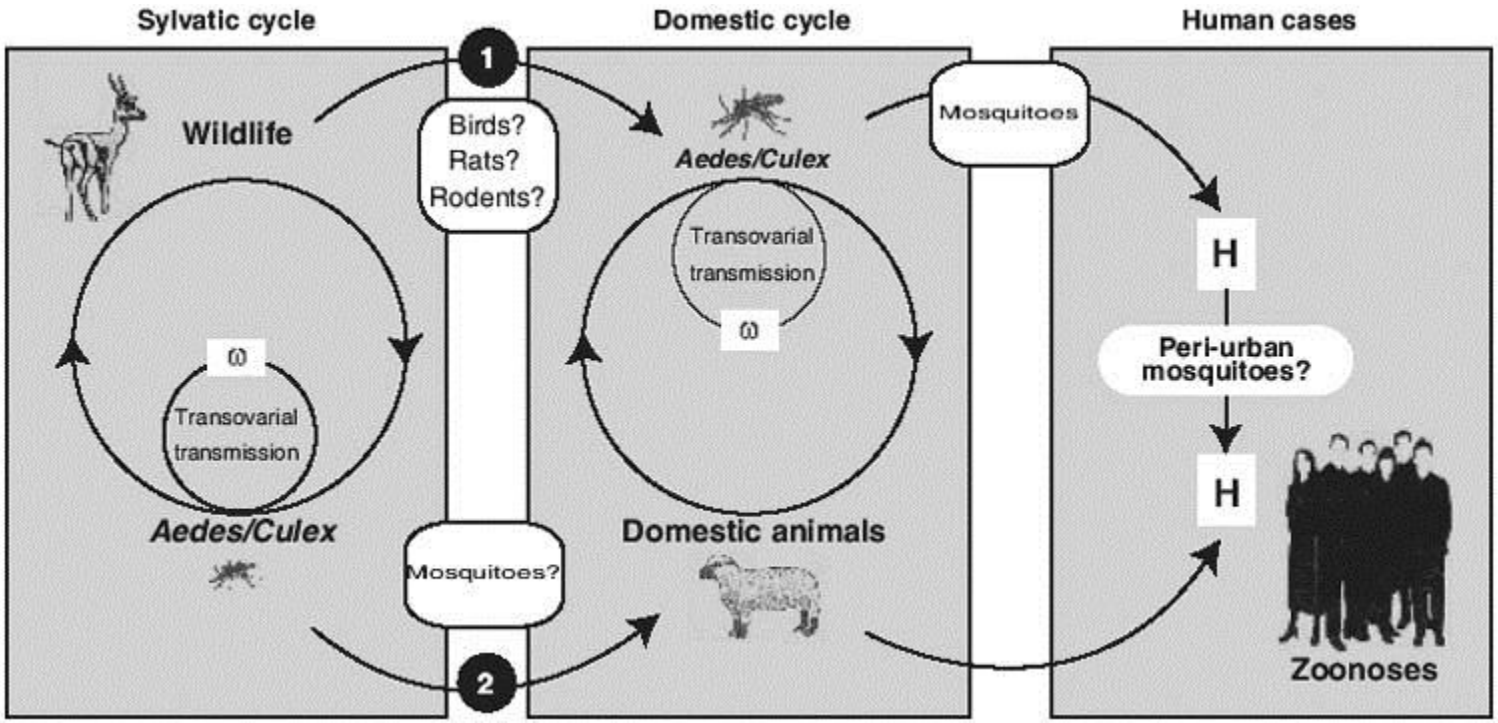


Figure 2.2 *The transmission cycle of RVFV. The figure shows the Sylvatic and Domestic cycles. The sylvatic cycle purely involves transmission of RVFV between the sylvatic mosquitoes and wildlifw while the domestic cycle involves transmission between mosquitoes and livestock with incidental transmission to humans. Adopted from Davies F. G. and Highton R. B. (1980). Possible vector of Rift Valley fever in Kenya. Transactions of The Royal Society of Tropical Medicine and Hygiene, 74: 815-25.*

2.4 RVF outbreaks and the climate

The first Rift Valley fever (RVF)-like disease was reported in livestock in 1912 near Lake Naivasha in the Rift valley province (Montgomery & Stordy, 1912; Stordy, 1913). Since then, RVF outbreaks have occurred in several parts of the country. One of the biggest epidemics occurred in 1997 in the NE Kenya where nearly 27,500 people were infected (CDC, 1998). The most recent one occurred between 2006 and 2007, mainly in the same region but also in Baringo in the Rift valley province where the virus was reported for the first time (Nguku *et al*., 2010; Sang *et al.*, 2010). The largest RVF outbreak ever recorded was in Egypt along the Nile River (1977–1979), which afflicted approximately 200,000 persons and caused 594 deaths (Meegan, 1979).

Outbreaks have been closely associated with *dambos* and unusually heavy rainfall in endemic areas (Linthicum *et al*., 1983; Woods *et al*., 2002). They occur at intervals of 5-15 years (Linthicum *et al*., 1999). When the *dambos* get flooded, the floodwater *Aedes* mosquito populations, primarily *Ae. mcintoshi* and *Ae. ochraceus* (Gargan, Jupp & Novak, 1988a), some already infected with RVFV, explode (Davies, Linthicum & James, 1985; Gerdes, 2004). Therefore flooded *dambos* are perfect habitats for floodwater mosquitoes (Linthicum *et al.,* 1983) conveniently bringing vectors in close proximity with vertebrate hosts as they serve as water sources for livestock and wildlife. The primary vectors transmit the virus to nearby animals (Davies & Highton, 1980) which then amplify the virus to sufficient titres to infect even inefficient vectors. The secondary vectors which belong to a wide variety of species then perpetuate infection setting off outbreaks.

2.4.1 Social and economic impact of RVF outbreaks

Outbreaks of RVF are often associated with high case fatality rates of up to 30% and between 80 and 100% of abortions in domestic animals (Kaschula, 1957; Easterday, 1965). Past outbreaks in South Africa (1951), Egypt (1977-1978), Kenya (1997), and Saudi Arabia (1998–2000) also resulted in the cumulative loss of thousands of human lives (Rich & Wanyoike, 2010). The 1997–1998 outbreak was the largest documented in

the Horn of Africa with an estimated loss of approximately 100,000 domestic animals, and approximately 90,000 human infections (Little, Teka & Azeze, 2001; Woods *et al*., 2002). Therefore, RVFV infection of sheep, cattle, goats, or camels results in substantial economic loss to the farmers, and the country, due to restriction of exportation of animals to other countries (Meegan & Bailey, 1988). For example the 2000 outbreak in Saudi Arabia (CDC, 2000a) led to the trade bans of live animals from Ethiopia, Somalia, and Kenya resulting in economic losses estimated in millions of dollars. A socio-economic study conducted after the 2006–2007 RVF outbreak in Kenya demonstrated huge financial losses incurred by the livestock production and marketing chains. Livestock producers lost an estimated Ksh 758,000 (USD 8,713) per camel producer from loss of milk due to abortions, up to Ksh 180,000 (USD 2,069) per livestock trader due to animal deaths; between Ksh 132,000 (USD 1,517) and Ksh 1.44 million (USD 16,552) per slaughterhouse due to closures of slaughterhouses, and Ksh 125,000 (USD 1437) per butcher due to closure of business or reduced numbers of animals killed, (Rich & Wanyoike, 2010). The outbreaks' negative impacts on agriculture and other sectors (transport, services, etc.) resulted in the loss of approximately Ksh 2.1 billion (USD 24 million) to the Kenyan economy (Rich & Wanyoike, 2010).

2.5 RVF outbreak prediction, prevention and control

2.5.1 Prediction of RVF outbreaks

Because of the socio-economic impact of RVF in most African countries, the absence of drugs against RVF and the lack of approved vaccine for humans, it is important to prevent an outbreak before it occurs. The geographical and seasonal distributions of many infectious diseases, including RVF, are linked to climate. RVF outbreaks in particular are associated with anomalous heavy rainfall (Davies, Linthicum & James, 1985; Woods *et al*., 2002; King *et al*., 2010) hence meteorological forecasting of extreme weather is useful in predicting and providing an early warning for an outbreak (Linthicum *et al*., 1999; Anyamba *et al*., 2009). An accurate detection of parameters, such as ground moisture, that determine flooding could provide local

officials with sufficient warning of a potential outbreak to allow for timely deployment of vector surveillance and control teams as well as widespread livestock vaccination to prevent an outbreak or spread of the disease (Linthicum *et al*., 1990). However, because RVF outbreaks are not generally identified/ recognized until human cases occur, a well-developed strategy that uses animals as sentinels may help to detect the disease early enough.

2.5.2 Prevention and control of RVF outbreaks

2.5.2.1 Vaccination of livestock

RVF is a model disease for implementing a "One Health" approach to disease prevention and control. The term "One Health" was first coined by Rudolf Virchow who stated: "*Between animal and human medicine there is no dividing line nor should there be*", and is further explained in this statement by USDA-APHIS: "*the health of animals, the health of people, and the viability of ecosystems are inextricable linked*". RVF is a complex disease which readily cycles between mosquitoes, wildlife, livestock, and humans. Mosquito host choice is therefore an important factor affecting the dynamics of mosquito-borne pathogen transmission, including RVFV, and has implications on the efficacy of different control measures (MacDonald, 1957).

Prevention and control of RVF in humans therefore rely on preventing the disease in domestic animals in the peridomestic environment (Gubler, 2002). Vaccination of livestock is essential to prevent major outbreaks (Pepin *et al*., 2010). The best and most efficient way to control the outbreak is to vaccinate all susceptible ruminants before a predicted outbreak. Several killed and live attenuated vaccines are available for livestock and provide immunity for 3 years. The first vaccine against RVFV was developed by Smithburn (Smithburn, 1949). However, because this vaccine induces abortions and teratogenesis in ewes, cows and goats (Botros *et al*., 2006, Kamal, 2009), its use is restricted during outbreaks and is only given to non-pregnant female animals (Barnard, 1979; Swanpoel & Coetzer, 2004). MP-12 vaccine which was derived by mutagenesis of the virulent Egyptian RVFV strain (ZH548) that was isolated during

the 1977 outbreak is efficacious in livestock (Morrill & Peters, 2003). The live attenuated MP-12 vaccine (developed by the USAMRIID) and the C-13 vaccines (developed at the Laboratoire des Bunyavirides, Institut Pasteur, Paris, France) are also promising and have been tested in animals (Muller *et al*., 1995). Although there is no RVFV vaccine approved by the Food and Drug Administration (FDA) for human use, several vaccine candidates are undergoing trials. A killed virus vaccine, TSI-GSD-200 (The Salk Institute–Government Service Division–200), is available primarily for laboratory personnel, and has produced good results for over 12 years of follow-up with only 3.5% of the subjects showing side effects (Pittman *et al*., 1999).

2.5.2.2 Vector control

Mosquito species differ in their breeding habitats, biting behavior, flight range and in many other ways. Knowledge about the biology and ecology of vectors is therefore one of the most important prerequisites for successfully implementing mosquito control (Becker, 1989). Different strategies are needed to control different species of mosquitoes. However, the best mosquito control program is an integrated program that includes point source reduction of breeding areas, routine larviciding in those breeding areas that cannot be eliminated, and adulticiding, especially when large mosquito populations emerge.

Insect growth regulators (IGR) kill insects by interfering with their normal process of growth and development. For example Methoprene (Altosid) mostly affects mosquitoes and not other organisms (Creekmur *et al.,* 1982) and can be applied to any larval stage. However, results are not immediate since the exposed larvae still pupate but the pupae die and adults do not emerge since this chemical inhibits ecdysis of the mosquito pupa to the adult stage (Kramer & Beesley, 1991). Therefore Methoprene is only effective when applied to the larval stage. Any pupae that are present in the water during application will not die and will successfully emerge as adults. Pre-treating of mosquito breeding grounds with larvicidals such as methoprene can effectively interrupt the mosquito life cycle especially when water-clogged bodies such as *dambos* are targeted (Logan *et al*., 1990). One of the challenges of treating of *dambos* with IGRs is

that the exercise may need to be repeated in cases of prolonged rains, since the efficacy of these chemicals is limited to a few days after application (Linthicum *et al*., 1989), and also because not all eggs hatch in a single flooding (Logan *et al.*, 1991a).

While larviciding is considered the best course of action for mosquito control after source reduction, it poses the greatest challenge in the wild especially where uncountable numbers of *dambos* occur, even if every body of standing water need not be larvicided. Additionally, while organophosphate compounds such as temephos remain economical for larviciding on a large-scale level (FCCMC, 1998), they are toxic to wetlands wildlife, such as fish and birds (Knight *et al*., 2003). Two mosquito-specific bacteria are currently used for mosquito control. *Bacillus thuringiensis israeliensis* (*Bti*), which was isolated from a dead mosquito larva in Israel in 1977, and registered for mosquito control in 1981, and *Bacillus sphaericus* (*Bs*) which was registered in 1991 (Knight *et al*., 2003), are very effective against mosquito larvae and have no adverse effect to humans and other non-target organisms. *Bs* has a narrower host range and is less effective against most *Aedes* species (Knight *et al*., 2003). However it is more effective to early stage larvae, which also require low dosage, than late stages.

Mosquitocidal oils, such as Bonide Mosquito Larvicide, kill mosquito larvae and pupae by suffocating them. These oils, which vaporize within 48 hr when exposed to sunlight, are typically reserved for emergency use, and when pupae are present and the emergence of adults is imminent.

In regions where rainfall is common, or during above normal rains that cause flooding, adulticiding becomes the only means of controlling mosquitoes. This method is preferred as an effective means of quickly eliminating infected mosquito populations when there is increased risk of disease outbreak, during which time larviciding is ineffective. Adulticides such as Pyrethrin, Malathion and Permethrin, applied at the recommended dosages are not acutely toxic to common freshwater insects and aquatic vertebrates (Lawler, Jensen & Dritz, 1997).

Unfortunately, the use of chemicals for adult vector control has never proven to be effective in decreasing the incidence of the disease because each potential vector

species has a unique life cycle and distinct behaviuor (Meegan & Bailey, 1984). This is particularly true of RVFV which is associated with over 40 mosquito species. Therefore integrated pest management (IPM) approaches which combine source reduction, biological control agents and application of mosquito-specific larvicides are always recommended, especially in the case of RVFV which is transmitted by multiple vector species. Use of bed nets, full-length clothes (preferably light colored) and mosquito repellents such as N, N-Diethylmethyltoluamide to prevent bites is recommended to local inhabitants and travelers (Kapoor, 2008). Mosquito control is moving away from application of conventional chemical pesticides to more environmentally friendly methods such as mosquito-specific bacteria in the genus *Bacillus* and IGRs. Although long-term effects on aquatic food webs of the latter mosquito control agents have been suggested (Hershey *et al*., 1998), subsequent studies (Schmude, Balcer & Lima, 1998; Balcer *et al*., 1999) failed to confirm that either mosquito control agent causes the food web effects observed in the study of Hershey and his colleagues. Nevertheless, judicious use of any control agent is advisable.

One of the challenges of controlling RVFV vectors is that eggs of these mosquitoes undergo synchronized or "installment hatching" (Breeland & Pickard, 1967; Logan *et al.*, 1991a). Because the eggs hatch at different times, a single treatment with an insecticide is inadequate to control mosquitoes for more than a single flooding (Linthicum *et al*., 1989, Logan *et al*., 1990).

2.6 Mosquito species succession

Mosquito species succession is an observed change in the population structure of mosquito species in an area over time. Although species succession of mosquitoes has been studied to some extent in Kenya, there is still more information to be generated especially on how it is influenced by different ecological factors. In a previous study on species succession in a forest in Kenya, it was shown that floodwater *Aedes* mosquitoes (*Ae. cumminsii, Ae.* (*Aed.*) *dentatus, Ae.* (*Mucidus*) *sudanensis, Ae. mcintoshi, Ae. circumluteolus,* etc.) are the first to emerge from flooded *dambos,* followed by *Culex* and *Anopheles* spp. (Linthicum *et al.,* 1983). A study conducted in a permanent marsh

habitat also recorded floodwater *Aedes* mosquitoes (*Ae. quasiunivittatus, Ae. dentatus, Ae. mcintoshi*) as the first to emerge followed by members of the genus *Culex* (Logan *et al*., 1991b). These two studies give a sneak preview into the nature of oviposition practices and egg characteristics of the different mosquito genera. While floodwater *Aedes* mosquitoes lay drought tolerant eggs on the drying margins of flooded *dambos*, *Culex* and *Anopheles* mosquitoes lay drought intolerant eggs on the water surface, which then hatch almost immediately. In this case, adults must discover the flooded area to lay eggs, and because the *Aedes* eggs are already present when flooding occurs, they have a head start developmentally.

2.6.1 Synchronized or "installment" hatching of floodwater mosquito eggs

Synchronized or "installment hatching describes a case in which eggs subjected to the same conditions do not all hatch simultaneously (Becker, 1989). This is a characteristic of floodwater mosquitoes, especially multivotine ones (those able to produce multiple generations each year) such as *Ae. vexans*, which lay their eggs in areas that are subject to flooding following rainfall. These eggs remain dormant until sufficient inundation occurs to initiate hatching, (synchronized, partial or "installment hatching") at any one inundation (Gillett, 1955a; Mallack *et al*., 1964; Breeland & Pickard, 1967; Logan *et al.*, 1991a). In this case, serial inundations are required to induce some eggs to hatch (Wilson & Horsfall, 1970). There is therefore variation in parentage and age of the population of eggs present as well as in the duration and nature of environmental pressures to which the eggs have been exposed before submergence (Wilson & Horsfall, 1970). Although widespread among floodwater mosquitoes, "installment hatching" varies greatly among species, within populations of the same species and even between egg batches of the same female (Gillett, 1955a, 1955b; Becker, 1989; Andreadis, 1990; Clements, 1992). For example the eggs of *Ae. sollicitans* and *Ae. taeniorhynchus*, repeatedly flooded and dried, continue to hatch through the 3rd and 5th floodings respectively (Travis, 1953).

While “installment hatching” is a reproductive adaptation for long-term survival of species that develop in temporary water bodies and subjected to irregular fluctuations (Andreadis, 1990; Clements, 1992) it has implications for vector control, as a single treatment with an insecticide is inadequate to control mosquitoes for more than a single flooding (Linthicum *et al*., 1989; Logan *et al*., 1990). Because subsequent flooding could allow maturation of *Aedes* mosquitoes and disease transmission, a routine mosquito control operation within floodplain areas, with attention focused on the frequency of flooding, may be necessary. Therefore understanding vector biology and the habitat of potential vectors is important to best control RVFV transmission (Linthicum *et al*., 1988).

2.6.2 Breeding sites of primary vectors of RVFV

Different mosquito species, both larval and adult stages, vary in their habitat requirements (WHO, 1982). The characteristics of water bodies, including vegetation, play a role in determining which mosquito species inhabit an area (WHO, 1989). The density of mosquitoes in any area is largely determined by the availability of their breeding habitats which, in turn, is directly affected by environmental factors (Apiwathnasorn *et al.,* 2006). Floodwater mosquitoes, associated with RVF outbreaks, prefer breeding sites that are characterized by a favourable alternating sequence of dry and wet spells (Knight & Baker, 1962; Olson & Meek, 1977; Cassani & Bland, 1978). This allows adequate time for embryonation, drying of eggs and therefore many generations of mosquitoes to develop (Becker, 1989). In the NE region of Kenya these sites include *dambos*, which are shallow stream-less depressions on land (Mackel, 1974; Young, 1976). Additionally, riverine areas along River Tana in the same region are also important breeding sites for floodwater mosquitoes such as *Ae. mcintoshi*, *Ae. ochraceus* and *Ae. sudanensis*. On the other hand, *Mansonia* spp., including *Ma. uniformis* and *Ma. africana,* which are predominant and associated with RVFV in Marigat District, Baringo County in the Great Rift Valley, require extensive vegetation cover and breed in open swampy and relatively permanent water bodies with organic material (Wharton, 1962). These mosquitoes are also associated with gently flowing water and oviposit on the

underside of the leaves and on the submerged roots and stems of African water lilies and floating vegetation (Horsfall, 1972). In particular, *Ma. uniformis* prefers to breed in open swampy areas unshaded by trees (Wharton, 1962).

2.6.2.1 *Dambos*

Dambos (Ackermann, 1936; Mackel, 1974; Young, 1976) are shallow depressions in the general topography, with a poorly porous stratum, that retain water after heavy rains (Whitlow, 1984), hence acting as hydrologic stores beyond the rainy season (Roberts, 1988), (Plates 2.1 and 2.2).

Plate 2.1 *Flooded dambo in Sangailu, Ijara District, Garissa County, following heavy rains*

Plate 2.2 *Dry dambo in Sangailu, Ijara District, Garissa County, after the rains. (Photos by Joel Lutomiah, 06.04.2013)*

Dambos are usually of different shapes and sizes, distributed throughout the bushveld-savanna mosaic and higher altitude grasslands of sub-Saharan Africa, and are typically associated with sedges and grasses (Pepin *et al*., 2010). *Dambos* are usually found in areas prone to flooding and are subject to great fluctuations in their water levels (Knight & Baker, 1962; Clements, 1963; Olson & Meek 1977; Cassani & Bland, 1978). *Dambo* soil can also have a wide range of pH values from under pH 5 to over pH 8 (Roberts, 1988). Because of the constant fluctuation of the prevailing abiotic conditions in the *dambo* water, floodwater mosquitoes require highly specialized egg-laying behaviour for species survival (Becker, 1989).

2.6.2.2 Riverine sites

These are temporary water bodies along large rivers and lakes that are subject to water fluctuations. They also serve as breeding sites for floodwater mosquitoes such as *Ae. vexans, Ae. sticticus, Ae. vexans,* and *Ae. rossicus* (Knight & Baker, 1962; Clements, 1963; Olson & Meek, 1977; Cassani & Bland, 1978). In NE Kenya, these riverine sites are situated along river Tana.

2.6.2.3 Swampy habitats

During the rain season the open swamp floods with water due to the swelling of Lake Baringo providing an ideal habitat for mosquito breeding. In the dry season the water level of Lake Baringo recedes, the open swamp dries up and becomes a grazing field for livestock and wildlife. Mosquito breeding, which occurs year round, is restricted to the area occupied by the papyrus reeds, which usually remains wet, during the dry season (Plate 2.3).

Plate 2.3 *An expansive open swamp with papyrus reeds in the background in Marigat District, Baringo County. This is an ideal breeding habitat for Mansonia spp. (Photo by Joel Lutomiah, 16.08.2013).*

2.7 Oviposition site selection and habitat properties that influence larval development

Dambo characteristics including soil type, water pH, availability of nutrients, oxygen concentration, optical density, reflectance, and water temperature are known to influence the choice of breeding sites by floodwater mosquitoes (Strickman, 1980a; 1982; Apiwathnasorn *et al.,* 2006; Sérandour *et al*., 2010). Various environmental factors, particularly rainfall and temperature, control the population dynamics of mosquitoes inhabiting natural breeding habitats (Apiwathnasorn *et al.,* 2006). For example, rainfall creates more breeding habitats, increases evapotranspiration and vegetation coverage, cools the environment and therefore directly affects reproduction and survival of mosquitoes (Wharton, 1962).

Mosquitoes are able to discriminate between suitable habitats for egg deposition, thus determining the distribution of larval population (Sérandour *et al*., 2010) based on the quality of the breeding site such as soil conditions, vegetation structure, and sufficient water fluctuation (Strickman, 1980a). Some floodwater mosquitoes such as *Ae. sollicitans* and *Ae. taeniorhynchus* are attracted to soil with high content of inorganic ions (Horsfall 1963, Knight 1965; Petersen & Rees, 1966). Soils in most flood prone areas consist of a high percentage of clay and low percentage of humus or organic materials (Ikeshoji and Mulla, 1970; Strickman, 1980a; b). Vegetation density also influences larval density in a *dambo* (Rydzanicz, Kącki & Jawień, 2011) by creating shaded surfaces that reduce light reflection from the ground (Strickman, 1982).

While female mosquitoes deposit eggs in areas where flooding will last long enough for completion of larval development (Strickman, 1982), environmental factors such as optical density, temperature, and reflectance are also critical for the choice of breeding sites by female mosquitoes (Sérandour *et al*., 2010). Pond water temperature affects both spawning and growth of larvae and pupae (Sanford, 2005). Because *dambos* usually hold water temporarily, high temperatures permit rapid larval development, thus preventing deaths due to unexpected changes in the water level (Becker, 1989). The mean

water temperature range of 16°C - 32°C has been described as the best for the breeding of most mosquito species in the tropics (Bradley & Kutz, 2006).

Various chemical properties of the larval habitat ranging from pH also affect larval development and survival (Mutero *et al.,* 2004). Water of a near neutral pH of 6.8 – 7.2 is preferable for breeding of many species of mosquitoes (Pelizza, *et al.*, 2007; CDC, 2004). pH readings outside this range slows growth of mosquito eggs, larvae and pupae (CDC, 2007; Curtis, 1996) while values below 4.5 or above 10 result in mortalities. Therefore oviposition site selection is an important aspect of the behavioral ecology of floodwater *Aedes* spp. as it determines the larval habitat (Jenkins, 1946) and survival and development of eggs and larvae to adults (Strickman, 1982; Becker, 1989) and is considerably species dependent (Bentley & Day, 1989).

2.8 Inter-epizootic transmission of RVFV

The mechanism of RVFV maintenance during IEP is still unclear (Fafetine *et al*., 2013) although it is believed that the virus is maintained in floodwater aedine mosquito eggs through TOT (Linthicum *et al*., 1985b, Davies *et al*., 1985, Davies *et al*., 1992, Peters, 1997). This is aided by low level transmission of the virus to livestock (Mellor & Leak, 2000). While RVFV has been isolated only once from male mosquitoes collected as adults outside of an epidemic (Mohamed *et al*., 2013) virus-specific antibodies have been detected in humans, livestock and wildlife (Sumaye *et al*., 2013). Evidence of inter-epidemic transmission of RVFV in humans in Kenya abounds through detection of Immunoglobulin G (IgG) antibodies in young children born after the 1997-1998 outbreak in a high-risk setting of Northeastern Kenya (LaBeaud *et al*., 2008) and in other areas outside of the NE Kenya (Johnson *et al*,. 1982, 1983a; Morill *et al*., 1991). Inter-epidemic transmission of RVFV to humans has also been documented in Tanzania based on observed increase of RVFV seroprevalence by age (Henrich *et al*., 2012; Sumaye *et al*., 2013) implying a constant exposure to infectious mosquito bites. However, the mechanism by which humans become infected during IEPs is unknown (LaBeaud *et al*., 2008).

Evidence of inter-epidemic transmission, including 1999–2006 IEP, of RVFV has also been documented in wildlife including African buffalo (*Syncerus caffer*), elephant (*Loxodonta africana*), warthog (*Phacochoerus africanus*), rhino (*Diceros bicornis*), zebra (*Equus burchelli*), waterbucks (*Kobus ellipsiprymnus*) and several antelope species. It is however not clear if these animals play a role in RVFV maintenance between outbreaks (Evans *et al*., 2008; LaBeaud *et al*., 2011b; Britch *et al*., 2013). Inter-epidemic transmission of RVFV most likely passes undetected due to lack of surveillance in the livestock or human populations (Sumaye *et al*., 2013) since most of the infections are either subclinical or mistaken for other diseases (Pourrut *et al*., 2010; Heinrich *et al*., 2012).

2.9 Blood-feeding behaviour of mosquitoes

The blood-feeding behaviour of a vector is critical in the transmission and maintenance of vector-borne pathogens in nature (Kent, 2009). While several studies have been conducted on the blood-feeding behaviour of vectors, the role of mammals in the maintenance of RVFV, and the existence of a wild mammal reservoir in the epidemiologic cycle of RVFV, remains largely unknown (Olive, Goodman & Reynes, 2012). A study of the blood feeding behaviour of mosquitoes from *dambos* revealed that *Ae. mcintoshi, Ae. cumminsii, Ae. dentatus, Ae. sudanensis,* and *Ae. circumluteolus* preferentially feeds on cows (Linthicum *et al.,* 1984b). Dispersal and survival studies have also shown that *Ae. mcintoshi* do not travel far from the *dambos* (Linthicum *et al.,* 1985c) probably due to abundance of hosts (cattle and wildlife) in the vicinity of the breeding habitats. This suggests that these mosquitoes may not have a wide variety of vertebrate species to feed on beyond what is present. Convergence of vectors, vertebrate hosts and pathogens result in efficient transmission of arboviruses (Kent, 2009), a factor that influences the ecology of aboviruses. Determination of the range of vertebrate hosts a vector feeds on is important to understand their roles as amplifiers of arboviruses (Townzen, Brower & Judd, 2008). Similarly, the degree of contact between the vector and the vertebrate reservoir is an important variable in determining the vectorial capacity of mosquito species for arthropod-borne infections (Apperson *et al*., 2004).

2.10 Co-circulation of RVFV with other viruses during RVF outbreaks

Co-circulation of viruses during the 2006-2007 RVF outbreak was demonstrated through isolation of seven different viruses including Ndumu virus (NDUV) from *Ae. mcintoshi, Ae. ochraceus* and *Ma. uniformis;* West Nile virus (WNV) from *Ae. sudanensis*; Bunyamwera virus (BUNV) from *Ae. ochraceus;* Pongola virus (PGAV) from *Ae. mcintoshi*; Babanki virus (BABV) from *Ae. mcintoshi* and *Ae. sudanensis*; Sindbis virus (SINV) from *Ae. sudanensis* and *Cx. quinquefasciatus* and Semliki forest virus (SFV) from *Ae. mcintoshi* and *Ae. ochraceus* (Crabtree *et al*., 2009). LaBeaud *et al*., (2011a) also demonstrated co-circulation of RVFV and WNV in mosquitoes in Ijara District. Previously, Turell and his colleagues (2002) isolated WNV and Sindbis virus (SINV) from *Cx. antennatus* and *Cx. perexiguus* respectively, while Ngari virus (NRI) was isolated from patients with acute hemorrhagic fever during the 1997-1998 RVF outbreak in Garissa, Kenya, and Somalia (Bowen *et al.*, 2001; Gerrard *et al*., 2004). NRI was also isolated from *An. funestus* sampled from Tana-delta and from *Ae. mcintoshi* from Garissa during an inter-epidemic survey (Ochieng *et al*., 2013).

Given that some of these viruses cause diseases with symptoms similar to RVF, it is possible to have other arboviruses circulating and causing diseases concurrently with a known RVF outbreak, a situation that may be confounding since they serve as alternative causes of fever (Turell *et al.,* 2002; LaBeaud *et al*., 2011a), including haemorrhagic fevers. RVFV is transmitted by many vectors, some of which also happen to transmit other disease-causing arboviruses. This, and the close proximity of infected mosquitoes to susceptible amplifying hosts and humans during an RVF outbreak calls for further investigation of transmission dynamics of these arboviruses (LaBeaud *et al*., 2011a) and diversification of accurate diagnostics to avoid missing out on other likely viruses for provision of targeted medication. Given that most of these viruses share the same vectors with RVFV, any control measures against RVF would have an obvious positive impact on other diseases as well.

Despite repeated isolation of multiple viruses from the same locality, and same field-collected mosquito species, the role of co-circulation of viruses among mosquito

vectors during an outbreak is unknown. Most of the viruses isolated during RVF outbreaks are known to cause diseases in humans with symptoms similar to RVFV. PGAV has been isolated from a human patient with febrile illness in Uganda, and PGAV-specific antibodies identified in humans in several African countries (Kalunda *et al*. 1985; Karabatsos, 1985). WNV is associated with mild febrile illness with rash, although more severe symptoms including hepatitis and encephalitis have been reported (Burt *et al*., 2002). NDUV, a member of Family: *Togaviridae* and Genus: *Alphavirus*, was first isolated in 1959 from *Ma. uniformis* in South Africa (Korkenot, McIntoshi & Worth 1961), and later in Kenya from *Ae. mcintoshi, Ae. ochraceus, Ae. tricholabis* and *Cx. rubinotus* (Crabtree *et al*., 2009; Ochieng *et al*., 2013).

Although NDUV occurs throughout most of Africa, and antibodies to the virus have been identified in humans from several African countries, the virus has not been associated with human morbidity this far (Korkenot, McIntoshi & Worth 1961; Karabatsos 1985). However, the Alphavirus genus comprises over 31 viruses (Forrester *et al*., 2012) some of which cause human morbidity (Masembe *et al*., 2012), including Chikungunya virus (CHIKV) which was responsible for recent severe outbreaks of human disease in Eastern Africa (Sergon *et al*., 2007, 2008). Human infection with SINV is characterized by fever, arthritis, and rash (Karabatsos, 1985) while BABV, a strain of SINV, is associated with human febrile illness, rash and arthritis, and has been isolated from humans in Cameroon, Madagascar, and the Central African Republic (Karabatsos, 1985; Anonymous, 2000).

CHAPTER THREE
MATERIALS AND METHODS

3.0 Study Design

This was a mixed methods study that used archived samples which were collected during the 2006-2007 RVF outbreak. Prospective sampling was also conducted in April and November 2013 in Sangailu Location, Ijara District, Garissa County and in May 2013 in Marigat District, Baringo County.

3.1 Study Sites

Two ecologically distinct regions of Kenya where RVFV activity was detected in humans and livestock during the 2006-2007 outbreak were selected for study. These are Garissa County (in the NE) and Baringo County (in the Rift Valley) which are separated by a distance of over 400 km. In the NE Kenya, the archived bloodfed mosquitoes were collected from El-Humow in Garissa County during the 2006-2007 RVF outbreak. Sampling of larvae, adult and bloodfed mosquitoes during the IEP was conducted in Marey and Wakab-Harey Sublocations in Ijara District of Garissa County in April and November 2013. This area was chosen because of the presence of numerous and clearly defined natural breeding habitats (*dambos*). In the Rift Valley, IEP sampling of bloodfed mosquitoes was conducted in Sirata Sub-location, Marigat District in Baringo County.

3.1.1 Garissa County in the Northeastern region

In general, Garissa County borders Tana River to the west and Somalia to the east. Garissa County is an arid area with Somali *Acacia-Commiphora* bushlands and thickets, sporadic rainfall that averages 200–500 mm per year and occasional torrential storms causing extensive flooding. The average temperatures range from 20°C to 38°C and the altitude varies from 70 to 400 m above sea level. The soil in NE Kenya is generally sandy with scattered areas of dark clay that retain water after the rains and serve as watering holes for livestock and wild animals. The population in this region is generally sparse

with ≈7 people/ km^2 (Statoids, 2007) and is largely pastoral, keeping large herds of livestock.

3.1.2 Baringo County in the Rift Valley region

Baringo County is over 400 km to the West of Garissa County, lies 250 km northwest of Nairobi and covers approximately 8,646 km^2 with ≈31 people/ km^2 (Statoids, 2007). The low-lying arid part of Baringo consists of northern *Acacia-Commiphora* bushlands and thickets. The local inhabitants are agro-pastoralists with limited crop production and livestock rearing. The collection of bloodfed mosquitoes was conducted around flooded marshlands near Lake Baringo (elevation ~980 m) where the annual rainfall ranges from 300 to 700 mm, and the daily temperature varies between 16°C and 42°C (Fig. 3.1).

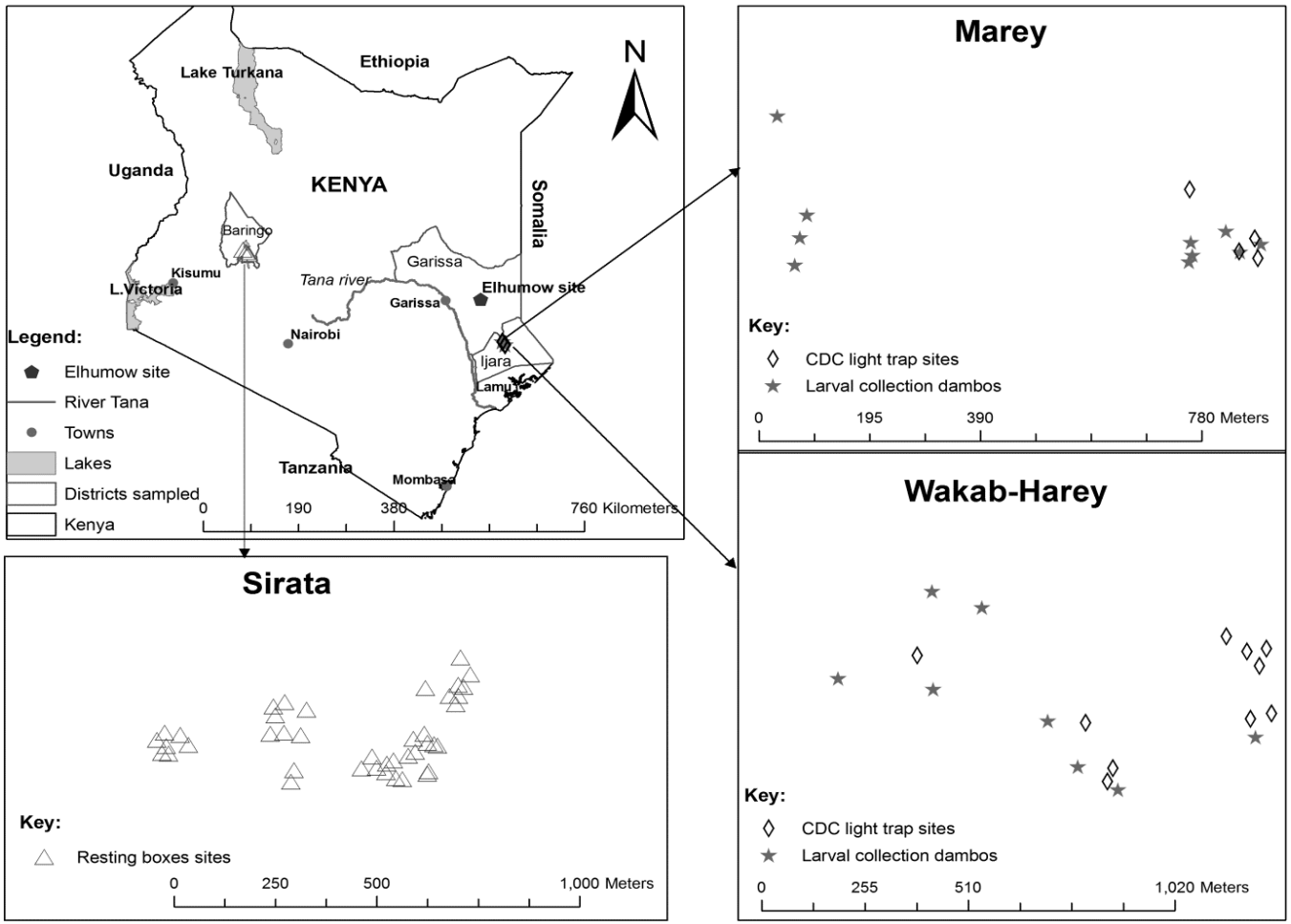


Figure 3.1: *A map of Kenya showing collection sites for blood-fed mosquitoes: El-Humow (Garissa County) during the 2006-2007 RVF outbreak; Marey and Wakab-Harey in Sangailu, Ijara District (Garissa County) and Sirata (Baringo County) during the IEP2013. It also shows locations of larval sampling sites (dambos) in marey and Wakab-Harey*

3.2 Mosquito species of interest

Bloodfed *Ae. ochraceus, Ae. mcintoshi* and *Ae. sudanensis* were collected from El-Humow in Garissa County during the 2006-2007 outbreak. These mosquitoes occur in large densities, except *Ae. sudanensis,* in NE Kenya and are believed to play a primary role in RVFV transmission in this region.

3.3 Sample size determination

The minimum sample size for blood-fed mosquitoes collected from El-Humow during the 2006-2007 outbreak was calculated using the formula by Daniel, (1999) $\mathbf{n=Z^2_{(1-\alpha/2)}\ P\ (1-P)/d^2}$ where:

n = sample size,

$Z_{(1-\alpha/2)}$ = Z statistic for a level of confidence, taken to be 1.96 in the case of this study;

P = the expected prevalence or proportion, taken as 3% (Nguku *et al*., 2012).

d = precision in proportion of one, taken to be half of 3% since the expected prevalence/ proportion was below 10% (*d*=0.015).

Plugging these values in the above formula gives a minimum sample size of 497 specimens. This was further increased by 5% to account for possible contingencies resulting into a sample size of 522 samples.

3.4 Sampling of mosquitoes

3.4.1 Archived samples collected during the 2006-2007 RVF outbreak

The archived bloodfed mosquito samples were previously collected during the 2006-2007 RVF outbreak from El-Humow in Garissa County. The collection had been done using the CO_2-baited CDC miniature light traps (John W. Hock Company, Gainesville, FL, USA) placed in villages where RVF human cases were reported as described by Sang *et al*., (2010). Individual traps, which included Igloos containing 0.5 kg of dry ice, were set at 17:00 hrs and collected at 06:00 hrs the following day. The

mosquitoes had been transferred to a field laboratory where they were immobilized using Triethylamine (TEA) (Sigma-Aldrich, St. Louis, MO). They were preserved in 1.5-mL cryogenic vials or 15-ml centrifuge tubes in LN_2 for transportation to the Kenya Medical Research Institute (KEMRI) Viral Haemorrhagic Fever (VHF) laboratory for preservation at -80°C.

3.4.2 Collection of adult and blood-fed mosquitoes during IEP

Adult mosquitoes, including the bloodfed, were collected using the CO_2-baited CDC miniature light traps. In Sangailu Location (Garissa County), the traps were set in Marey and Wakab-Harey Sublocations; in the same localities as *dambos* where larval sampling was conducted while in Marigat (Baringo County), they were placed in Sirata which was mostly affected during the 2006-2007 outbreak. Blood-fed mosquitoes were also collected using resting boxes measuring 30 cm x 35 cm x 45 cm. The boxes were made of 1 inch x 1 inch wooden frames with two removable clothing materials: a red one on the inside for ease of visibility of engorged mosquitoes during aspiration, and a black one on the outside to attract blood fed mosquitoes looking for a resting place after a blood meal (Plate 3.1).

Plate 3.1 *A sample of collapsible resting box*

Ten resting boxes were placed in shaded areas, at least 30 m from each other, and cotton wool soaked in water on a petri dish placed inside to create a humid environment favourable for mosquito survival. Between 1000 hrs and 1400 hrs each day, mosquitoes found resting in the boxes were aspirated using a procopack aspirator and the collection cups with the mosquitoes taken to the site laboratory for preservation and transportation as described (section 3.5.1).

3.4.3 Larval sampling and rearing

The larvae were sampled at Marey and Wakab-Harey in Ijara District, Garissa County, from the flooded natural breeding habitats (*dambos*) every alternate day using 350-ml standard larval dippers (Bioquip Inc., Compton, MA), from the start to the end of the rain season. The number of dips for larvae undertaken in any one dambo varied depending on the size of the *dambo* but was in multiples of 10 per day. Larvae collected from the *dambos* were transferred, in well labelled (date of collection and *dambo* number) whirl-pak bags (Bioquip), to a field laboratory where they were reared to adults in similarly labelled 4-liter capacity plastic cages with the tops screened with netting material. The emerging adults were immobilized using TEA, pooled in vials by date of collection and *dambo* number, transported and preserved as described (section 3.5.1).

3.4.4 Mosquito identification, pooling and preservation

All preserved mosquitoes collected as adults and those emerging from pupae were identified to species by morphological methods using the keys of Edwards (1941) and Gillies & de Meillon (1968). They were pooled by species, sex, site and date of collection, dambo number (for those collected as larvae) up to 25 mosquitoes per pool. Bloodfed mosquitoes were separated, identified and preserved individually. All the samples were preserved in 1.5-mL vials at -80°C until assayed.

3.5 Data collection procedures

3.5.1 Determination of mosquito species succession during and after flooding

3.5.1.1 Timing of mosquito sampling

Mosquito species succession was examined by sampling of larvae in *dambos* in the course of the rain season. Sampling coincided with heavy rains that cause flooding of *dambos* resulting in large numbers of floodwater *Aedes* and other mosquito species. Sampling in Marey (Sangailu, Garissa County) was conducted in April 2013 during the long rain season that is usually experienced from March to May of each year. Wakab-Harey (Sangailu, Garissa County) was sampled in November the same year during the short rain season that occurs between October and December. The two sites could not be sampled at the same time because the long rains did not occur in Wakab-Harey while the short rains were only experienced in Wakabharey and not Marey, both separated by approximately 10 km.

3.5.1.2 Site mapping

Ten *dambos* were selected in Marey and seven in Wakab-Harey for larval sampling based on their state of inundation at the time of field visit. The location of the *dambos* were recorded using a GPS (eTrex® HC series, Garmin, Taiwan) and given unique identifiers.

3.5.1.3 Larval counts

The numbers of larvae sampled from each *dambo* per day were recorded by species after identification. The totals for each larval species collected from all the *dambos* by site were obtained to determine the change in species populations over time during the sampling period (mosquito species succession).

3.5.2 Evaluation of the relationship between physical and environmental variables and larval species composition and densities

3.5.2.1 Measured parameters

The depths of the *dambos* were measured, at the center, in centimetres using a one meter rule, while the temperature and pH were measured using a Multi-Parameter PCTestr^{TM} 35 (Eutech Instruments/ Oakton) (Plate 3.2).

Plate 3.2 *The PCTestr^{TM} 35 Temperature/PH meter that was used to measure the dambo water pH and temperature. (Photo by Joel Lutomiah, 06.04.2013)*

The pH was measured once daily while the daily temperatures of the *dambo* waters were taken between 0830 hrs and 0900 hrs, 1200 hrs and 1230 hrs and 1700 and 1730 hrs. The mean pH and temperatures of each *dambo* were then calculated and species composition and density for each dambo determined (section 3.6.1.3) and compared.

3.5.3 Determination of Transovarial Transmission (TOT) of RVFV and other arboviruses in mosquitoes collected as larvae

3.5.3.1 Sample preparation and virus isolation in cell culture

To determine TOT of RVFV and other arboviruses, all pools of adult mosquitoes collected as larvae (Section 3.5.3) and identified to species (section 3.5.4.) were homogenized, using copper beads, in 1 mL of homogenizing media comprising of Minimum Essential Medium Eagle (MEM) containing Earle's salts and reduced $NaHCO_3$ (Sigma-Aldrich, St. Louis, MO) supplemented with 15% heat-inactivated foetal bovine serum (FBS) (Sigma-Aldrich), 2% L-Glutamine (Sigma-Aldrich) and 2% antibiotic/ antimycotic solution with 10,000 units penicillin, 10mg streptomycin and 25µg amphotericin B per mL (Sigma-Aldrich). The homogenates were clarified by centrifugation at 12,000 rpm (Eppendorf centrifuge 5417R) for 10 min at 4°C and the supernatants transferred into 1.5-mL Cryogenic vials. Each mosquito pool supernatant (50 µL) was inoculated per well onto confluent monolayers of Vero cells in 24-well plates grown in MEM supplemented with 10% heat-inactivated FBS, 2% L-Glutamine, and 2% Antibiotic/ antimycotic solution (100x). Infected cultures were incubated for 45 min to allow for virus adsorption after which 1 mL of maintenance media comprising of MEM supplemented with FBS (2%), L-Glutamine (2%) and antibiotic/antimycotic solution (2%) was added to each well. The cultures were incubated at 37°C in 5% CO_2 and monitored daily, through day 10, for cytopathic effects (CPE) as an indication of virus infection.

3.5.3.2 Total RNA isolation

Total RNA was isolated from the supernatant of each culture exhibiting CPE by Trizol-chloroform method (Chomczynski & Sacchi, 1987). Briefly, 750 µL of Trizol LS reagent (Invitrogen, Carlsbad, California, USA) was mixed with 250 µL of harvested CPE positive cultures for dissociation of nucleoprotein complexes. Chloroform (200 µL) was added to allow phase separation after which 550 µL of the upper aqueous phase was pipetted and mixed with 1 µL of glycogen. The RNA was precipitated by mixing with

500 µL of isopropyl alcohol, pelleted by centrifuging at 12000 rpm (Eppendorf centrifuge 5417R) for 10 min at 4°C, washed with 500 µL of 75% ethanol and recovered by centrifugation at 5000 rpm for 5 min at 4°C and the excess liquid removed with a pipette. The RNA was then eluded in 11 µL of nuclease free water, left to stand at room temperature for 1 min and mixed by vortexing.

3.5.3.3 RT-PCR amplification of RNA

The presence of RVFV in the RNA was determined by RT-PCR using 735-bp RVFV-specific primers (Table 1), reference strain RVFV 200803170 (*Accession no JF311385.1, 2011*). Briefly, Extracted RNA was reverse transcribed to cDNA using SuperScript® III Reverse Transcriptase (Invitrogen, Van Allen Way, Carlsbad, CA92008, USA) in a 20 µL reaction volume, following the manufacturer's instructions. The RNA (10 µL) was placed in a 0.2 µL PCR tube, 100 ng of random primers added before denaturing at 70°C for 10 min and cooled to 4°C for 5 min. Four µL of 5x first strand buffer (Invitrogen), 2 µL of 0.1 M DTT (Invitrogen), 10 mM dNTP mix (Invitrogen), 10 units of RNaseOut Recombinant RNase inhibitor (Invitrogen), 200U of SuperScript® III RT (Invitrogen) was then added. The mixture was heated to 25°C for 5 min, 50°C for 45 min and the termination of first strand synthesis achieved by heating the mixture at 70°C for 15 min. Two µL of the cDNA was amplified in a 25 µL reaction in the presence of 1 x PCR buffer (Invitrogen), 200 µM dNTP mix (Invitrogen), 1 unit of recombinant Taq polymerase (Invitrogen) and 25 picomoles each of RVFV specific forward and reverse primers (Table 1). The thermocycling conditions included initial denaturation at 94°C for 3 min and 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 68°C for 45 sec. Final cycle was completed with 7 min of extension at 72°C. Amplification products were resolved in 1% agarose gel in Tris-borate EDTA buffer stained with ethidium bromide (Ibrahim *et al.,* 1997; Jupp *et al.,* 2000). The PCR product bands were visualized by a UV transilluminator and recorded using a gel photo imaging system.

Because the isolates did not amplify with RVFV primers, they were further analyzed using a panel of genera (*alphavirus* and *flavivirus*) and specific primers for

NDUV, Babanki (BBKV) and Sindbis virus (SINV) (Table 3.1). For NDUV, 2 µL of the synthesised cDNA was amplified in a 25 µL reaction in the presence of 1 x PCR buffer, 200 µM dNTP mix, 1 unit of recombinant Taq polymerase and 25 picomoles each of NDUV specific forward and reverse primers (Table 3.1). The thermocycling conditions included initial denaturation at 95°C for 10 min and 35 cycles of 95°C for 30 sec, 50°C for 30 sec and 72°C for 7 min and the PCR products held at 4°C. Amplification products were resolved and visualized as described above.

3.5.3.4 Sequencing

The PCR products were directly purified using Wizard® SV Gel and PCR Clean-Up System kit (Promega). Sequencing was outsourced (Macrogen Inc., South Korea) and performed using ABI Prism 3700 DNA analyzer (Perkin-Elmer Applied Biosystems, Foster City, CA) as described by Sanger, Nicklen, & Coulson, (1977). The resulting sequences were compared with available sequences of other members of the *alphavirus* genus from Genbank using Basic Local Alignment Search Tool (BLAST. http://blast.ncbi.nlm.nih.gov/Blast.cgi.) and the GenBank database (Genbank. http://www.ncbi.nlm.nih.gov/genbank/) to confirm the identity of the virus isolates. The sequences were aligned using ClustalW (Larkin *et al*., 2007).

Table 3.1 *DNA sequences of the primers used, their target genes/proteins and positions*

	Virus or genus	**Gene Target**	**Primer Sequence**	**Position**	**Reference**
1.	RVFV	M Segment	F - RVF1: (5'-GAC TAC CAG TCA GCT CAT TAC C-3′)	777-798	Kuno *et al*., (1996)
			R - RVF2: (5′-TGT GAA CAA TAG GCA TTG G-3′)	1309-1327	
2.	Flavivirus	NS5	F - FU 1; (5'-TAC AAC ATG ATG GGA AAG AGA GAG AA-3')	9007-9032	Bryant *et al*., (2005)
	"	"	R - CFD2; (5'-GTG TCC CAG CCG GCG GTG TCA TCA GC-3')	9308-9283	
3.	Bunyavirus	nucleocapsid protein	F - BCS82C; (5'-ATG ACT GAG TTG GAG TTT CAT GAT GTC GC-3')	86-114	Kuno *et al*., (1996)
	"	"	R - BCS332V; (5'-TGT TCC TGT TGC CAG GAA AAT-3')	309-329	
4.	Alphavirus	NSP4	F - VIR 2052F;(5'-TGG CGC TAT GAT GAA ATC TGG AAT GTT-3')	6971–6997	Eshoo *et al*., (2007)
	"	"	R - VIR 2052R; (5'-TAC GAT GTT GTC GTC GCC GAT GAA-3')	7086–7109	
5.	Ndumu	envelope (E1) gene	F - ND 124F; (5'-CAC CCT AAA AGT GAC GTT-3')	124-141	Bryant *et al*., (2005)
	"	"	R - ND 615R; (5'-ATT GCA GAT GGG ATA CCG-3')	615-632	
6.	Babanki	E1 envelope glycoprotein	F - Bab 3368F; (5'-CAG CAG ATT GCG CGA CTG ACC-3')	3368-3388	Bryant *et al*., (2005)
	"	"	R - Bab 4203R; (5'- GCT CAC GAT ATG GTC AGC AGG-3')	4184-4203	
7.	Sindbis	Non Structural Protein	F - SINV1; (5´-TTT AGC GGA TCG GAC AAT TC-3´)	5194-5213	Bryant *et al*., (2005)
	"	"	R - SINV2; (5´-GCG GTG ACG AAC TCA GTA G-3')	6482-6500	

3.5.4 Determination of blood-feeding pattern of key vectors of RVFV

3.5.4.1 Mosquito dissection and homogenization

To determine the source of blood meals and the preferred vertebrate hosts, the archived blood-fed mosquitoes (section 3.5.1) and those collected and preserved during the IEP (sections 3.5.2 and 3.5.4) were dissected individually. To avoid cross-contamination of the samples, each mosquito was dissected on a new microscope slide, placed on icepacks, using sterile fine forceps and dissecting pins. The abdomen and heads were separated from the thoraces and transferred into sterile 1.5-mL eppendorf tubes using a sterile pair of forceps. The heads were preserved at -70°C for assay, based on the cell culture and PCR results of the abdomens. Bloodmeals were removed from the abdomens of individual mosquitoes by rupturing the abdominal cuticle using sterile 200 µL pipette tips and suspended in 300 µL of 1x PBS (Sigma-Aldrich). The homogenates were clarified by centrifugation at 5000 rpm for 5 min at 4°C and each supernatant divided into two aliquots of 250 µL and 50 µL. The 250 µL aliquot was used for blood meal source analysis while the 50 µL was used for virus infection detection assays.

3.5.4.2 DNA isolation from the blood meals

Genomic DNA was extracted from the blood meals in mosquito vectors using QIAGEN DNeasy Blood and tissue extraction kit (Qiagen Inc., Maryland, CA, USA), following the manufacturer's instructions. Briefly proteinase K (20 µL) was added to the homogenate (220 µL) followed by 200 µL of lysis buffer (Buffer AL). The mixture was vortexed for 30 seconds then incubated for 10 min in a water bath maintained at 56° C for 10 min. After incubation, 200 µL of absolute ethanol was added and vortexed thoroughly for 1 min and the mixture pipetted into a DNeasy Mini spin column in a 2 ml collection tube followed by centrifugation at 8000 rpm for 1 min. The flow-through was discarded together with the collection tube and the spin column placed in a new 2 ml collection tube. Wash buffer AW1 (500 µL) was added and the sample centrifuged at 8000 rpm for 1 min. The spin column was removed carefully so that it did not come in contact with the flow-through which was then discarded and placed in a new collection tube. Wash buffer AW2 (500 µL) was added to the column and centrifuged at 14000

rpm for 3 min. The spin column was removed carefully, the flow through discarded and the collection tube replaced. The sample was dry span at 14000 rpm for 1min and the spin column transferred into a new 2 mL micro centrifuge tube and 30 µL of elution buffer AE added. The sample was incubated for 1 min at room temperature and centrifuged at 8000 rpm for 1 min. For maximum yield, centrifugation was repeated ones without replacing the collection tube.

3.5.4.3 PCR amplification

Extracted DNA was used as templates in the subsequent cytochrome c oxidase subunit 1 (*COI*) PCR analysis for source identification, selectively targeting a 750-base pair (bp) region near the 5' end of this gene (Ratnasingham & Hebert, 2007; Waugh, 2007). Published *COI* Primer sequences VF1d_t1 forward (5'-TGTAAAACGACGGCCAGTTCTCAACCAACCACAARGAYATYGG-3') and VR1d_dt reverse (5'-CAGGAAACAGCTATGACTAGACTTCTGGGTGGCCRAARAAYCA-3') (Ivanova *et al*., 2007), specific for vertebrates were first used for PCR amplification (Molaei *et al.*, 2006) using the outlined parameters for each primer pair. The PCR cycling conditions included an initial denaturation at 98° C for 1 min followed by 40 cycles of 98° C for 10 sec, annealing at 57° C for 30 sec and extension at 72° C for 30 sec per cycle. The final cycle was completed with 7 min of extension at 72° C and then the products stored at 4° C until resolved in 1.5% agarose gels stained with ethidium bromide.

If the *COI*_ primer pair did not amplify the blood meal DNA, the samples were further analyzed for Cytochrome b (*cytb*) using degenerate primers: L14841 Forward (5'-CCATCCAACATCTCAGCATGATGAAA-3') and H151494 Reverse (5'-GCCCCTCAGAATGATATTTGTCCTCA-3') which flank a 358-bp sequence (Kocher *et al*., 1989). The PCR was run using thermocycling profile of one cycle of 1 min at 98° C, 40 cycles of 98° C for 30 sec, 61° C for 20 sec and 72° C for 30 sec each. Samples were then held at 72° C for 7 min and stored at 4° C until resolved in 1.5% agarose gel in Tris-Borate EDTA buffer stained with ethidium bromide.

3.5.4.4 DNA purification

DNA fragments were purified using GeneJET™ Gel Extraction Kit (Fermentas) following the manufacturer's instructions. Briefly, DNA fragments were excised from gels, as close to the DNA as possible, using a sterile scalpel. Each gel slice was placed in a pre-weighed 1.5-mL tube and weight again and the weight of the gel slice recorded. A 1:1 (volume: weight) volume of binding buffer was added to the gel slice (100 µL of binding buffer for every 100 mg of agarose gel). The gel mixture was incubated at 50° C - 60° C for 10 min or until the gel slice was completely dissolved. The tubes were mixed by inversion every few minutes to facilitate the melting process and ensure that the gel is completely dissolved. The colour of the solution was checked to ensure that an optimal pH (yellow colour) for DNA binding was maintained. Up to 800 µL of the solubilized gel solution was transferred to the GeneJET™ purification column and centrifuged at 12000 rpm for 1 min. The flow-through was discarded and the column placed back into the same collection tube. One hundred microlitres of Binding Buffer was added to the purification column and centrifuged for 1 min, the flow-through discarded and the column again placed back into the same collection tube. Seven hundred µL of Wash Buffer (diluted with ethanol) was added to the purification column and centrifuged for 1 min, the flow-through discarded and the column placed back into the same collection tube. The empty purification column was centrifuged for an additional 1 min to completely remove residual wash buffer, transferred into a clean 1.5-mL microcentrifuge, 50 µL of Elution Buffer added to the centre of the purification column membrane and centrifuged for 1 min. The purification column was discarded and the purified DNA stored at -20° C until sequenced.

Purified *Cytb* and *CO1* amplicons were sequenced using each primer pair in both directions (Macrogen Inc. South Korea) as described by Sanger, Nicklen, & Coulson, (1977). The chromatograms for each individual sequence were edited in Molecular Evolutionary Genetic Analysis software (MEGA) (Kumar, Tamura & Nei, 2004). Edited sequences were used to query GenBank DNA sequence database, (National Center for Biotechnology Information –NCBI www.ncbi.nlm.nih.gov/) using BLAST (Altschul *et*

al., 1990) and the Barcode of Life Data System (BOLD), (www.barcodinglife.org) using the default identification engine (Ratnasingham & Hebert, 2007). For GenBank, an e-value of <1.0e-1671.0e-167 was used to determine a positive identification of a bloodmeal, and a 96-100% identity of vertebrate host used for BOLD (Sarkar & Trizna, 2011).

3.5.5 Determination of the prevalence of RVFV and other arbovruses in blood-fed field collected mosquitoes

3.5.5.1 Presence of RVFV and other arboviruses in mosquito blood meals

To determine the prevalence and infection rates of RVFV and other arboviruses in blood-fed archived mosquitoes and those collected during IEP, the 50 µL preserved homogenate (Section 3.6.4.1) was inoculated in confluent monolayers of Vero cells in 24-well plates and observed for CPE through day 10 post inoculation as described (section 3.6.3.1).

3.5.5.2 Dissemination of RVFV and other arboviruses in blood-fed mosquitoes

The heads of virus positive abdomens were homogenized individually, using glass grinders, in 1.5-mL tubes containing 500 µL of homogenizing media comprising of MEM, supplemented with 15% FBS, 2% L-Glutamine, and 2% Antibiotic/ antimycotic solution (100x) and inoculated in confluent monolayers of Vero cells in 24-well plates and monitored as described (section 3.5.1).

3.5.5.3 Identification of RVFV and other arboviruses in cell cultures exhibiting CPE

The process of total RNA isolation and virus identification are described (sections 3.5.2 and 3.5.3) using the primers described (Table 3.1). Only cell cultures exhibiting CPE were analyzed by RT-PCR to identify the infecting virus. All supernatants of mosquito heads with corresponding positive blood meals were not

subjected to RT-PCR because it was presumed that the CPE was due to dissemination of the confirmed virus in the blood meals to the heads.

Detection of virus in mid gut tissue was an indication of a mid gut infection (MI) and the mid gut infection rate (MIR) was calculated as the number of positive mosquito mid guts divided by the number of mosquito midguts assayed. Detection of virus in the head tissue revealed a disseminated infection (DI), and the disseminated infection rate (DIR) was calculated as the number of mosquitoes with the virus in the head tissue divided by the number of mosquitoes with MI. Proportions of mosquito species that fed on specific hosts was compiled to identify preferred hosts of each mosquito species. Mosquitoes with disseminated virus infection were considered to, probably, have transmitted the virus to the host, while those without disseminated infection were considered to have acquired the virus from the host.

3.6 Data Management and Analysis

Data were entered into a password-protected Excel (Microsoft Corporation, Redmond, WA, USA) spreadsheet. The relative abundance of the mosquito species was calculated as the number of each larval species divided by the total number of mosquito larvae sampled. The species abundance and composition in Marey and Wakab-Harey were compared using the analysis of similarity (ANOSIM) statistics. The relationship between the species composition and the environmental characteristics were analysed using a Mantel test based on Pearson's product-moment correlation. These comparisons were performed on ecological distances for community/ species matrix (Kulczynski distance) and environmental variables (Euclidean distance). To ensure that differences in total abundance among *dambos* do not influence the results, the data were standardized by dividing species abundance by the total abundance for each *dambo*. This was done for data from both sites combined, and separately for Marey and Wakab-Harey. Spatial/ geographical heterogeneity in mosquito species composition was analyzed by analysis of variance (ANOVA) to examine whether the relative abundance of these species varied between the *dambos*. Vector species diversity and relative abundance within and between dambos were analysed using Renyi's diversity index. Effect of physical and

environmental variables on occurrence and mosquito species composition in *dambos* was analysed by multiple quasi-Poisson regression model. Chromatograms for each individual sequence were edited in MEGA software. Edited sequences for isolated viruses and *cytb* were identified by comparison to the GenBank DNA sequence database, NCBI www.**ncbi**.nlm.nih.gov/) using BLAST while *COI* sequences were identified using BOLD, (www. barcodinglife.org).

3.7 Ethical Considerations

The community was informed by the local administration (Chief/Sub-Chief), via a local village meeting (*baraza*), of the investigators presence, the purpose of their visit and the nature of work to be done. No human subjects/ human samples nor experimental animals were used in this study. The investigators involved in mosquito sampling were provided with mosquito repellents such as N,N-Diethyl-meta-toluamide (DEET) to apply on clothing, before going out to set and collect traps to protect them against mosquito bites. This protocol was approved on October 25th 2011 by the KEMRI Ethical Review Committee, SSC 2121.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.0 RESULTS

4.1 Change in population structure of mosquito species (species succession) during and after flooding

4.1.1 Rainfall pattern and environmental temperature in Marey and Wakab-Harey in Sangailu, Garissa County, North eastern region

Marey received heavy rains that flooded the *dambos* between March 31st and April 1st 2013. The study team arrived on April 6th 2013. In Wakab-Harey, the first phase of the short rain season started on November 2nd 2013 but immediately flattened out until December 5th when the 2nd phase began. This 2nd phase of rains also lasted just for one day and recorded $\leq$ 50 mm Relative humidity remained fairly constant throughout (Fig. 4.1).

Figure 4.1 *Daily rainfalls, relative humidity and temperature for September to Decemer 19th 2013 in Wakab-Harey, Sangailu* (*Garissa County*).

4.1.2 Adult mosquito species sampled in Marey and Wakab-Harey

The first collection was made on April 6th 2013 (7 DPF) and yielded mainly floodwater *Aedes* Spp. comprising *Ae. tricholabis, Ae. ochraceus, Ae. mcintoshi* and *Ae. sudanensis*, but no *Culex* or *Anopheles* spp. Between 7 and 15 DPF, a total of 35,515 mosquitoes belonging to three genera and six species were collected. *Ae. tricholabis* was the predominant species (84%; n=29,817), followed by *Ae. ochraceus, Ae. mcintoshi, Cx. pipiens, Ae. sudanensis* and *Anopheles* spp. The daily adult collection did not vary greatly over the collection period. However, as expected, the composition of the mosquito spp. was uneven. Trap per night collection of adult mosquitoes for Marey is presented (Table 4.1).

Table 4.1 Abundance by species of adult mosquitoes collected in Marey between 7 and 15 days post-flooding

	Days post-flooding in April 2013						**Relative abundance**
Species	**7**	**9**	**11**	**13**	**15**	**Total**	**(%)**
Ae. tricholabis	7,850	5,231	5,899	4,458	6,379	29,817	84.00
Ae. ochraceus	596	775	1,136	687	725	3,919	11.00
Ae. mcintoshi	341	285	344	312	421	1,703	4.80
Cx. pipiens	0	0	23	11	1	35	0.09
Ae. sudanensis	7	13	5	2	2	29	0.08
Anopheles spp.	0	5	3	0	4	12	0.03
Total	8,794	6,309	7,410	5,470	7,532	35,515	100.00

In Wakab-Harey, the first adult trapping on November 6th 2013 (6 DPF) collected *Ae. mcintoshi* (n=47), *Cx. pipiens* (n=3) and *Cx. poicilipes* (n=5). There was gradual increase of *Ae. tricholabis*, *Ae. mcintoshi* and *Ae. ochraceus* up to 18 DPF, the last collection day. *Ae. tricholabis* was the most predominant (98.7%; n=46,755) followed by *Ae. mcintoshi* (0.62%; n=289), (Table 4.2).

Table 4.2 Abundance by species of adult mosquitoes collected in Wakab-Harey, between 6 and 18 days post-flooding

Species	Days post-flooding in November 2013								Relative abundance (%)
	6	8	10	12	14	16	18	Total	
Aedes tricholabis	1	218	7200	5810	7400	12051	14075	46755	98.69
Ae. mcintoshi	47	1	21	4	51	25	143	292	0.62
Ae. ochraceus	0	0	0	5	33	17	186	241	0.5
Ae. sudanensis	1	0	2	0	7	5	15	30	0.06
Ae. metallicus	0	0	0	0	0	0	11	11	0.02
Cx. pipiens	3	1	9	3	4	10	8	38	0.08
Cx. poicilipes	5	0	0	0	0	0	0	5	0.01
Cx. univittatus	0	0	1	0	1	2	0	4	0.01
Mansonia uniformis	0	0	0	0	2	0	0	2	0.004
Total	57	220	7233	5822	7498	12110	14438	47378	100

4.1.3 Cumulative mosquito species collected as larvae in Marey and Wakab-Harey

Larval sampling in Marey was conducted from April 6th (7 DPF) every alternate day until April 16th 2013 (17 DPF) after which all the *dambos* dried up. Mosquito species succession and fluctuations in numbers were observed in the 10 dambos. Nine species were collected during the study period. *Cx. pipiens*, *Cx. zombaensis*, and *Cx. univittatus* progressively increased in number over the sampling period (Fig. 4.2).

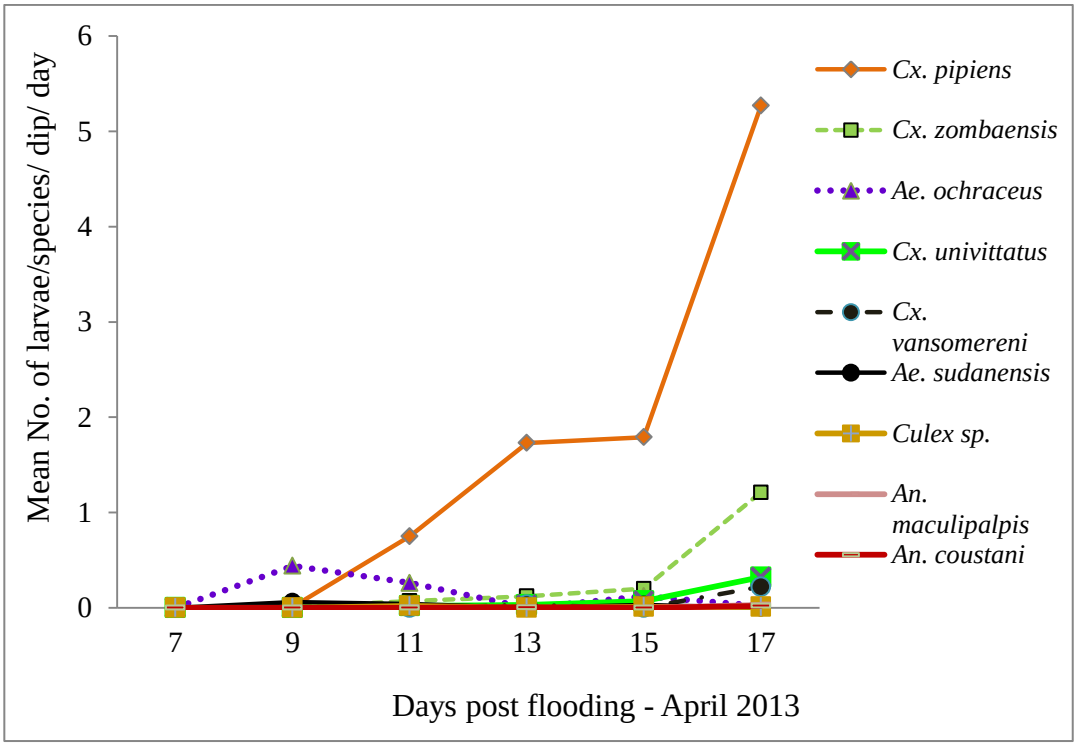


Figure 4.2 *Cumulative abundance of mosquito species collected as larvae per dip for each day sampled in Marey.*

In Wakab-Harey, larval sampling was conducted from November 6th (6 DPF) to November 18th 2013 (18 DPF) during which thirteen species were collected. *Cx. univittatus* and *Cx. pipiens* progressively increased in number peaking at 14 DPF before drastically decreasing (Fig. 4.3).

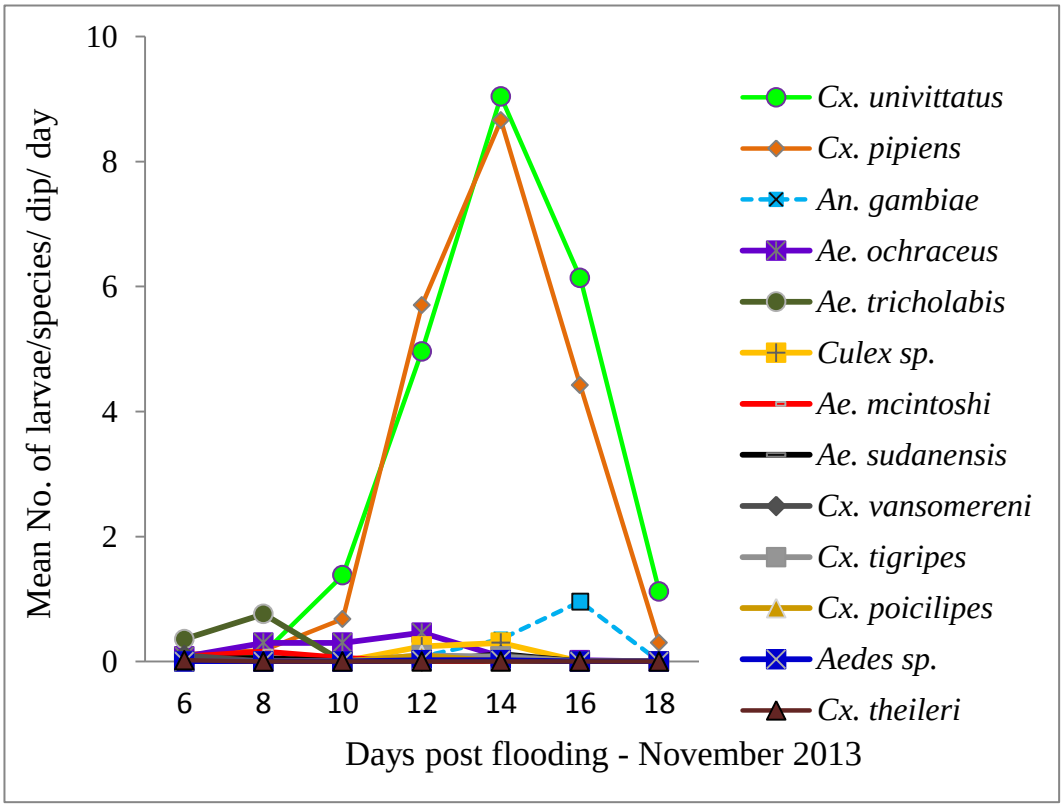


Figure 4.3 *Cumulative abundance of mosquito species collected as larvae per dip for each day sampled in Wakab-Harey.*

A total of 2,565 mosquitoes were collected from 10 *dambos* sampled in Marey. Emerging adults were identified into 3 genera and 9 species of varying abundances. The first larvae were sampled on April 7th (8 DPF). At the time of sampling, Marey was dominated by *Cx. pipiens* (74.8%). While members of *Culex* spp. collection increased with subsequent sampling days, both *Ae. ochraceus* and *Ae. sudanensis* seemed to decrease. *Ae. tricholabis* was not sampled as larvae (Table 4.3).

Table 4.3 Cumulative number of mosquito species collected as larvae for all the *dambos* in Marey from day 7 to 17 post-flooding

	Days post flooding							Relative abundance
Species	**7**	**9**	**11**	**13**	**15**	**17**	**Total**	**(%)**
Cx. pipiens	0	3	193	379	390	941	1906	74.3
Cx. zombaensis	0	3	16	25	42	208	294	11.5
Ae. ochraceus	0	128	65	0	25	2	220	8.6
Cx. univittatus	0	0	3	4	11	47	65	2.5
Cx. vansomereni	0	0	0	5	0	39	44	1.7
Ae. sudanensis	0	10	5	0	3	0	18	0.7
Culex spp.	0	0	5	0	2	2	9	0.4
An. maculipalpis	0	0	0	0	0	5	5	0.2
An. coustani	0	0	0	1	0	3	4	0.1
Total	0	144	287	414	473	1247	2565	100.0

A total of 2,118 mosquitoes were collected from 7 *dambos* sampled in Wakab-Harey. Emerging adults were identified into 3 genera and 13 species of varying abundances. At the time of sampling, Wakab-Harey was dominated by *Cx. univittatus* (45.3%) and *Cx. pipiens* (42.2%). Like in Marey, *Culex* spp. collection increased with subsequent sampling days. *Ae. tricholabis* seemed to decrease over time while *Ae. ochraceus* increased before starting to decrease at 14 DPF although the collections were very low (Table 4.4).

Table 4.4 Cumulative number of mosquito species collected as larvae for all the dambos in Wakab-Harey from day 6 to 18 post flooding

	Days post flooding								Relative
	6	8	10	12	14	16	18	Total	abundance (%)
Cx. univittatus	0	6	57	203	393	258	40	957	45.1
Cx. pipiens	6	9	34	251	390	188	13	891	42.1
An. gambiae	0	0	0	5	18	46	0	69	3.3
Ae. ochraceus	5	18	18	19	4	1	0	65	3.1
Ae. tricholabis	13	27	19	3	0	0	0	62	2.9
Culex spp.	0	0	0	9	13	0	0	22	1.0
Ae. mcintoshi	4	8	3	0	0	0	0	15	0.7
Ae. sudanensis	2	3	0	0	5	0	0	10	0.5
Cx. tigripes	0	0	0	3	5	0	0	8	0.4
Cx. vansomereni	3	2	1	1	3	0	2	12	0.6
Cx. poicilipes	0	0	0	5	0	0	0	5	0.3
Aedes spp.	0	0	0	0	1	0	0	1	0.0
Cx. theileri	1	0	0	0	0	0	0	1	0.0
Total	34	73	132	499	832	493	55	2118	100.0

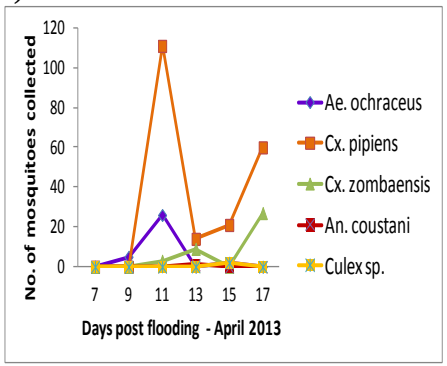
4.1.4 Species diversity for mosquitoes collected as larvae in Marey and Wakab-Harey

There was a significant difference in species composition between Marey and Wakabharey (ANOSIM statistics $r=0.3$, $p=0.005$). The Shannon diversity indices for Marey and Wakab-Harey were 0.91 and 1.22 respectively.

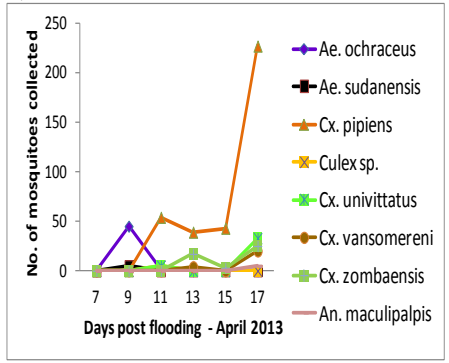
In Marey, *dambo* 4 was dry by 17 DPF, *dambo* 6 and 7 by 13 and 11 DPF respectively. The highest number of species was recorded in *dambo* 2 and the least in *dambo* 7. The only floodwater mosquitoes, primary vectors of RVFV, collected in Marey were *Ae. ochraceus* and *Ae. sudanensis*, both sampled in *dambo* 2, 3, 4, 6 and 7. *Ae. ochraceus* was also sampled in *dambo* 1, 5 and 9. The secondary vectors including

Cx. pipiens, (predominant in most of the *dambos*) *Cx. univittatus* and *Cx. zombaensis* were collected in *dambos* 2, 8, 9 and 10. The collection of *Cx. pipiens* in virtually all the *dambos*, except *dambo* 7 which only produced *Ae. ochraceus* and *Ae. sudanensis*, was a significant observation. *Cx. zombaensis* was also collected in *dambos* 1 and 5 (Fig. 4.4).

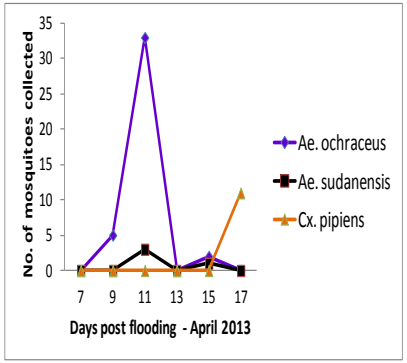
a) Dambo 1



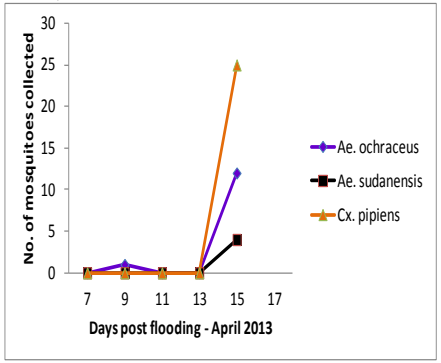
b) Dambo 2



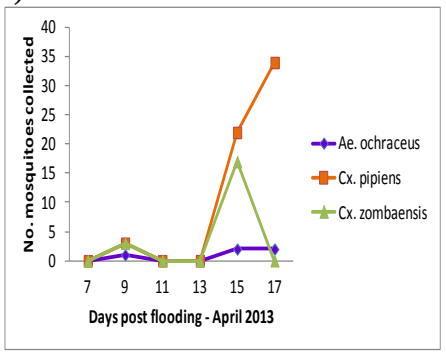
c) Dambo 3



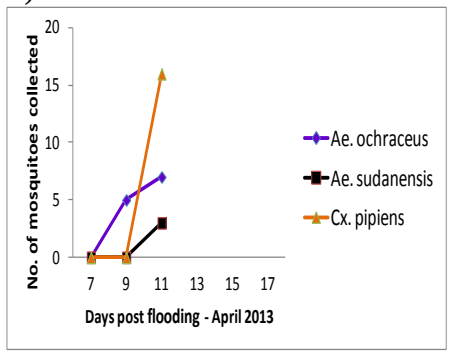
d) Dambo 4



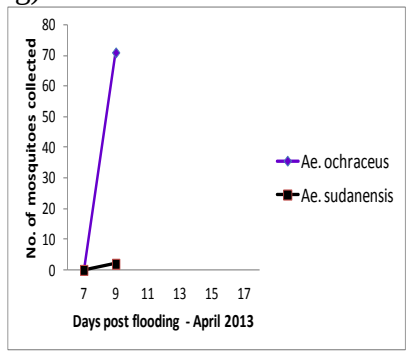
e) Dambo 5



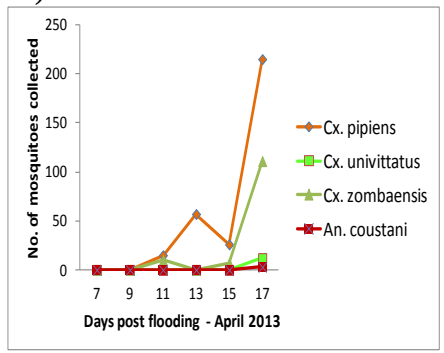
f) Dambo 6



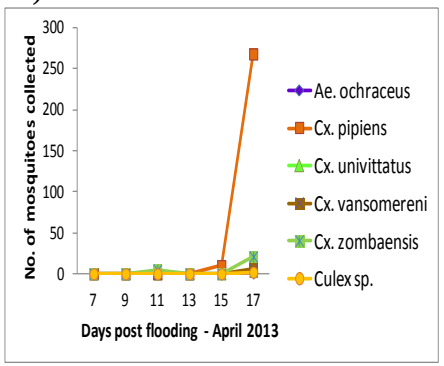
g) Dambo 7



h) Dambo 8



i) Dambo 9



j) Dambo 10

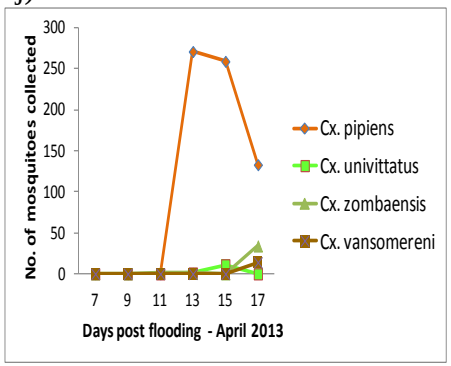


Figure 4.4: Comparison of the abundance and diversity of mosquito species collected per day across the 10 dambos in Marey. Dambo 6 and 7 were sampled for 3 and 2 days respectively before the dambos dried up

Dambos 2 and 7 were the least and most diverse (Shannon's diversity indices: 1.142 and 0.126 respectively) with 8 and 2 species respectively (Fig. 4.5).

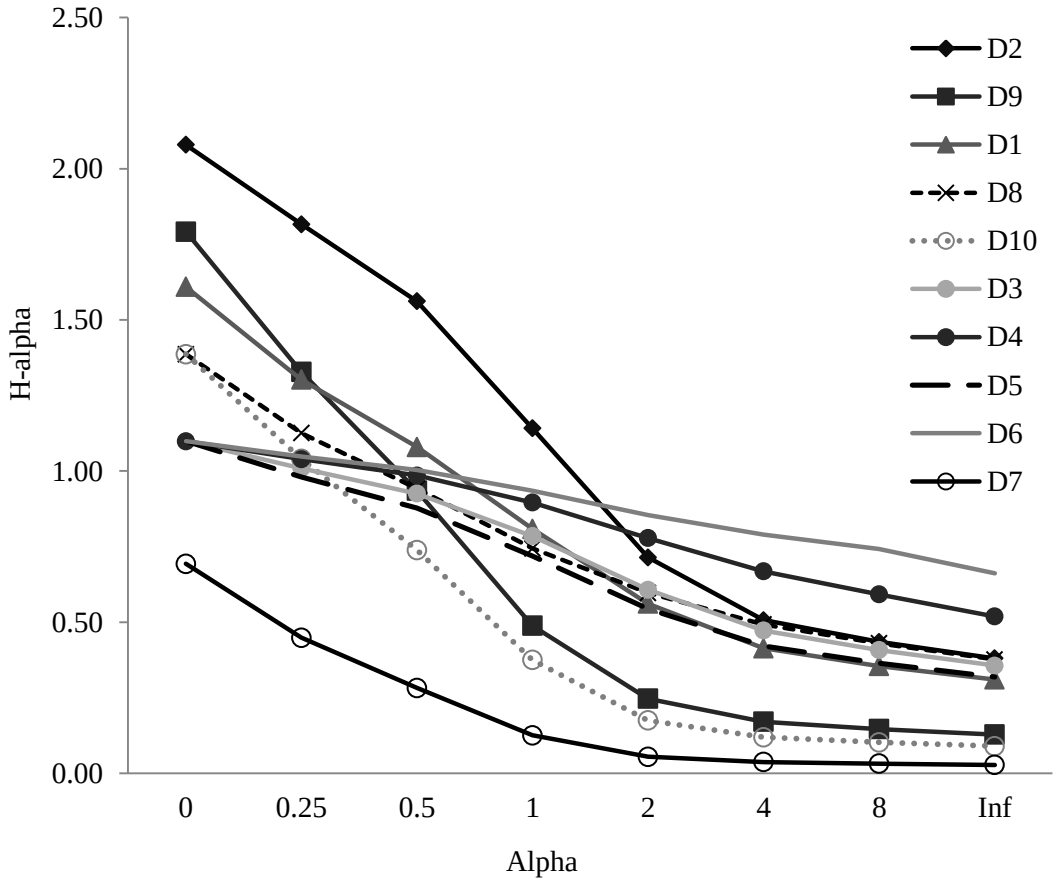
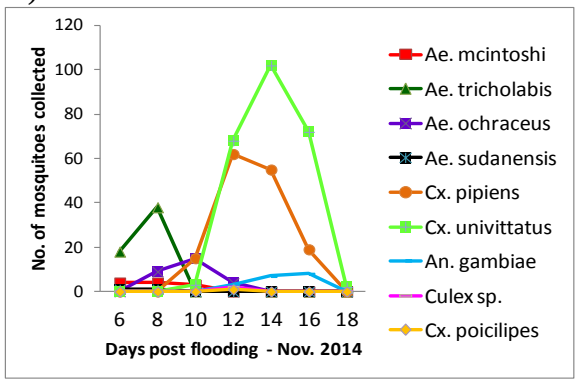


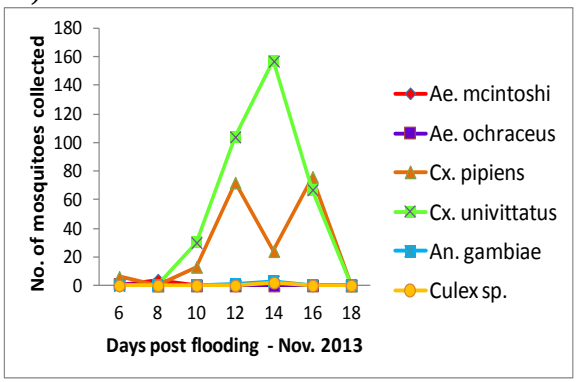
Figure 4.5 *Renyi diversity profiles for the dambos sampled in Marey.*

In Wakab-Harey, *Dambo* 4 was dry by 15 DPF, *dambo* 6 by 14 and *dambo* 7 by 16 DPF. The floodwater mosquitoes sampled in *dambo* 1 were *Ae. mcintoshi*, *Ae. ochraceus*, *Ae. sudanensis* and *Ae. tricholabis*. *Ae. ochraceus* was sampled in all the *dambos* while *Ae. mcintoshi* was sampled in *dambo* 1 and 2, *Ae. tricholabis* in 1 and 7 and *Ae. sudanensis* in 1, 3, 4 and 7. The secondary vectors, *Cx. pipiens* and *Cx. univittatus*, which predominated in five of the seven *dambo*, were sampled in all the *dambo* while no *Cx. zombaensis* was sampled. *Cx. poicilipes* was sampled in *dambo* 1, 3 and 7 while *Cx. vansomereni* was sampled in *dambo* 3. *An. gambiae*, which is a vector of malaria, was sampled in *dambo* 1, 2 and 3 (Fig. 4.6).

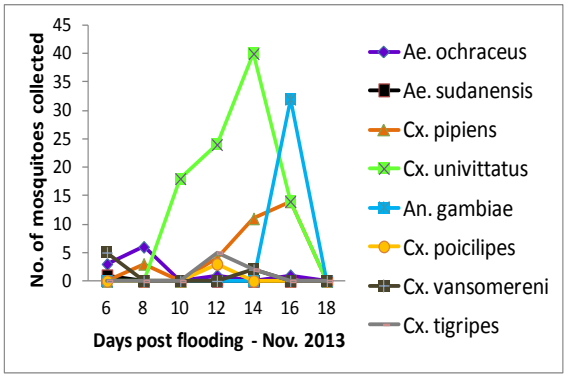
a) Dambo 1



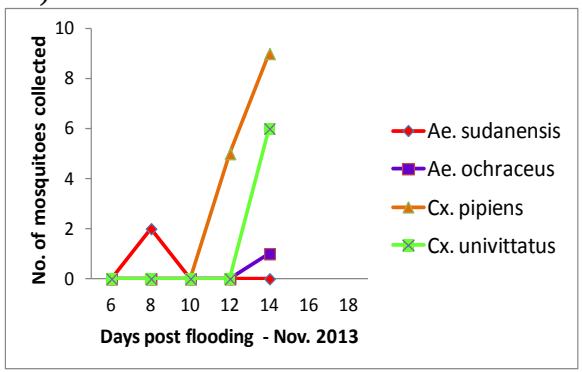
b) Dambo 2



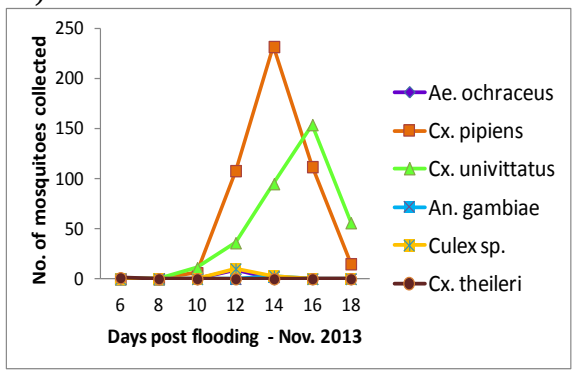
c) Dambo 3



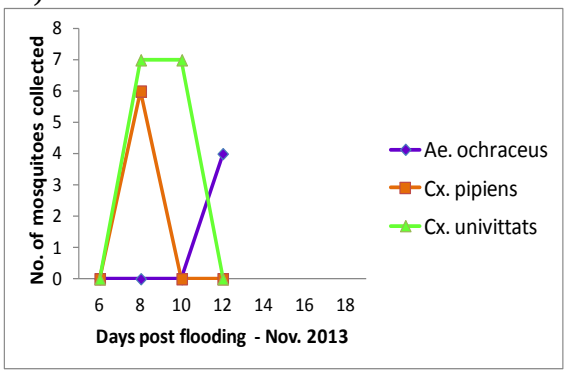
d) Dambo 4



e) Dambo 5



f) Dambo 6



g) Dambo 7

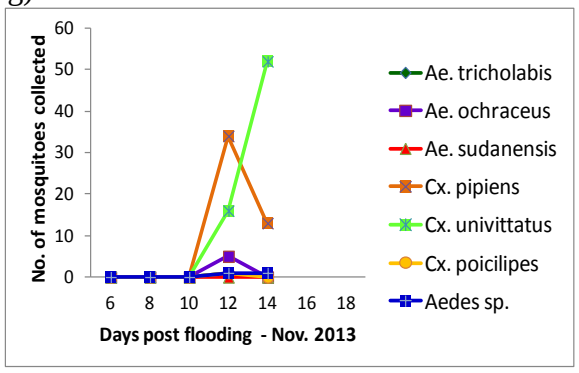


Figure 4.6 Comparison of the abundance and species diversity across the 7 dambos in Wakab-Harey.

Dambo 3 was the most diverse with 10 species and Shannon diversity index of 1.628 while *dambo* 6 was the least diverse with only one species and Shannon diversity index of 0.0. (Fig. 4.7).

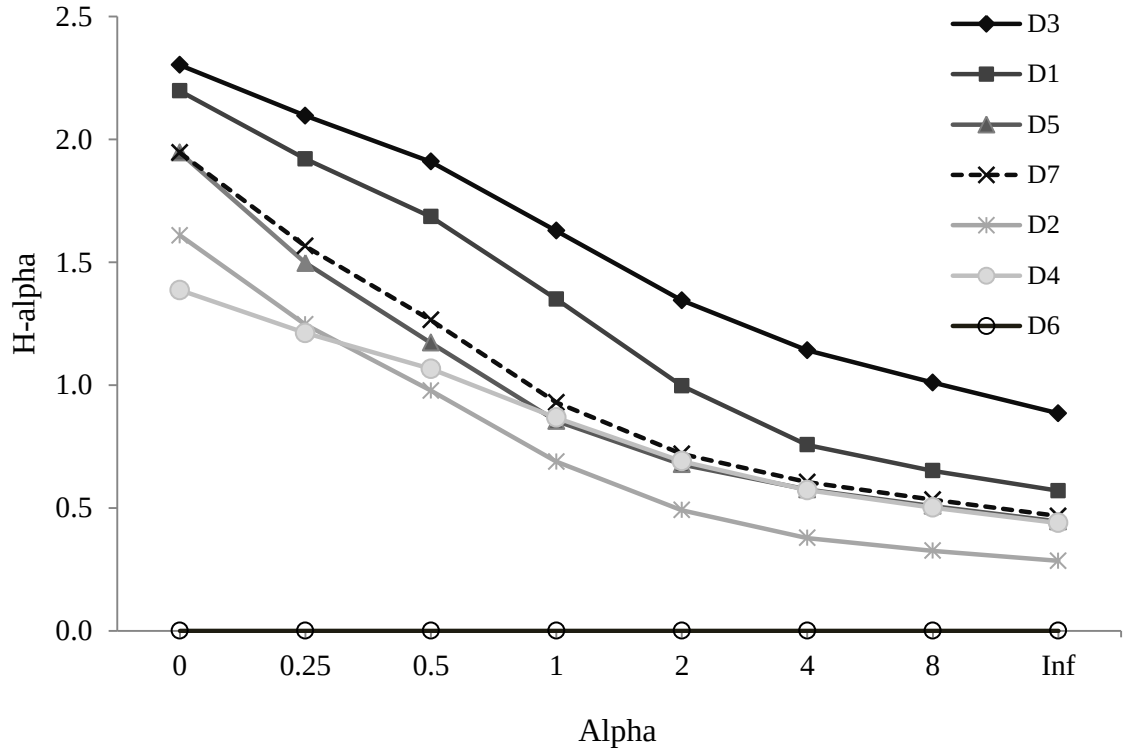


Figure 4.7 *Renyi diversity profiles for the dambos sampled* in *Wakab-Harey*.

4.2 Relationship between physical and environmental characteristics of *dambos* and the species composition and density

4.2.1 Physical and environmental characteristics of the dambos in Marey and Wakab-Harey

In Marey, *dambo* 8 was the deepest and *dambos* 1, 4 and 7 the shallowest. The pH ranged from 8.7 – 10.8 while the daily mean temperature of the *dambos* ranged form 32.3°C – 37.4°C. The highest pH was recorded in *dambo* 4 (10.8) and the highest daily mean temperature in *dambo* 2 (Table 4.5).

Table 4.5 Physical and environmental characteristics of *dambos* in Marey

Co-ordinates	Dambo	Surface area (m^2)	Depth (cm)	Daily mean temp (°C)	pH	Vegetation cover
S01°14'46.8" E040°39'30.4"	1	24	10	36.6	10.3	Open, no canopy, no vegetation cover
S01°14'45.5" E040°39'29.6"	2	36	20	37.4	9.8	Grass and water plants
S01°14'46.2" E040°39'27.6"	3	20	20	35.3	10	Open with grass on edges
S01°14'47.0" E040°39'27.7"	4	9	10	36.2	10.8	Grass and twigs on edges
S01°14'47.4" E040°39'27.5"	5	56	15	35.4	10.7	Open, no plant canopy, no vegetation cover
S01°14'44.5" E040°39'05.7"	6	64	18	32.6	9.7	Canopy present, grass on edges and center
S01°14'45.9" E040°39'05.3"	7	40	10	36.1	8.7	Open with grass on the edges
S01°14'38.4" E040°39'04.0"	8	20	22	35	9.1	Open with grass on the edges only
S01°14'46.3" E040°39'31.6"	9	15	20	36.9	10.1	Open, no plant canopy, no vegetation cover
S01°14'47.6" E040°39'05.0"	10	100	17	32.3	9.9	Plant canopy, with grass all over

Depths of dambos were measured at the center using a meter rule

In Wakab-Harey, the surface area of the *dambos* ranged from 100 – 8,750 m^2, the depth ranged from 15 – 45 cm while the pH range was 11.7 – 14 and the daily mean *dambo* temperature range was 35.1°C – 38.8°C (Table 4.6).

Table 4.6 Physical and environmental characteristics of *dambos* in Wakab-Harey

Co-ordinates	Dambo	Surface area (m^2)	Depth (cm)	Daily mean temp (°C)	pH	Vegetation cover
-1.30557622 40.70826198	1	2,800	15	37.2	12.7	Plant canopy
-1.30683518 40.70520585	2	700	45	38.8	13.8	Plant canopy
-1.30628809 40.70432131	3	6,400	25	37.3	12.7	Plant canopy
-1.30442773 40.70111155	4	1,960	20	35.7	12.1	Plant canopy
-1.30518738 40.7036526	5	7,850	30	38.6	12.7	Plant canopy
-1.30416831 40.69900023	6	100	15	35.1	14	Plant canopy
-1.30207418 40.70108623	7	615	20	37.8	11.7	Plant canopy

Depths of dambos were measured at the center using a meter rule

Both Marey and Wakab-Harey differed significantly with respect to physical and environmental characteristics. The mean (standard error) surface area, depth, daily temperature and pH for all the *dambos* sampled were 1224.06 m^2 (574.24), 19.53 cm (2.05), 36.14°C (0.44) and 11.11 (0.40) respectively. The mean depths of the *dambos* were 16.2 and 24.3 cm for Marey and Wakab-Harey respectively. The pH values show that all the *dambos* in both sites were alkaline, although more so in Wakab-Harey than Marey. The mean daily temperature for the *dambos* in Marey was 35.38°C and 37.21°C in Wakab-Harey. The two-sample t-test comparisons were based on log transformed values, but data presented in the table are on their original scale (Table 4.7).

Table 4.7 Summary statistics for the physical and environmental characteristics of the *dambos* sampled in Marey and Wakab-Harey

	Marey		Wakab-Harey			
Variable	**Mean**	**s.e**	**Mean**	**s.e.**	**t-value**	**p-value**
Surface area (m^2)	38.40	8.86	2,917.86	1150.00	-6.97	<0.001
Depth (cm)	16.20	1.48	24.29	4.00	-2.18	0.046
Daily mean temp. ($^{\circ}C$)	35.38	0.54	37.21	0.52	-2.34	0.034
pH	9.91	0.21	12.81	0.32	-7.93	<0.001

s.e. = standard error

4.2.2 Association between physical and environmental characteristics of *dambos* and the species composition

Results for both sites combined indicated a significant association between the levels of pH and species composition (*r=0.45, p=0.001*), but not surface area (*r=0.06, p=0.280*), depth (*r=0.18, p=0.175*), and the water temperature *(r=0.01, p=0.425*). For Marey, none of the factors was significant: *dambo* surface area (*r=-0.19, p=0.900*), depth (*r=-0.01, p=0.400*), *dambo* water temperature (*r=-0.20, p=0.9*). However the association pH and species composition was positive but not significant (*r=0.24, p=0.100*). For Wakab-Harey, there was a significant positive association between pH and species composition (*r=0.41, p=0.040*). The association between depth and species composition (*r=0.01, p=0.300*), and *dambo* water temperature and species composition (*r=0.38, p=0.100*) were also positve, but not significant while the negative association between surface area and species composition (*r=-0.16*) was also not significant (*p=0.600*).

4.2.3 Association between environmental variables and species abundance

To study the association between the environmental characteristics and species abundance, a quasi-Poisson model was fitted with the log number of dips as an *offset*, with the site also controlled for. Of the 22 species, only the 10 most abundant were

considered. For all the species combined, the larval abundance was significant associated with all the factors ($p<0.001$) except daily water temperature ($p=0.221$). The model results for the association between the environmental variables for the *dambos* and the abundance of all the species combined (top row) and each of the species separately (subsequent rows) are presented. All the factors were significantly associated with the abundance of *Cx. pipiens* ($p<0.001$) but no factor was significantly associated with *Ae. ochraceus* and *Ae. sudanensis.* These model results should, however, be interpreted with a lot of caution because of the very small numbers of larvae recorded for each species. The model did not converge for *Cx. zombaensis, An. gambiae,* and *Ae. trihcolabis* (Table 4.8).

Table 4.8 Quasi-Poisson regression model results for the association between physical and environmental variables of the *dambos* and the species abundance. All species abundance includes data on all the 22 species (combined top row) recorded. Only species for which the models converged are presented.

		Variable				
Species		Site	Surface area (m^2)	Depth (cm)	Daily mean temperature	pH
All	RR (95% CI)	0.46 (0.39-0.55)	1.01 (1.01-1.01)	1.06 (1.05-1.06)	1.01 (0.99-1.04)	0.73 (0.7-0.77)
	P value	<0.001	<0.001	<0.001	0.221	<0.001
Cx. pipiens	RR (95% CI)	0.21 (0.16-0.27)	1.02 (1.02-1.02)	1.07 (1.06-1.07)	0.93 (0.91-0.95)	0.73 (0.68-0.78)
	P value	<0.001	<0.001	<0.001	<0.001	<0.001
Cx. univittatus	RR (95% CI)	1.98 (1.26-3.12)	1 .00 (0.99-1.00)	1.00 (0.98-1.01)	1.89 (1.68-2.14)	1.17 (1.01-1.35)
	P value	0.003	0.928	0.838	<0.001	0.033
Ae. ochraceus	RR (95% CI)	0.52 (0.06-3.93)	1.01 (0.97-1.04)	0.93 (0.85-1.01)	1.45 (1-2.30)	0.61 (0.33-1.11)
	P value	0.553	0.609	0.132	0.104	0.131
Cx. vansomereni	RR (95% CI)	0.002 (0.00-0.04)	1.05 (1.02-1.09)	1.12 (1.05-1.22)	1.10 (0.91-1.33)	1.02 (0.58-1.9)
	P value	0.001	0.005	0.002	0.336	0.949
Culex spp.	RR (95% CI)	0.001(0.00-0.03)	1.00 (0.98-1.02)	0.86 (0.71-0.98)	39.15 (7.55-512.99)	3.55 (1.32-11.46)
	P value	0.002	0.950	0.078	0.005	0.038
Ae. sudanensis	RR (95% CI)	1.45 (0.07-28.41)	0.99 (0.93-1.03)	0.97 (0.85-1.07)	1.28 (0.79-2.43)	0.63 (0.24-1.55)
	P value	0.810	0.605	0.580	0.395	0.344
Ae. mcintoshi	RR (95% CI)		1.05 (1.01-1.12)	0.93 (0.73-1.07)	0.73 (0.35-1.52)	1.4 (0.74-2.76)
	P value		0.049	0.441	0.381	0.295

RR = Relative risk

4.3 Establishing TOT of RVFV and other arboviruses in mosquitoes collected as larvae and pupae from Sangailu, Ijara District, Garissa County

4.3.1 Mosquitoes collected, cell culture and PCR results

A total of 4,683 mosquito larvae were collected. In Marey, 2,565 mosquito larvae were collected between 7th and 15th of April 2013 while 2,118 were collected in Wakab-Harey between 6th and 18th of November 2013.These collections were made in the same *dambos* analysed in 4.1.4 and 4.1.5 above. The mosquitoes were reared to adults, identified and pooled into 325 pools of $\leq$ 25 mosquitoes each for virus isolation. The mosquitoes belonged to three genera and twelve different species (Table 4.9).

Table 4.9 Larval mosquito species abundance and virus isolation in reared adults from Marey and Wakab-Harey

	Mosquito collection and virus isolation in the two sites					
	Marey			Wakab-Harey		
Mosquito species	Total	No. of pools	Virus isolates (positive pools)	Total	No. of pools	Virus isolates
Ae. mcintoshi	0	0	_	15	4	_
Ae. ochraceus (F)	131	10	_	56	8	_
Ae. ochraceus (M)	89	6	UNIDENTIFIED (1)	9	4	_
Ae. sudanensis	18	3	_	10	5	_
Ae. trihcolabis	0	0	_	62	7	_
Aedes sp.	0	0	_	1	1	_
An. coustani	4	1	_	0	0	_
An. gambiae	0	0	_	69	12	_
An. maculipalpis	5	1	_	0	0	_
Cx. pipiens (F)	1,813	80	NDUV (2)	653	42	_
Cx. pipiens (M)	93	8	_	238	14	_
Cx. poicilipes	0	0	_	5	3	_
Cx. theileri	0	0	_	1	1	_
Cx. tigripes	0	0	_	8	4	_
Cx. univittatus	65	6	_	957	72	_
Cx. vansomereni	44	4	_	12	3	_
Cx. zombaensis	294	17	_	0	0	_
Culex spp.	9	4	_	22	5	_
Total	2,565	140	3	2,118	185	0

F = female M = male

Out of 325 pools inoculated in Vero cell cultures, 3 pools (IJR26, IJR37 and IJR73) caused CPE. The Vero cells inoculated with IJR26 developed CPE on day 3 post inoculation (p.i.) and were harvested on day 4 pi after CPE was observed in 75% of the monolayer. Vero cells inoculated with IJR37 and IJR73 developed CPE on day 3 and 2 p.i. respectively and both were harvested on day 5 pi because of the slow progression of the CPE. When the cell cultures that developed CPE were analysed by RT-PCR, the virus agent was confirmed to be Ndumu virus (NDUV) for two pools IJR37 (n=18) and IJR73 (n=3) of female *Cx. pipiens*, while that in IJR26 (male *Ae. ochraceus;* n=2) has not been determined (Plate 4.1). The IJR26, male *Ae. ochraceus* mosquitoes, were collected on 08.04.2013 from *dambo* 1 while IJR37 and IJR73 were collected on 15.04.2013, from *dambo* 9 and *dambo* 2 all of them from Marey from. This study did not detect RVFV in adults reared from larvae; thus not verifying TOT of RVFV.

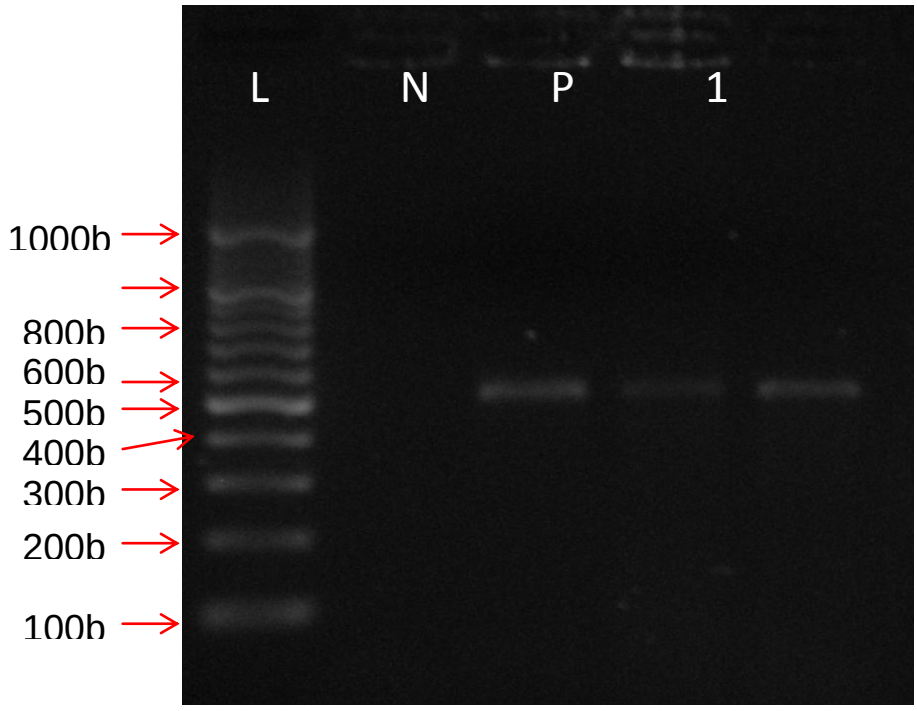


Plate 4.1 *Agarose gel image of PCR results of mosquitoes that were positive for NDUV. L - GeneRuler 100bp DNA ladder, ready to use, N – negative control, P – positive control, 1 – IJR037 (Cx. pipiens), 2 – IJR073 (Cx. pipiens).*

4.4 Determining the blood-feeding patterns of key vectors of RVFV

4.4.1 The blood feeding patterns of mosquitoes collected from El-Humow during the 2006-2007 outbreak

A total of 577 blood-fed mosquitoes, comprising of *Ae. ochraceus* (n=452), *Ae. mcintoshi* (n=124) and *Ae. sudanensis* (n=1), were collected from Garissa and analysed for blood meal source identification. Most of the blood meals in *Ae. ochraceus* mosquitoes were from goats (40.9%; n=185), cattle (17.5%; n=79), donkey (11.9%; n=54), sheep (4.6%; n=21), camel (3.3%; n=15) and 6%; n=27 from humans while 15.2%; n=69, were unidentified. Similarly, 37.9% (n=47) of blood-meals in *Ae. mcintoshi* were from goat, cattle 16.9% (n=21), human 7.3% (n=9), donkey 4.8% (n=6), sheep 3.2% (n=4) and unidentified 26.6% (n=33). Only one *Ae. sudanensis*, with blood meal from sheep, was sampled (Table 4.10).

Table 4.10 Proportion of blood meals taken from different vertebrate hosts by different species of mosquitoes in El-Humow, Garissa County.

Mosquito species	No. tested	Number (%) of mosquitoes feeding on									
		Human	Cattle	Sheep	Goat	Donkey	Camel	Lesser Kuddu	Duiker	Gazelle	Unidentified host
Ae. ochraceus	452	27(6.0)	79(17.4)	21(4.6)	185(40.9)	54(11.9)	15(3.3)	1(0.2)	0	1(0.2)	69(15.2)
Ae. mcintoshi	124	9(7.3)	21(16.9)	4(3.2)	47(37.9)	6(4.8)	3(2.4)	0	1(0.8)	0	33(26.6)
Ae. sudanensis	1	0	0	1(100)	0	0	0	0	0	0	0
Total	577	36(6.2)	100(17.3)	26(4.5)	232(40.1)	60(10.4)	18(3.1)	1(0.2)	1(0.2)	1(0.2)	99(17.1)

4.5 The prevalence of RVFV and other arboviruses in bloodfed field collected mosquitoes

4.5.1 The prevalence of RVFV in bloodfed mosquitoes collected from El-Humow during the 2006-2007 RVF outbreak

Five out of the 577 mosquito abdomens tested were infected with the RVFV, resulting in an overall mid gut infection rate (MIR) of 0.9%. Four of the five mosquitoes with mid gut infections (MI) were *Ae. ochraceus*, and therefore the MIR for RVFV in these mosquitoes was 0.9% (4/452). RVFV was detected in the mid gut of one *Ae. mcintoshi* mosquito representing 0.8% (1/124). No virus was detected in the *Ae. sudanensis* tested. Analysis of RVFV in the heads of *Ae. ochraceus* mosquitoes with MI revealed that 2 had disseminated infection (DI) with the DIR of 50% while DIR in *Ae. mcintoshi* was 100%.

In relation to vertebrate hosts, one of the 4 *Ae. ochraceus* mosquitoes with MI had fed on cattle, sheep (2) and unidentified host (1). One of the 2 mosquitoes with a MI had fed on sheep and the other, which had fed on unidentified vertebrate host, had DI. The *Ae. mcintoshi* with a MI also had a DI and had blood meal from a donkey (Table 4.11).

Table 4.11 Mid gut infection and disseminated infection rates of RVFV in blood-fed mosquitoes from El-Humow (Garissa County) in relation to their vertebrate hosts

				Rift Valley fever virus infection and dissemination rates			
Mosquito species	No.	Vertebrate host	No. of blood meals	RVFV +ve blood meals	MIR (%)	RVFV +ve heads	DIR (%)
Ae. ochraceus	452	Human	27	0	0	0	0
		Cattle	79	1	1.3	0	0
		Sheep	21	2	9.5	1	50
		Goat	185	0	0	0	0
		Unidentified	69	1	1.4	1	100
		Others	71	0	0	0	0
Ae. mcintoshi	124	Donkey	6	1	16.7	1	100
		Goat	47	0	0	0	0
		Camel	3	0	0	0	0
		Others	68	0	0	0	0
Ae. sudanensis	1	Sheep	1	0	0	0	0
Total	577		577	5	0.9	3	60

MIR was defined as the percentage of all the mosquitoes tested that had RVFV detected in their blood meals, while DIR was defined as the percentage of mosquitoes with RVFV positive blood meals that had the virus also detected in their heads.

4.5.2 The prevalence of NDUV in bloodfed mosquitoes collected from El-Humow (Garissa County) during the 2006-2007 RVF outbreak

In addition to RVFV, NDUV was also isolated from 19 mosquitoes collected from Garissa (Plate 4.2).

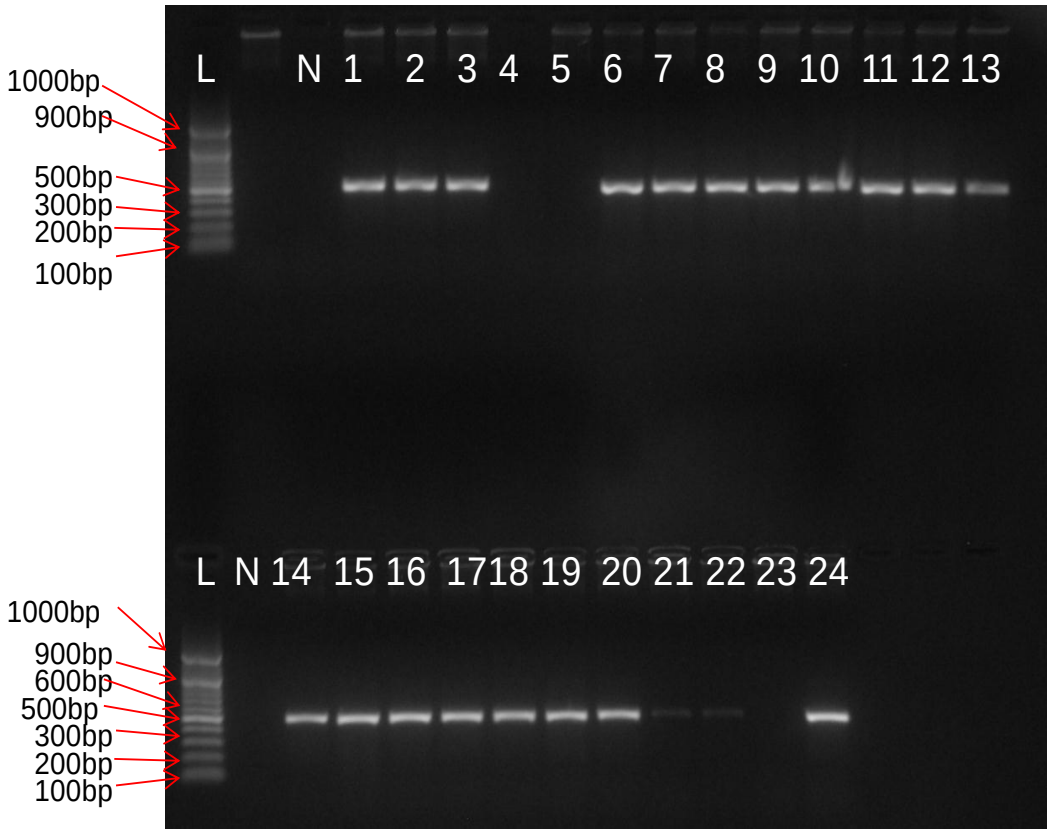


Plate 4.2 *Agarose gel image of 19 of the 24 blood meals that were infected with NDUV. L - GeneRuler 100bp DNA ladder, ready to use, N – negative control, 1-24 – sample.*

Thirteen of the NDUV isolates were from *Ae. ochraceus*, 5 from *Ae. mcintoshi* and 1 from *Ae. sudanensis*. Therefore, overall MIR and DIR of 3.3% and 1.4% respectively for NDUV were observed among all combined mosquitoes collected during the outbreak. In terms of specific mosquito species, MIR of 2.9% and DIR of 30.8% were observed in *Ae. ochraceus* mosquitoes, 4% and 80% in *Ae. mcintoshi* and a MIR of 100% in the one *Ae. sudanensis*.

In relation to vertebrate host, ten of the 13 *Ae. ochraceus* mosquitoes infected with NDUV had fed on goat and 3 had disseminated infection. One that had fed on sheep, had non-disseminated infection while one of the two with unidentified blood meal source also had disseminated infection. Infected *Ae. mcintoshi* mosquitoes which had fed on goat (n=2) camel (n=1) and donkey (n=1) had disseminated infection while the only infected *Ae. sudanensis* had a non-disseminated infection (Table 4.12).

Table 4.12 Mid gut infection and disseminated infection rates of NDUV in blood-fed mosquites collected from El-Humow, Garissa County, in relation to their vertebrate hosts

				Ndumu virus infection and dissemination rates			
Mosquito species	No.	Vertebrate host	No. of blood meals	NDUV +ve blood meals	MIR (%)	NDUV +ve heads	DIR (%)
Ae. ochraceus	452	Human	27	0	0	0	0
		Cattle	79	0	0	0	0
		Sheep	21	1	4.8	0	0
		Goat	185	10	5.4	3	30
		Unidentified	69	2	2.9	1	50
		Others	71	0	0	0	0
Ae. mcintoshi	124	Donkey	6	1	16.7	1	100
		Goat	47	3	6.4	2	66.7
		Camel	3	1	33	1	100
		Others	68	0	0	0	0
Ae. sudanensis	1	Sheep	1	1	100	0	0
Total	577		577	19	3.3	8	42

MIR was defined as the percentage of all the mosquitoes tested that had NDUV detected in their blood meals from a particular vertebrate host, while DIR was defined as the percentage of mosquitoes with NDUV positive blood meals that had the virus also detected in their heads.

4.5.3 The prevalence of RVFV and other arboviruses in bloodfed mosquitoes collected from Ijara and Baringo during the IEP, 2013

A total of 822 bloodfed mosquitoes were collected from Marigat in Baringo (280) and Sangailu in Ijara (542) in May and November 2013 respectively. These mosquitoes comprised of 3 genera and 11 species including *Ae. tricholabis, Ae. ochraceus* and *Ae. mcintoshi* from Ijara *and Ma. uniformis, Ma. africana* and *Cx. univittatus,* among others (Table 4.13). No virus was isolated from these samples.

Table 4.13 Bloodfed mosquito species sampled in Sangailu and Marigat during the IEP

	Sites			Virus
Species	Sangailu	Marigat	Total	isolation
Ae. tricholabis	350	0	350	_
Ae. ochraceus	185	2	187	_
Ae. mcintoshi	7	2	9	_
Ma. uniformis	0	95	95	_
Ma. africana	0	129	129	_
An. ocustani	0	13	13	_
An. gambiae	0	23	23	_
Cx. poicilipes	0	6	6	_
Cx. bitaeniorhynchus	0	2	2	_
Cx. pipiens	0	7	7	_
Cx. univittatus	0	1	1	_
Total	542	280	822	_

4.1 DISCUSSION

This study has demonstrated differential mosquito species succession and proportions in various *dambos* even those proximal to each other. It has also demonstrated the first evidence of TOT of NDUV in mosquitoes, as well as co-circulation of NDUV alongside RVFV during the 2006-2007 outbreak. The host seeking pattern of key vectors of RVFV is dynamic and influenced by the composition and proportion of the host animal species present in an area.

Floodwater mosquito breeding habitats (*dambos*) are usually subjected to fluctuating water levels depending on the amount and duration of the rainfall. High temperatures, a characteristic of semiarid areas such as Marey and Wakab-Harey in Ijara, may cause rapid evaporation and lead to drying of the *dambos* soon after a downpour while the acidic or alkaline conditions are unfavourable for the survival of immature stages of mosquitoes. One or a combination of these factors may cause the deaths of immature mosquito stages. In general, the rainfall in NE Kenya is sporadic, averaging 200–500 mm per year, with occasional torrential storms that cause extensive flooding (Sang *et al*., 2010). The rainfall pattern during both short and long rain seasons is bi-phasic. Both phases may comprise heavy rainfall of varied intensity, mostly lasting for one to several days, and are usually separated by a few days to about a month of minimal to no rains. In Marey and Wakab-Harey the first phase, during which sampling was conducted, lasted for two and one days respectively. These rains flooded the *dambos* resulting in hatching of floodwater mosquito eggs, but there was minimal or no subsequent rain for upto a month and so the *dambos* quickly dried up. This was caused by the high rate of evaporation due to the extremely high environmental temperatures prevailing at the time, as well as the *dambo* water being used for domestic purposes and watering of the numerous livestock and wildlife in the area.

It is difficult to tell if the *dambo* characteristics observed in Marey and Wakab-Harey are permanent or were unique to this sampling period. However floodwater mosquitoes, and the secondary vectors that subsequently take over to breed in these temporary habitats, would reflect several adaptations to survive in these extreme

conditions (Becker, 1989; Schäfer & Lundström, 2006). In most cases, all these factors collectively influence the choice of oviposition sites by gravid mosquitoes.

The predominance of adult floodwater *Aedes* mosquitoes in both Marey and Wakab-Harey during the first few days post flooding (DPF) and in the same localities where larval sampling was conducted suggests that these mosquitoes are usually the first to colonize the *dambos* upon flooding. The fact that no larvae were sampled in Marey on the first day that adults were collected means that these floodwater *Aedes* spp. had emerged 1-2 days earlier (4-5 DPF) and that hatching is synchronized (Breeland & Pickard, 1967; Logan *et al.,* 1991a). *Ae. tricholabis* was the predominant *Aedes* species collected as adults in both Marey and Wakab-Harey at 84 and 98.8% respectively, whereas *Ae. mcintoshi*, *Ae. ochraceus* and *Ae. sudanensis* collectively comprised 16 and 1.2% in both areas respectively. This is consistent with the previous findings of Lutomiah *et al.,* (unpublished data) where 87.7 and 85.2% of all adult floodwater *Aedes* mosquitoes collected in Marey and Wakab-Harey respectively were *Ae. tricholabis*. Therefore this suggests that this species is the most predominant among the floodwater *Aedes* spp. in the two Sub-locations.

The number of species was much lower than previously observed (Lutomiah *et al*., unpublished data). This suggests that during the two sampling periods, the rains were probably insufficient to allow for prolonged flooding which could have allowed a wider variety of mosquito species to breed or complete their development. Prolonged rains may therefore important for species proliferation.

The adult *Culex* and *Anopheles* spp. (secondary vectors) comprised 0.2 and 0.1% of adult collections in Marey and Wakab-Harey respectively. On the other hand, larval sampling data showed that floodwater *Aedes* comprised 9.3% and 7.2% of the total collection in Marey and Wakab-Harey respectively while the secondary vectors constituted over 90% in both areas. Although the percentages of the secondary vectors seemed high, the actual numbers were very low compared to those of floodwater *Aedes* spp. collected as adults. This is attributable to the early drying of *dambos,* due to insufficient rains and high temperatures, which may have prevented the secondary vectors

such as *Culex* spp. from colonizing and breeding in sufficient numbers as would be expected. The few floodwater *Aedes* mosquitoes observed from 8 and 6 DPF for Marey and Wakab-Harey respectively were perhaps at the tail-end of the synchronized hatching of floodwater *Aedes* spp. that typically follows the inundation of *dambos*.

If some of the hatching floodwater *Aedes* eggs are already infected with RVFV through TOT, as documented (Linthicum *et al*., 1985b), some of the mosquitoes that emerge during this first phase will already be carrying the virus. This is likely to result in the transmission of the virus to the smaller ruminants such as goats and sheep which are usually present in the villages throughout the year. This may explain the documented detection of RVFV antibodies in livestock and wildlife (Olive *et al*., 2012; Sumaye *et al*., 2013; Fafetine *et al*., 2013) and even humans (LaBeaud *et al*., 2008) during IEP. Hence, RVFV transmission and disease risk can occur during the early days of the rain season, as previously observed (Mondet *et al*., 2005). Therefore this first phase of rainfall during the rainy season may be significant in the epidemiology of RVF, both during and between epidemics.

Some of the floodwater *Aedes* spp. likely to initiate RVFV transmission are *Ae. mcintoshi* and *Ae. ochraceus. Ae. mcintoshi* is a known vector albeit a poor one (Turell *et al*., 2008) in which vertical transmission of RVFV has been demonstrated (Linthicum *et al*., 1985b). RVFV has only been isolated from field collected *Ae. ochraceus* mosquitoes (Fontenille *et al*., 1998; Sang *et al*., 2010) but its competence as a vector hasn't been evaluated. Although *Ae. tricholabis* is very abundant in the NE epizootic zones, it has never been associated with RVFV transmission either experimentally or through virus isolation from field collected samples. Therefore it is particularly important that the role of *Ae. ochraceus* and *Ae. tricholabis* in RVFV transmission be investigated. This is because it is difficult to fathom how *Ae. mcintoshi*, described as an inefficient vector, and *Ae. ochraceus* whose vectorial competence for RVFV is unknown, could have initiated an RVF outbreak of the 2006-2007 magnitude, whereas *Ae. tricholabis* that occurs in such abundance plays no role. For unknown reasons this species was interestingly collected in very small numbers during the 2006-2007 outbreak but has subsequently

been collected in large numbers in the same localities. Several other virus isolates have also been obtained from it (Ochieng *et al*., 2013).

Although RVFV transmission may be initiated during the early part of the first phase of the rains, the secondary vectors are usually insufficient, if the *dambos* run dry, to perpetuate virus transmission among the amplifying vertebrate hosts in the desired magnitutude to cause an outbreak. However if the second phase of rainfall follows shortly, the potential for an outbreak increases since the *dambos* are inundated again, and probably for long, thus allowing more primary vectors to hatch and the secondary vectors to breed in sufficient numbers. The second batch of the primary vectors will further seed the virus while the secondary vectors will disseminate the virus to livestock including cattle, which will have returned from the grazing fields, as well as wildlife. Usually, cattle travel with the herders to distant places in search of pasture during the dry season while the smaller ruminants remain in the homesteads to provide milk for the families.

Based on the onset of rains and the time that sampling began, it was estimated that it took between 4-5 days for the floodwater mosquitoes to undergo full development. This was probably influenced by the noted high temperatures of the *dambo* waters. Therefore the observations made in this study are consistent with those of Becker (1989), Fontanarrosa *et al*., (2000) and Mondet *et al*., (2005) that high temperatures influence the life cycle of floodwater mosquitoes. The high development rate prevents deaths due to unexpected drying of the *dambos*, which is the main cause of deaths of immature stages of mosquitoes (Pritchard & Scholefield, 1983).

The over 84% floodwater *Aedes* spp. collected as adults in both sites compared to those collected as larvae provide further evidence that floodwater mosquito eggs are the first to hatch following inundation. This is because the drought-resistant eggs, which are able to survive for several years (O'Malley, 1990) are already deposited in the soil following the previous rain season. It also provides further evidence of mass hatching of floodwater mosquito eggs based on r-strategy, and "installment hatching" (Rydzanicz, Kącki & Jawień, 2011). Installment hatching is a reproductive strategy for long-term survival of populations in case initial larvae die from drying of breeding habitats

(Andreadis, 1990). One interesting observation was the absence of larvae/ pupae in all the *dambos* in Marey 7 DPF, suggesting that those eggs which hatch when the *dambos* flood mature faster and almost at the same time resulting in an instant explosion of adult populations.

There was no positive association between the physical characteristics of *dambos* and species composition and density in both Marey and Wakab-Harey. This is despite the *dambos* varying greatly in depth and surface area. For example in Marey, *Ae. ochraceus* and *Ae. sudanensis* were sampled in 8 and 5 of the ten *dambos* respectively and overlapped in 5 out of 10. In Wakabharey, *Ae. ochraceus* and *Ae. mcintoshi* were both sampled in 3 of the 7 *dambos.* It has been suggested that mosquitoes are likely to choose deep water as an oviposition site to minimize the risk of deaths of immature stages due to habitat drying (Reiskind & Zarrabi, 2012). However laboratory studies have also shown that shallow *dambos* induce faster maturation of larvae (Juliano & Stoffregen, 1994) to avoid larval deaths arising from drying *dambos* thus ensuring survival and continuity of the species. This has been described for *Oc. sticticus* (Schäfer & Lundström, 2006) although the same study determined that *Ae. vexans* adults delayed to emerge with decreasing water levels. This suggests that different mosquitoes respond differently to drying larval habitats.

The vegetation cover and plant canopy did not have an effect on species composition and densities.

In both areas, there was no significant positive association between the water temperature and species composition. This is probably because water temperatures of the *dambos* were well above the optimal mean range of 16 - 32°C for mosquito breeding in the tropics (Bradley & Kutz, 2006). This suggests that the mosquito species in our study areas are well adapted to breeding in the "hot" *dambo* waters resulting from the high temperatures characteristic of the semiarid nature of Ijara. Environmental temperatures in these areas can rise as high as 40°C and influence the temperatures and volumes of the *dambo* waters. The rapidly decreasing water volumes in turn warms up much faster and increases the rate of larval development as an adaptive behavior which, in itself, is

temperature-dependent as demonstrated with *Oc. sticticus* (Schäfer & Lundström, 2006). This reduces larval mortalities due to drying of the habitats (Slater & Pritchard, 1979). The high environmental temperature also increases the rate of virus replication in the mosquitoes, and transmission, if some of the emerging mosquitoes are already infected.

Apart from Marey, it was interesting to note positive association between *dambo* water pH and the diversity of mosquito species in Wakab-Harey (*p=0.040*). However, larval abundance in both Marey and Wakab-Harey was associated with pH. This can be interpreted as an adaptation of mosquito spp. in this area to alkaline breeding habitats. The mean pH of all the *dambo* waters combined (9.91) was above the EPA recommended range of MPL (pH of 6-9). Any pH reading above MPL is unsuitable for mosquito habitation (Gilvear & Bradley, 2000; Tiimub, Adu & Obiri-Danso, 2012). Near neutral pH range of 6.8-7.2 is preferable for breeding of many species of mosquitoes (Pelizza, *et al.,* 2007; CDC, 2004). pH readings outside this range reduce the development of, and even kill mosquito eggs, larvae and pupae (CDC, 2007; Curtis, 1996). It is therefore clear that mosquitoes adapt to the prevailing pH conditions of the breeding habitats in specific geographical regions and it cannot be absolute that all mosquitoes breed within a specified narrow pH range. Variation in water pH of *dambos* may be associated with soil type. In the NE region, the soil is generally sandy with scattered areas of dark clay (Sang *et al*., 2010) that are usually alkaline in nature.

Although TOT of RVFV was not established in this study, this was established for NDUV in *Cx. pipiens*. TOT is a fairly common phenomenon among arboviruses and is thought to play a role in their maintenance in nature thus ensuring their survival during adverse conditions (Tesh, 1984). TOT of RVFV has been demonstrated twice (Linthicum *et al*., 1985b; Mohamed *et al*., 2013). It was expected that RVFV would most likely be isolated from floodwater mosquitoes including *Ae. mcintoshi* and *Ae. ochraceus* which are the primary vectors. However, these species were collected in small numbers as larvae and therefore limited the chances of isolating the virus.

In contrast to other arbovirus groups, there is little field or experimental evidence to suggest TOT of alphaviruses (Tesh, 1984). The few alphaviruses for which TOT has

been demonstrated include Eastern equine encephalitis virus (EEEV) which was isolated from *Culiseta melanura* larvae and a pool of male mosquitoes of the same species (Chamberlain & Sudia, 1961), and Ross River virus in experimentally infected *Ae. vigilax* mosquitoes (Kay, 1982). Buggy Creek Virus (Togaviridae: Alphavirus) has also been isolated from field-collected eggs of *Oeciacus vicarius* (Hemiptera: Cimicidae) (Brown *et al*., 2009). Therefore isolation of NDUV from field-collected *Cx. pipiens* represents the first evidence that this virus is maintained through TOT in this semiarid region that is characterized by prolonged drought conditions and limited breeding habitats for mosquitoes during the dry season. This is also an important finding regarding the natural maintenance of alphaviruses in general and probably other arboviruses including RVFV.

Because of the limited breeding sites during dry season, it is difficult to understand how the few *Culex* mosquitoes available could sustain transmission of the virus between themselves and the available vertebrate hosts especially given that laying of drought resistant eggs has not been demonstrated for this species. This observation is also interesting because NDUV has not been associated with *Cx. pipiens* before. In previous studies, this virus was isolated from several mosquito spp. including *Cx. rubinotus* and the floodwater mosquitoes; *Ae. tricholabis, Ae. mcintoshi* and *Ae. ochraceus;* and *Ma. africana* (Crabtree *et al*., 2009; Ochieng *et al*., 2013). Since NDUV was not being cultured in the laboratory at the time of processing of the collected mosquitoes, and because all necessary measures were taken to avoid cross contamination between pools of mosquitoes during processing, we can conclude that this isolation of NDUV from *Cx. pipiens* is not due to contamination of the specimens.

Since *Cx. pipiens* feeds on avians, it is possible that avians are important in the circulation of NDUV. However this virus has also been isolated from numerous mosquito species that feed on a wide range of vertebrate hosts that could also play a role in its circulation. Recently, Masembe *et al*., (2012) demonstrated the domestic pig (*Sus scrofa*) as a potential vertebrate host for NDUV while Lwande *et al*., (2013) isolated NDUV from *Rhipicephalus pulchellus* found infesting cattle (*Bos taurus*) and warthog (*Phacochoerus*

africanus). This signifies the potential for NDUV to be maintained by livestock as well as wild animals.

NDUV occurs throughout Africa including South Africa where it was first isolated from *Ma. uniformis* (Korkenot, McIntoshi & Worth 1961) and Kenya where over ten mosquito spp. including *Ae. mcintoshi, Ae. ochraceus, Ae. tricholabis, Ae. sudanensis* and *Cx. rubinotus* have been incriminated (Crabtree *et al.*, 2009; Ochieng *et al.*, 2013; Lutomiah *et al.*, 2014). Although antibodies to the virus have been identified in humans, no morbidity has been attributed to NDUV infection (Korkenot, McIntoshi & Worth 1961; Karabatsos, 1985) but mice experimentally infected with NDUV do not survive the infection (Korkenot, McIntoshi & Worth 1961). However, the genus Alphavirus comprises 31 members (Forrester *et al*., 2012) some of which cause diseases in humans. These include chikungunya virus which was responsible for the 2004-2005 severe epidemics along the East African coast (Chretien *et al*., 2007) as well as Ross river virus (RRV) which was associated with the 1979 epidemic in Fiji (Aaskov *et al*., 1981). Some of the symptoms associated with alphavirus infections range from fevers and rashes, to transient or debilitating arthritis, or encephalitis (Tesh, 1982; Whitley, 1990). This finding therefore provides further evidence to support the importance of TOT as a mechanism of maintaining arboviruses in nature.

Engorged *Ae. ochraceus* were the most collected mosquitoes during the 2006-2007 outbreak compared to *Ae. mcintoshi*. However, unengorged members of these two mosquito spp. collected during the same period comprised mostly of *Ae. mcintoshi* relative to *Ae. ochraceus* (Sang *et al*., 2010). Assuming that blood seeking *Ae. ochraceus* and *Ae. mcintoshi* are equally attracted to the CDC light traps, these results may suggest that bloodfed *Ae. ochraceus* survived for longer compared to *Ae. mcintoshi*, a factor that may influence their vectorial capacity for RVFV and other arboviruses including NDUV. Other factors affecting the observed low percentages include proximity of the mosquitoes to traps and the preferred host availability.

Despite these differences, both species obtained bloodmeals from the same vertebrate hosts in almost equal proportions with goats (*Capra hicus*), cattle (*Bos taurus*)

and donkeys (*Equus asinus*) being the common sources. This contrasts with previous observations that *Ae. mcintoshi* preferentially feeds on cattle (Anderson, 1967; Metselaar *et al*., 1973, Linthicum *et al*., 1985a; Beier *et al*., 1990). The high number of blood meals from goats may, however, be attributed to the fact that there are usually more goats around homesteads than cattle. These findings suggest that the host-seeking pattern of mosquitoes is dynamic and is influenced by the composition and proportion of host animal species present. However, the observed difference between this and earlier studies could also be attributed to improved methods of bloodmeal source identification. Previously, identification of mosquito bloodmeals was based on serologic tests, such as the precipitin test and enzyme-linked immunosorbent assays (ELISAs) (Tempelis, 1975; Washino & Tempelis, 1983). These methods were limited by the availability of species-specific antisera required for testing, and identifications were often only at the level of family or order. However the DNA barcoding used in this study can genetically identify bloodmeal source to the genus and species level.

The percentage of *Ae. ochraceus* and *Ae. mcintoshi* with human blood was nearly identical. This is quite significant considering the low human population, 5 people/km^2, in Sangailu (Statoids, 2007). Although it may appear that both species preferentially feed on goats over humans (*Homo sapiens*), goats are more common, located outdoors and always available to nocturnal-feeding mosquitoes (Beier *et al*., 1990). Similarly, some of the mosquitoes may have been collected several hours post-engorgement resulting in poor PCR amplification of human DNA, as the probability of obtaining good amplification decreases with time since ingestion (Mukabana *et al*., 2012). Among domestic animals, camels (*Camelus dromedarius*) whose population was low compared to cattle, goats and sheep during the outbreak (Murithi *et al*., 2007) were the least fed upon. Antibodies to RVFV have been detected in camels in Kenya prior to and after the 2006-2007 outbreak (Britch *et al*., 2013).

There were only a few instances of mosquitoes feeding on wildlife such as Lesser Kudu (*Tragelaphus imberbis*), Gazelle (*Gazella* spp.) birds (various spp.) and Duiker (*Cephalophus harveyi*). This could be due to the generally low density of wildlife

compared to livestock or the low preference for wildlife. Collection methods were also biased against mosquitoes that fed on wildlife since the traps were positioned proximal to human habitation, usually avoided by wildlife. Trap placement and availability of vertebrate host species influence the collection of engorged mosquitoes (Thiemann & Reisen, 2012). Because there is no current data on wildlife census in this area, it is difficult to speculate on the significance of the relative representation of a species or lack thereof in the bloodmeal host identifications. While our results may suggest that wildlife could be of limited significance in the epidemiology of RVFV during outbreaks, other studies have shown that the African buffalo and a number of other species have evidence of exposure to RVFV (Davies & Martin, 2003, Evans *et al*., 2008; Britch *et al*., 2013). Therefore it is possible that there is an independently maintained cycle in the wild which periodically interacts with livestock and humans resulting in outbreaks.

The rarity of *Ae. sudanensis* may be due to its predatory habit during larval stage (Knight, 1947). Therefore this species may become infected by feeding on transovarially infected larvae of other mosquito species. Although it has been observed feeding on humans (Knight, 1947) it may be less important regarding RVFV transmission since it usually occurs in low numbers. Therefore it is no wonder that *Ae. sudanensis* has never been associated with RVFV. The significance of the bloodmeals originating from unidentified hosts is unknown. Lack of host identification may have been due to co-amplification of mosquito genes, which prevents positive host identification (Townzen, Brower & Judd, 2008). The method used, including the database, may also have had limitations especially for gene variation, as well as sequence errors.

An interesting observation was the lack of blood-fed *Culex* mosquitoes sampled, yet they represented a large proportion of unengorged mosquitoes collected during the outbreak (Sang *et al.*, 2010). This suggests that when engorged, they are probably less attracted to CO_2-baited CDC light traps which have been demonstrated to be inefficient for capturing blood-fed mosquitoes (Watts, Fitzpatrick & Maruniak, 2009). However, several *Culex* spp. are important in RVFV transmission (Linthicum *et al*., 1985b) and are

highly efficient vectors in experimental studies (Turell *et al*., 2008). In Kenya, RVFV was isolated from several *Culex* spp. during the 2006-2007 outbreak (Sang *et al*., 2010).

Ae. ochraceus and *Ae. mcintoshi* occur most abundantly in Garissa County compared to any other part of the country (Lutomiah *et al*., 2013). Although we have no data on blood-fed specimens from Baringo, RVFV was mostly isolated from pools of *Ma. uniformis* during the 2006-2007 outbreak (Sang *et al*., 2010) suggesting that the two areas, separated by over 400 km, have distinct mosquito spp. that drive RVF outbreaks. In NE Kenya, there is an abundance of *dambos*, which are the preferred aestivation and breeding sites for floodwater mosquitoes (Linthicum *et al*., 1983, Linthicum, Davies & Kairo, 1984b) and are thought to be the source of virus infected mosquitoes (Linthicum *et al*., 1985b). And in Baringo, the expansive marshland provides suitable breeding habitats for the *Mansonia* spp. The availability of preferred breeding habitats specific to these mosquitoes in both areas means that there will always be abundance of RVFV vectors, hence the NE and Baringo in the Rift Valley will remain epizootic to RVF for many decades.

Despite the large number of mosquitoes tested in this study, no mixed blood meals were detected in individual mosquitoes. This is probably because of the limited variety of vertebrate hosts at any one time in adequate densities within the flight range of the mosquitoes to allow for multiple feeding (Tempelis, 1975). Another possible explanation is the feeding pattern of the mosquitoes, most of which take a single blood meal in a single gonotrophic cycle. Even in the case of multiple blood-feeding within a single gonotrophic cycle, a mosquito is likely to have uninterrupted feeding from a single vertebrate host and may seek a second bloodmeal only after the first is digested. This second blood meal may come from any vertebrate host species available but probably the most preferred if multiple host species are present in adequate proportions.

In general, the proportion of infected bloodfed *Ae. ochraceus* and *Ae. mcintoshi* was the same as those recorded for unengorged mosquitoes (Sang *et al*., 2010). Based on these proportions, it is possible that both species are equally important in RVFV transmission during outbreaks given that they also have preference for the same

vertebrate hosts including goats, cattle and sheep (*Ovis aries*); which are the main amplification hosts of the virus (Fischer *et al*., 2013). DIRs were highest in *Ae. mcintoshi* mosquitoes although this is based on only one specimen that had a midgut infection. Vector competence studies of *Ae. mcintoshi* have shown the species to posses a significant salivary gland barrier (SGB) with an estimated transmissio rate of 5% (Turell *et al*., 2008). This is still fairly efficient given the abundance of the species in this region. Detection of disseminated infections in the heads of *Ae. ochraceus* suggests that this species has the potential to transmit RVFV, unless they have a SGB, and may have played a major role during 2006-2007 outbreak. The non-disseminated infection in the two *Ae. ochraceus* mosquitoes may have been due to a mid gut escape barrier (MEB) or these mosquitoes had a recent infection.

Midgut infection rates (MIRs) in *Ae. ochraceus* were highest in specimens that had fed on sheep while DIRs were highest in specimens that had fed on unidentified hosts. The only specimen that had fed on cattle, and one that had fed on sheep, had non disseminated infection. This suggests that these specific *Ae. ochraceus* mosquitoes may have picked the infection from an already infected sheep and cattle respectively during the outbreak, and that both cattle and sheep were probably involved in virus amplification. This is interesting since an overall seroprevalence rate of 9.2% in sheep and 11.6% in goat has been documented (Fafetine *et al*., 2013). The two *Ae. ochraceus* mosquitoes with disseminated infection that each fed on sheep and unidentified hosts may have been transmitting the virus to these specific animal species. Hence the role of the unidentified host and donkeys in RVFV amplification remains unclear. The only mosquito with a blood meal from donkey in our study had a disseminated infection, hence may have transmitted the virus to donkey; however, equines are generally resistant to RVFV infection (Daubney, 1931) and there is no evidence that donkeys develop viremias after exposure. Lack of isolation of RVFV from mosquitoes which fed on camels was most likely due to the low number of specimens with camel blood but also probably due to camels being poor amplifiers of the virus (EFSA Journal, 2005). Based on our findings, both *Ae. ochraceus* and *Ae. mcintoshi* were the species that may have been significantly

involved in RVFV transmission in Garissa during the outbreak. This is supported by the almost equal number of isolates obtained from unengorged specimens during the outbreak (Sang *et al*., 2010).

The isolation of NDUV supports existing evidence that other viruses were circulating alongside RVFV during the outbreak (Crabtree *et al*., 2009). NDUV infection rates in *Ae. mcintoshi* and *Ae. ochraceus* were comparable. Most of these mosquitoes had bloodmeals from goats, of which 5/13 had disseminated infection, indicating that 8/13 blood meals may have been from infected goats. This suggests that goats are important in the natural maintenance and amplification of NDUV. However, it is also possible that some of the engorged mosquitoes with non disseminated infection were already infected with NDUV from a previous host at the time of ingesting the blood meals that were identified. Hence the virus in the blood meal may have been due to a "contamination" from the mosquito tissues during processing.

It was also interesting to observe that no mixed infection was detected despite both viruses co-circulating in the same area and being isolated from the same vector species. This may be a case of virus interference in nature, a phenomenon whose significance is unknown, but probably prevents or reduces the overall impact of an outbreak of the most lethal disease through "competition" between different viruses for the same vectors and amplifying hosts. The low infection rates observed for the circulating viruses, coupled with the small numbers of engorged mosquitoes tested in this study, may also explain the lack of mixed infection.

To demonstrate virus interference in the wild is a daunting task but in experimental studies, this phenomenon has been demonstrated for Kamiti river virus (KRV) and West Nile virus (WNV) (Lutomiah *et al*., unpublished data). Inhibition of California serogroup bunyaviruses in *Ae. triseriatus* has also been observed for mosquitoes previously infected with La Crosse (LAC) virus (Beaty *et al*., 1983). Porcine kidney cell line (PS cells) infected with *Dugbe virus* develope resistance to superinfection with *Bwamba virus* and *Nairobi sheep disease virus* (David-West & Porterfield, 1974).

No virus was isolated from bloodmeals from wildlife, probably due to the low number of mosquito specimens that fed on wildlife; and the inability of wildlife to develop viremias sufficient to infect mosquitoes. For instance, antelopes develop low prevalence of antibodies to RVFV (Davies, 1975); although recent work by Britch *et al*., (2013) suggests that seroprevalence increases after outbreaks. Birds are refractory to RVFV while susceptibility of deer-species remains unknown (Fischer *et al*., 2013) but natural infection of rodents has been documented (Imam *et al*., 1979).

This study did not detect RVFV or any other arbovirus infection in freshly collected blood-fed mosquitoes from Ijara or Marigat. This may have been due to the small number of samples assayed, since we only targeted bloodfed mosquitoes and mosquito abundance has been associated with the transmission of arboviruses even during IEP (Ochieng *et al*., 2013). Interepidemic transmission of RVFV has been described in mosquitoes, livestock and humans (Linthicum *et al*., 1985b; LaBeaud *et al.,* 2007; Sumaye *et al*., 2013, Mohamed *et al*., 2013). The difficulty in isolating arboviruses from mosquitoes outside an epizootic has been aptly demonstrated by the many unsuccessful attempts by various researchers including Metselaar *et al*., (1974) and Ochieng *et al*., (2013). It has been suggested that the prospects of detecting RVFV are high when the prevailing conditions are similar to those when epizootics are experienced (Linthicum *et al*., 1985b). In this study, sampling was conducted when the predisposing conditions, which constitute widespread and prolonged rainfall (Davies *et al*., 1985), were not prevailing since the rainfall pattern in Ijara during both sampling occasions was below normal.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.0 Summary

This study sought to determine the environmental drivers of densities of key RVFV vectors and the role of host-vector interaction in the maintenance of viruses in the natural ecosystem. Chapter four of this document provides a summary of the results and their interpretations. In general, this study has demonstrated differential mosquito species compositions and proportions in various breeding habitats as, probably, influenced by the physical and environmental variables of the habitats. It has also provided the first evidence of TOT of NDUV in mosquitoes, as well as showed that the key vectors of RVFV fed on a wide variety of hosts although they showed a preference for goats.

5.1 Conclusions

Five hypotheses were evaluated. The null hypothesis that population structure of mosquito species does not change during and after flooding was rejected. This is based on the observed differential mosquito spp. compositions and proportions in various *dambos*.

The hypothesis that there is no association between physical and environmental variables of *dambos* and the composition and densities of key RVFV vectors is also rejected. This study demonstrated an association between pH and species composition.

The hypothesis that arboviruses are not maintained in nature by TOT was rejected because TOT of NDUV in *Cx. pipiens* was demonstrated.

The hypothesis that blood fed RVFV vectors collected during the 2006-2007 outbreak did not feed on specific preferred vertebrate hosts was accepted. *Ae. mcintoshi* and *Ae. ochraceus* demonstrated equal preference for the same vertebrate hosts. However their host-seeking pattern was dynamic, probably influenced by the composition and proportion of host animal species present in an area.

The null hypothesis that bloodfed field-collected mosquitoes are not infected with arboviruses was rejected. This study established that NDUV was circulating alongside RVFV during the 2006-2007 RVF outbreak.

5.2 Recommendations

Studies on species succession should be conducted targetting prolonged rain seasons and also extended to other RVFV epidemic areas.

There is also need to conduct more studies on the effect of a wide range of physical and environmental characteristics of mosquito breeding habitats on the diversity and density of mosquito species in diverse RVFV epidemic regions.

More studies to determine if RVFV is maintained in nature by TOT are still needed.

To continue with studies on the prevalence of RVFV and other arboviruses in blood fed field-collected mosquitoes in RVF epizootic regions during wet seasons.

REFERENCES

Aaskov, J. G., Mataika, J. U., Lawrence, G. W., Rabukawaqa, V., Tucker, M. M., … Dalglish, D. A. (1981). An epidemic of Ross River virus infection in Fiji, 1979. *American Journal of Tropical Medicine and Hygiene,* 30**,** 1053–1059.

Abu-Elyazeed, R., el-Sharkawy, S., Olson, J., Botros, B, Soliman, A, … Arthur, R. (1996). Prevalence of anti-Rift-Valley-fever IgM antibody in abattoir workers in the Nile Delta during the 1993 outbreak in Egypt. *Bulletin of the World Health Oorganization,* 74**,** 155–158.

Ackermann, E. (1936). Dambos in Nordrhodesien, Wissenschaftliche Vero¨ffenlichungen, *Museum fur Landerkunde der Leipzig,* 4, 147–157.

Altshcul, S. F., Gish M., Miller, W., Myers, E. W. and Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology,* **215,** 403-410.

Anderson, G. W. Jr, Saluzzo, J. F., Ksiazek, T. G., Smith, J. F., Ennis W., … Digoutte J. P. (1989). Comparison of in vitro and in vivo systems for propagation of Rift Valley fever virus from clinical specimens. *Research in Virology,* 140, 129–138.

Anderson, D. (1967). Ecological studies on Sindbis and West Nile viruses in South Africa. III. Host preferences of mosquitoes as determined by the precipitin test. *South African Journal of Medical Science,* 32**,** 31-39.

Andreadis, T. G. (1990). Observations on installment egg hatching in the Brown Saltmarsh Mosquito, *Aedes cantator. Journal of the American Mosquito Contontrol Association,* 6**,** 727-729.

Andrewes, C. H. & Horstmann, D. M. (1949). The susceptibility of viruses to ethyl ether. *Journal of General Virology,* 3, 290–297.

Anonymous. (2000). Annual report of the WHO collaborating centre for reference and research on arboviruses and haemorrhagic fever viruses. Institut Pasteur de Dakar, Dakar, Senegal.

Anyamba, A., Chretien, J. P., Small, J., Tucker, C. J., Formenty, P. B. … Linthicum, K. J. (2009). Prediction of a Rift Valley fever outbreak. *Proceedings of the National Academy of Science,* 106, 955-9.

Apperson, C. S., Hassan, H. K., Harrison, B. A., Savage, H. M., Aspen, S. E., … Unnasch, T. R. (2004). Host Feeding Patterns of Established and PotentialMosquito Vectors of West Nile Virus in the Eastern United States. *Vector-Borne Zoonotic Diseases*, 4**,** 71-82.

Apiwathnasorn, C., Asavanich, A., Komalamisra, N., Samung, Y., Prummongkol, S., & Kanjanopas, K. (2006). The relationship between the abundance of *Mansonia* mosquitoes inhabiting a peat swamp Forest and remotely sensed data. *Southeast Asian Journal of Tropical Medicine and Public Health,* 37**,** 463-467.

Archer, B. N., Weyer, J., Paweska, J., Nkosi, D., Leman, P., … Blumberg, L. (2011). Outbreak of Rift Valley fever affecting veterinarians and farmers in South Africa, 2008. *South African Medical Journal,* 101**,** 263–266.

Arishi, H. M., Aqeel, A. Y. & Al Hazmi, M. M. (2006). Vertical transmission of fatal Rift Valley fever in a newborn. *Annals of the Tropical Paediatrics,* 26, 251–253.

Balcer, M. D., Schmude, K. L., Snitgen, J. & Lima, A. R. (1999). Long-term effects of the mosquito control agents *Bti* (*Bacillus thuringiensis israelensis*) and methoprene on non-target macroinvertebrates in wetlands in Wright County, Minnesota (1997–1998). Final report. Metropolitan Mosquito Control District. St. Paul, MN. 140 pp.

Barnard, B. J. H. (1979). Rift Valley fever vaccine–antibody and immune response in cattle to a live and an inactivated vaccine. *Journal of the South African Veterinary Association,* 50, 155-157.

Beaty, B. J., Bishop, D. H. L., Gay, M. & Fuller, F. (1983). Interference between Bunyaviruses in Aedes triseriatus Mosquitoes. *Virology,* 127**,** 83-90.

Becker, N. (1989). Life strategies of mosquitoes as an adaptation to their habitats, *Bulletin of the Society of Vector Ecolology,* 14, 6–25.

Beier, J. C., Odago, O. W., Onyango, F. K., Asiago, C. M., Koech, D. K. & Roberts, C. R. (1990). Relative abundance and blood feeding behavior of nocturnally active culicine mosquitoes in western Kenya. *Journal of the American Mosquito Control Association,* 6, 207-212.

Bentley, M. D. B. & Day, F. J. (1989). Chemical ecology and behavioral aspects of mosquito oviposition. *Annual Review of Entomology,* 34, 401-21.

Bird, B. H., Ksiazek, T. G., Nichol S. T. & Maclachlan N. J. (2009). Rift Valley fever virus, *Journal of the American Veterinary and Medical Association***,** 234**,** 883–893.

Bird, B. H., Baweic, D. A., Ksiazek, T. G., Shoemaker, T. R. & Nichol, S. T. (2007). Highly sensitive and broadly reactive quantitative RT-PCR assay for high throughput detection of Rift Valley fever virus. *Journal of Clinical Microbiol*ogy, 45, 3506–3513.

Botros, B., Omar, A., Elian, K., Mohamed, G., Soliman, A., ... Earhart, K. (2006). Adverse response of nonindigenous cattle of European breeds to live attenuated Smithburn Rift Valley fever vaccine. *Journal of Medical Virology,* 78**,** 787–791.

Bouloy, M., Dufour, B., Andre´-Fontaine, G., Albina, E., Chevalier, V.,… Dorchies P. REPORT: Risque de propagation de la fie`vre de la valle´e du Rift (FVR) dans l'Oce´an Indien (La Re´union et Mayotte), AFSSA, Maisons-Alfort, France, www.afssa.fr, 2008, pp. 1–124.

Bowen, M. D., Trappier, S. G., Sanchez, A. J., Meyer, R. F., Goldsmith, C. S., … Nichol S. T. (2001). A reassortant bunyavirus isolated from acute hemorrhagic fever cases in Kenya and Somalia. *Virology* 291**,** 185-190.

Bradley, P. & Kutz, F. W. (2006). *Proceedings of the Workshop on Stormwater Management and Mosquito Control*. U.S. Environmental Protection Agency, Office of Research and Development. EPA /903/R-06/004.

Breeland, S. G. & Pickard, E. (1967). Field observations on 28 broods of floodwater mosquitoes resulting from controlled floodings of natural habitat in the Tennessee Valley. *Mosquito News,* 27, 343-358.

Britch, S. C., Binepal, Y. S., Ruder, M. G., Kariithi, H. M., Linthicum, K. J., … Wilson W. C. (2013). Rift valley fever risk map model and seroprevalence in selected wild ungulatesand camels from Kenya. Retrieved from: PLOS 2013; 8:e66626.doi:10.1371/journal.pone.0066626.

Brown, C. R., Moore, A. T., Young, G. R., Padhi, A. & Komar N. (2009). Isolation of Buggy Creek Virus (Togaviridae: Alphavirus) From Field-Collected Eggs of *Oeciacus vicarius* (Hemiptera: Cimicidae). *Journal of Medical Entomology*, 46**,** 375–379.

Bryant, J. E., Crabtree, M. B., Nam, V. S., Yen, N. T., Duc, H. M. & Miller, B. R. (2005). Isolation of arboviruses from mosquitoes collected in northern Vietnam. *American Journal of Tropical Medicine and Hygiene*, 73, 470–473.

Burt, F. J., Grobbelaar, A. A., Leman, P. A., Anthony, F. S., Gibson, G. V. & Swanepoel, R. (2002). Phylogenetic relationships of southern African West Nile virus isolates. *Emerging Infectious Diseases*, 8, 820-826.

Cassani, J. R. & Bland G. R. (1978). Distribution of floodwater mosquito eggs in partially wooded, central Michigan lowland. *Mosquito News,* 38, 566-569.

Centre for Disease Control and Prevention (2007). Infectious disease information: *Mosquito-Borne Diseases*. Retrieved from: http://www.cdc.gov/ncidod/diseases/list_mosquitoborne.hmt

Centre for Disease Control and Prevention (2004). Life stages of Anopheles mosquitoes. *Morbidity and Mortality Weekly Review*, 52, 989 – 997.

Centre for Disease Control and Prevention (2000a). Outbreak of Rift Valley fever – Saudi Arabia, August – October, 2000. *Morbidity and Mortality Weekly Review*, 49, 905-908.

Centre for Disease Control and Prevention (1998). Rift Valley fever - East Africa, 1997–1998. *Morbidity and Mortality Weekly Review,* 47**,** 261–264.

Centre for Disease Control and Prevention (1995). Update: management of patients with suspected viral hemorrhagic fever—United States. *Morbidity and Mortality Weekly Review*, 44**,** 475–479.

Chamberlain, R. W. & Sudia, W. D. (1961). Mechanism of transmission of viruses by mosquitoes. *Annual Review of Entomology,* 6**,** 371-390.

Chevalier, V., Thiongane, Y. & Lancelot, R. (2009). Endemic transmission of Rift Valley fever in Senegal. *Transboundary and Emerging Diseases,* 56**,** 372–374.

Chevalier, V., Mondet, B., Diaite, A., Lancelot, R, Fall, A. G. & Ponon, N. (2004). Exposure of sheep to mosquito bites: possible consequences for the transmission risk of Rift Valley Fever in Senegal. *Medical and Veterinary Entomology,* 1, 247-255.

Chretien, J. P., Anyamba, A., Bedno, S. A., Breiman, R. F., Sang, R., … **Linthicum, K. J. (2007).** Drought-associated chikungunya emergence along coastal East Africa. *American Journal of Tropical Medicine and Hygiene,* 76, 405-407.

Chomczynski, P. & Sacchi, N. (1987). Single Step Method of RNA Isolation by Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction. *Annals of Biochemistry,* 162, 156-159.

Clements, A. N. (1992). *The Biology of Mosquitoes*. Vol. 1. *Development, Nutrition and Reproduction*. Chapman & Hall, London. pp 509.

Clements, A. N. (1963). The Physiology of Mosquitoes. Oxford, Pergamon Press, pp395.

Coetzer, J. A. (1982). The pathology of Rift Valley fever. II. Lesions occurring in field cases in adult cattle, calves and aborted foetuses. *Onderstepoort Journal of Veterinary Res*earch, 49, 11–17.

Coetzer, J. A. (1977). The pathology of Rift Valley fever. I. Lesions occurring in natural cases in new-born lambs. *Onderstepoort Journal of Veterinary Res*earch, 44, 205–211.

Crabtree, M., Sang, R., Lutomiah, J., Richardson, J. & Miller, B. (2009). Arbovirus surveillance of mosquitoes collected at sites of active Rift Valley fever virus transmission: Kenya, 2006-2007. *Journal of medical entomology*, 46, 961-964.

Craig, D. E., Thomas, W. J. & DeSanctis, A. N. (1967). Stability of Rift Valley fever virus at 4°C. *Applied Microbiology*, 15, 446–447.

Creekmur, G. D., Russell, P. M. & Hazelrigg, J. E. (1982). Field evaluation of the effects of slow-release wet table powder formulation of Altosid nontarget organisms. *Proceedings of the California Mosquito and Vector Control Assoc*iation, 49, 95-97.

Curtis, C. F. (1996). An overview of mosquito biology, behavior and importance. *Ciba Foundation Symposium, 3-7.*

Daniel, W. W. (1999). Determination of sample size for estimating proportions. In: Biostatistics: Foundation for Analysis in the Health Sciences. John Wiley & Sons, Inc. New York, pp 183.

Daubney, R. l., Hudson, J. R. & Graham, P. C. (1931). Enzootic hepatitis or RiftValley Fever. An undescribed disease of sheep, cattle, and man in East Africa. *Journal of Pathology and Bacteriology*, 34, 545-579.

David-West, T. S. & Porterfield, J. S. (1974). Dugbe Virus: a Tick-borne Arbovirus from Nigeria. *Journal of General Virology*, 23**,** 297-307.

Davies, F. G. & Martin, V. (2003). Recognizing Rift Valley fever. FAO Animal Health Manual No. 17. Rome: *Food and Agriculture Organization (FAO) of the United Nations*, 1–45.

Davies, F. G., Kilelu, E., Linthicum, K. J. & Pegram, R. G. (1992). Patterns of Rift Valley fever activity in Zambia. *Epidemiology and Infection*, 08, 1985–1991.

Davies, F. G., Linthicum, K. J. & James, A. D. (1985). Rainfall and epizootic Rift Valley fever. *Bulletin of the WHO*, 63, 941-943.

Davies, F. G. & Highton, R. B. (1980). Possible vector of Rift Valley fever in Kenya. *Trans of The Royal Society of Tropical Medicine and Hygiene,* 74, 815-25.

Davies, F. G. (1975). Observations on the epidemiology of Rift Valley fever in Kenya. *Journal of Hygiene*, 75, 219-230.

Day, J. F. & Shaman, J. (2011). Mosquito-Borne Arboviral Surveillance and the Prediction of Disease Outbreaks, Flavivirus Encephalitis, Dr. Daniel Ruzek (Ed.), ISBN: 978-953-307-669-0. Retrieved from: http://www.intechopen.com/books/flavivirus-encephalitis/mosquito-borne-arboviral-surveillance-and-the-prediction-of-disease-outbreak

Diallo, M., Nabeth, P., Ba, K., Sall, A. A., Ba, Y., … Mathiot, C. (2005). Mosquito vectors of the 1998-1999 outbreak of Rift Valley fever and other arboviruses (Bagaza, Sanar, Wesselsbron and West Nile) in Mauritania and Senegal. *Medical and Veterinary Entomology,* 19, 119-126.

Drosten, C., Go¨ ttig, S., Schilling, S., Asper, M., Panning, M., … Gu¨nther. S. (2002). Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *Journal of Clinical Microbiology*, 40, 2323–2330.

Easterday, B. C. (1965). Rift Valley fever. *Advances in Veterinary Science*, 10, 65.

Edwards, F. W. (1941). Mosquitoes of the Ethiopian Region. III. Culicine adults and pupae. London, British Museum (Natural History).

EFSA Journal (2005). **238**: 1-128. The risk of a Rift Valley fever incursion and its persistence within the Community.

Elliott, R.M., (1996). The Bunyaviridae, Plenum Press, New York & London.

Eshoo, M. W., Whitehouse, C. A., Zoll, S. T., Massire, C., Pennella, T. D., … Njenga, K. M. (2008). Prevalence of antibodies against Rift Valley fever virus in Kenyan wildlife. *Epidemiology and Infection,* 136, 1261–1269.

Fafetine, J., Neves, L., Thompson, N. P., Paweska, T. J., Victor, P. M. G. Rutten, P. … Coetzer, W. A. J. (2013). Serological Evidence of Rift Valley Fever Virus Circulation in Sheep and Goats in Zambe´ zia Province, Mozambique. *PLoS Neglected Tropical Diseases*, 7(2): e2065. doi:10.1371/journal.pntd.0002065.

FCCMC, (1998). Florida Mosquito Control: The State of the Mission as Defined by Mosquito Controllers, Regulators, and Environmental Managers. Florida Coordinating Council on Mosquito Control, Vero Beach, FL.

Fischer, E. A. J., Boender, G. J., Nodelijk, G., de Koeijer, A. A. & van Roermund H. J. W. (2013). The transmission potential of Rift Valley fever virus among livestock in the Netherlands: a modelling study. *Veterinary Research*, 44, 58.

Flick, R. & Bouloy, M. (2005). Rift Valley fever virus. *Current Molecular Medicine*, 5, 827-34.

Fontanarrosa, M. S., Marinone, M. C., Fischer, S., Orellano, P. W. & Schweigmann, N. (2000). Effects of Flooding and Temperature on *Aedes albifasciatus* Development Time and Larval Density in Two Rain Pools at Buenos Aires University City. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, 95, 787-793.

Fontenille, D., Traore-Lamizana, M., Diallo, M., Thonnon, J., Digoutte, J. P. & Zeller, H. G. (1998). New Vectors of Rift Valley Fever in West Africa. *Emerging Infectious Diseases*, 4, 289-293.

Food and Agriculture Organization (2003). The state of food insecurity in the world.

Forrester, N. L., Palacios, G., Tesh, R. B., Savji, N., Guzman, H., … Lipkin, W. I. (2012). Genome-Scale Phylogeny of the Alphavirus Genus Suggests a Marine Origin. *Journal of Virology*, 86, 2729–2738.

Garcia, S., Crance, J. M., Billecocq, A., Peinnequin, A., Jouan, A., …. Garin D. (2001). Quantitative realtime PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds, *Journal of Clinical Microbiology*, 39, 4456–4461.

Gargan, T. P. II, Jupp, P. G. & Novak, R. J. (1988a). Panveld oviposition sites of floodwater *Aedes* mosquitoes and attempts to detect transovarial transmission of Rift Valley fever virus in South Africa. *Medical and Veterinary Entomology*, 2, 231-236.

Gargan, T. P. II, Clark, G. G., Dohm, D. J., Turell, M. J. & Bailey, C. L. (1988b). Vector potential of selected North American mosquito species for Rift Valley fever virus. *American Journal of Tropical Medicine and Hygiene*, 38, 440-446.

Gerdes, G. H. (2004). Rift Valley Fever. *Reviews in Science and Technology, Office International des Epizooties (Paris)*, 23, 613-623.

Gerrard, S. R., Li, L., Barrett, A. D. & Nichol, S. T. (2004). Ngari virus is a Bunyamwera virus reassortment that can be associated with large outbreaks of hemorrhagic fever in Africa. *Journal of virology*, 78, 8922-8926.

Gillett, J. D. (1955a). Variation in the hatching-response of Aedes eggs (Diptera: Cuiicidae). *Bulletin of Entomological Research*, 46, 241-254.

Gillett, J. D. (1955b). The inherited basis of variation in the hatching of *Aedes* eggs (Diptera: Cuiicidae). *Bulletin of Entomological Research*, 46, 255-265.

Gillies, M. T. & De Meillon, B. (1968). The Anophelinae of Africa, South of the Sahara. Published by *The South African Institute of Medical Research*, No. 54, 343pp.

Gilvear, D. J, & Bradley, C. (2000). *Hydrological monitoring and surveillance for wetland conservation and management*; a UK perspective. *Physics and Chemistry of the Earth* Pt B, 25, 571-588.

Goddard, B. L., Roth, A. E., Reisen, W. K. & Weaver, T. S. (2002). Vector Competence of California Mosquitoes for West Nile virus. *Emerging Infectious Diseases*, 8, 1385–1391.

Gubler, D. J. (2002). The Global Emergence/Resurgence of Arboviral Diseases as Public Health Problems. *Archives of Medical Research*, 33, 330–342.

Gubler, D. J. (2001). Human Arbovirus Infections Worldwide. *Annals of the New York Academy of Science*, 951**,** 13 – 24.

Gubler, D. J. (1996). The global resurgence of arboviral diseases. *Transactions of The Royal Society of Tropical Medicine and Hygiene*, 90: 449–451.

Heinrich, N., Saathoff, E., Weller, N., Clowes, P., Kroidl, I., … Hoelscher, M. (2012). High serorevalence of Rift Valley fever and evidence for endemic circulation in Mbeya region, Tanzania, in a cross-sectional study. *PloS Neglected Tropical Diseases* 6, e1557. doi:10.1371/journal.pntd.0001557.

Herrera, F., Urdaneta, L., Rivero, J., Zoghbi, N., Ruiz, J., … Rojas, E. (2006). Population genetic structure of the dengue mosquito *Aedes aegypti* in Venezuela. *Memórias do Instituto Oswaldo Cruz*, Rio de Janeiro, 101, 625-633.

Hershey, A. E., Lima, A. R. Niemi, G. J. & Regal, R. R. (1998). Effects of *Bacillus thuringiensis israelensis* (BTI) and methoprene on nontarget macroinvertebrates in Minnesota wetlands. *Ecological Applications*, 8, 41–60.

Hoch, A. L., Gargan, T. P. & Bailey, C. L. (1985). Mechanical Transmission of Rift Valley Fever Virus by Hematophagous Diptera. *American Journal of Tropical Medicine and Hygiene,* 34, 188-193.

Hogenhout, S. A., Ammar, E., Whitfield, A. E. & Redinbaugh, M. G. (2008). Insect vector interactions with persistently transmitted viruses. *Annual Review of Phytopathology* 46**,** 327–359.

Horsfall, W. R. (1972). Mosquitoes: their bionomics and relation to disease, Hafner Pub. Co., New York, 1972.

Horsfall, W. R. (1963). Eggs of floodwater mosquitoes (Diptera: Culicidae) IX. Local distribution. Ann. Entomol. Soc. Amer. 56, 426-441.

Ibrahim, M. S., Turell, M. J., Knauert, F. K. & Lofts, R. S. (1997). Detection of Rift Valley fever virus in mosquitoes by RT-PCR. *Molecular and Cellular Probes,* 11, 49-53.

Ikeshoji, T & Mulla, S. M. (1970). Overcrowding Factors of Mosquito Larvae. 2. Growth-Retarding and Bacteriostatic Effects of the Overcrowding Factors of Mosquito Larvae. *Journal off Econmic Entomology,* 63, 1737-1743

Imam, I. Z. E., El-Karamany, R. & Darwish, M. A. (1979). An epidemic of Rift Valley fever in Egypt.II. Isolation of the virus from animals. Bulletin of the World Health Organization, 57, 441-443.

Ivanova, V. N., Zemlak, S. T., Hanner, H. R. & Hebert, D. N. P. (2007). Universal primer cocktails for fish DNA barcoding. Molecular Ecology, doi:10.1111/j.1471-8286.2007.01748.x

Jenkins, D. W. (1946). Ecology of the tree hole breeding mosquitoes of Nearctic North America. *Ecological Monographs,* 16, 33-47.

Johnson, B. K., Ochieng, D., Gichogo, A., Okiro, M., Libondo, D., … French, M. (1983a). Antibodies against haemorrhagic fever viruses in Kenya populations. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 77, 731–733.

Johnson, B. K., Gitau, L. G., Gichogo, A., Tukei, P. M., Else, J. G., … Sayer, P. D. (1982). Marburg, Ebola and Rift Valley Fever virus antibodies in East African primates. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 76, 307–310.

Juliano, S. A. & Stoffregen, T. L. (1994). Effects of habitat drying on size at and time to metamorphosis in the tree hole mosquito *Aedes triseriatus*. *Oecologia*, 97, 369-376.

Jup, P. G., Kemp, A., Grobbelaar, A., Leman, P., Burt, F. J., … Swanepoel, R. (2002). The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Medical and Veterinary Entomology*, 16, 245-252.

Jupp, P. G., Grobbelaar, A. A., Leman, P. A., Kemp, A., Dunton, R. F., … Swanepoel, R. (2000). Experimental detection of Rift Valley Fever virus by reverse transcription-polymerase chain reaction assay in large samples of mosquitoes. *Journal of Medical Entomology*, 37, 467-471.

Jupp, P. G. & Cornel, A. J. (1988). Vector competence tests with Rift Valley fever virus and five South African species of mosquitoes. *Journal of the American Mosquito Control Association*, 4, 4-8.

Kalunda, M., Lwanga-Ssozi, C., Lule, M. & Mukuye, A. (1985). Isolation of Chikungunya and Pongola viruses from patients in Uganda. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 79, 567.

Kamal, S. A. (2009). Pathological studies on postvaccinal reactions of Rift Valley fever in goats. *Virology Journal*, 6, 94.

Kapoor, S. (2008). Resurgence of Rift Valley Fever. *Infectious Diseases in Clinical Practice*, 16, 9-12.

Karabatsos, N. (ed.) (1985). International catalogue of arboviruses. *American Society of Tropical Medicine and Hygiene*, San Antonio, TX.

Kaschula, V. R. (1957). Rift Valley fever as a veterinary and medical problem. *Journal of the American Veterinary and Medical Association*, 203, 219.

Kay, B. H. (1982). Three modes of transmission of Ross River virus by *Aedes vigilax*. *The Australian journal of experimental biology and medical Science*, 60, 339-344.

Kent, R. J. (2009). Molecular methods for arthropod bloodmeal identification and applications to ecological and vector-borne disease studies. *Molecular and Ecological Resources*, 9**,** 4–18.

King, C. H., Kahlon, S. S., Muiruri, S. & Labeaud, A. D. (2010). Facets of the Rift Valley fever outbreak in Northeastern Province, Kenya, 2006-2007. *American Journal of Tropical Medicine and Hygiene*, 82, 363.

Knight, L. R., Walton, E. W., O'Meara, F. G., Reisen, K. W & Wass, R. (2003). Strategies for effective mosquito control in constructed treatment wetlands. *Ecological Engineering,* 21, 211–232.

Knight, K. L. (1965). Some physical and chemical characteristics of coastal soils underlying mosquito breeding. *Mosquito News,* 25, 154-159.

Knight, K. I. & Baker, E. F. (1962). The role of the substrate moisture contact in the selection of oviposition sites by A. taeniorhynchus and A. sollicitans. *Mosquito News,* 22, 247-254.

Knight, K. L. (1947). Aedes (Mucidus) mosquitoes of the Pacijic (Diptera:Culicidae). *Jounal of the Washington Academy*. 37, 315-325.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., … Wilson A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification with conserved primers. *Proceedings of the National Academy of Science. USA* 86, 6196–6200.

Korkenot, R. H., McIntoshi, B. M. & Worth, C. B. (1961). Ndumu virus, a hitherto unknown agent, isolated from culicine mosquitoes collected in northern Natal, Union of South Africa. *American Journal of Tropical Medicine and Hygiene,* 10, 383-386.

Kramer, L. V. & Beesley, C. (1991). Efficacy and persistence of sustained-release Methoprene pellets against *Aedes* mosquitoes in an Irrigated pasture. *Journal of the American Veterinary and Medical Association*, 7, 646-648.

Kumar, S., Tamura, K., & Nei, M. (2004). MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and Sequence alighnment. Briefings in Bioinformatics, 5, 150-163.

Kuno, G., Mitchell, C. J, Chang, G-J. J. & Smith, G. C. (1996). Detecting bunyaviruses of the Bunyamwera and California serogroups by a PCR technique. *Journal of Clinical Microbiology*, 34, 1184–1188.

LaBeaud, A. D., Sutherland, L. J., Muiruri, S., Muchiri, E. M., Gray, L. R., … King, C. H. (2011a). Arbovirus Prevalence in Mosquitoes, Kenya. *Emerging Infectious Diseases*, 17, 233-241.

LaBeaud, A. D., Cross, P. C., Getz, W. M., Glinka, A. & King, C. H. (2011b). Rift Valley fever virus infection in African Buffalo (*Syncerus caffer*) herds in rural South Africa: evidence of inter-epidemic transmission. *American Journal of Tropical Medicine and Hygiene*, 84, 641–646.

LaBeaud, A. D., Muchiri, E. M., Ndzovu, M., Mwanje, M. T., Muiruri, S., ... King C. H. (2008). Interepidemic Rift Valley fever virus seropositivity, northeastern Kenya. *Emerging Infectious Diseases,* 14**,** 1240–1246.

Labeaud, A. D., Ochiai, Y., Peters, C. J., Muchiri, E. M. & King, C. H. (2007). Spectrum of Rift Valley Fever Virus Transmission in Kenya: Insights from three Distinct Regions. *American Journal of Tropical Medicine and Hygiene,* 76**,** 795–800.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., … Higgins D.G. (2007). Clustal W and Clustal X version 2.0 *Bioinformatics,* 23, 2947-2948.

Lawler, S. P., Jensen, T. & Dritz, D. A. (1997). Non-target effects of mosquito larvicides used on national wildlife refuges. University of California Mosquito Control Research Annual Report. *University of California, Berkeley, CA,* pp. 34–35.

Le Roux, C. A., Kubo, T., Grobbelaar, A. A., van Vuren, P. J., Weyer, J., … Peters, C. J. (1999). Climate and satellite indicators to forecast Rift Valley fever epidemics in Kenya. *Science*, 285, 397-400.

Linthicum, K. J., Bailey, C. L., Tucker, C. J., Mitchell, K. D., Logan, T. M., … Wagateh, J. N. (1990). Application of polar-orbiting, meteorological satellite data to detect flooding of Rift Valley fever virus vector mosquito habitats in Kenya. *Medical and Veterinary Entomology,* 4, 433-438.

Linthicum, K. J., Logan, T. M., Thande, P. C., Wagateh, J. N., Kamau, C. W., … Davies, F. G. (1989). Efficacy of a sustained release methoprene formulation on vectors of Rift Valley fever virus in field studies in Kenya. *Journal of the American Mosquito Control Association,* 5, 603-605.

Linthicum, K. J., Bailey, C. L., Davies, F. G., Kairo, A. & Logan, T. M. (1988). The horizontal distribution of *Aedes* pupae and their subsequent adults within a flooded dambo in Kenya: implications for Rift Valley fever virus control. *Journal of the American Mosquito Control Association,* 4**,** 551-554.

Linthicum, K. J., Kaburia, H. F., Davies, F. G. & Lindqvist, K . J. (1985a). A blood meal analysis of engorged mosquitoes found in Rift Valley fever epizootics area in Kenya. *Journal of the American Mosquito Control Association,* 1, 93–95.

Linthicum, K. J., Davies, F. G., Kairo, A. & Bailey, C. L. (1985b). Rift Valley fever virus (family Bunyaviridae, genus *Phlebovirus*). Isolations from Diptera collected during an inter-epizootic period in Kenya. *Journal of Hygiene,* 95, 197-209.

Linthicum, K. J., Bailey, C. L., Davies, F. G. & Kairo, A. (1985c). Observations on the dispersal and survival of populations of *Aedes lineatopennis* (Ludlow) (Diptera: Culicidae) in Kenya. *Bulletin of Entomological Research,* 75**,** 661-670.

Linthicum, K. J., Davies, F. G. & Kairo, A. (1984b). Observations of the biting activity of mosquitoes at a flooded dambo in Kenya. *Mosquito News,* 44**,** 595-598.

Linthicum, K. J., Davies, F. G., Bailey, C. L. & Kairo A. (1983). Mosquito species succession in a *dambo* in an East African forest. *Mosquito News,* 43**,** 464-470.

Little, P. D., Teka, T. & Azeze, A. (2001). Cross-Border Livestock Trade and Food Security in the Horn of Africa: An Overview. Washington, DC: USAID/REDSO.

Logan, T. M., Linthicum, K. J., Thande, P. C., Wagateh, J. N. & Roberts, C. R. (1991b). Mosquito species collected from a marsh in western Kenya during the long rains. *Journal of the American Mosquito Control Association,* 7, 395-399.

Logan, T. M., Linthicum, K. J., Thande, P. C., Wagateh, J. N. & Roberts, C. R. (1991a). Egg hatching of *Aedes* mosquitoes during successive floodings in a Rift Valley fever Endemic area in Kenya. *Journal of the American Mosquito Control Association,* 7**,** 101-112.

Logan, T. M., Linthicum, K. J., Wagateh, J. N., Thande, P. C., Kamau, C. W. & Roberts, C. R. (1990). Pretreatment of floodwater *Aedes* habitats (dambos) in Kenya with a sustained-release formulation of methoprene. *Journal of the American Mosquito Control Association,* 6, 736-738.

Lutomiah, J., Omondi D., Masiga, D., Mutai, C., Mireji, P. O., Ongus, J., … Sang R. (2014). Bloodmeal analysis and virus detection in bloodfed mosquitoes collected during the 2006-2007 Rift Valley fever outbreak in Kenya. *Vector-Borne and Zoonotic Diseases,* 14, 656-64.

Lutomiah, J., Bast, J., Clark, J., Richardson, J., Yalwala, S.,... Sang R. (2013). Abundance, diversity, and distribution of mosquito vectors in selected ecological regions of Kenya: public health implications. *Journal of Vector Ecology,* 38**,** 134-142.

Lwande, O. W., Lutomiah, J., Obanda, V., Gakuya, F., Mutisya, J., … Sang, R. (2013). Isolation of Tick and Mosquito-Borne Arboviruses from Ticks Sampled from Livestock and Wild Animal Hosts in Ijara District, Kenya. *Vector-Borne and Zoonotic Diseases,* 13**,** 637-642.

MacDonald, G. (1957). The Epidemiology and Control of Malaria. Oxford University Press, London.

Mackel, R. (1974). Dambos a study of morphodynamic activity on the plateau regions of Zimbabwe. *Catena,* 1, 327-365

Madani, T. A., Al-Mazrou, Y. Y., Al-Jeffri, M. H., Mishkhas1, A. A., Al-Rabeah, A. M., … Shobokshi O. (2003). Rift Valley fever epidemic in Saudi Arabia: epidemiological, clinical, and laboratory characteristics. *Clinical and Infectious Diseases,* 37, 1084–1092.

Mallack, J., Smith, W. L. Jr., Berry, A. R. Jr. & Bickley, W. E. (1964). Hatching of eggs of three species of aedine mosquitoes in response to temperature and flooding. *Alabama Agricultural Experiment Station Auburn University Leaflet*, 138, l-21.

Masembe, C., Michuki, G., Onyango, M., Rumberia, C, Norling, M., … Fischer A. (2012). Viral metagenomics demonstrates that domestic pigs are a potential reservoir for Ndumu virus. *Virology Journal*, 9, 218.

McIntoshi, B. M., Jupp, P. G., Dos Santos, I. & Rowe, A. C. (1983). Field and laboratory evidence implicating *Culex zombaensis* and *Aedes circumluteolus* as vectors of Rift Valley fever virus in coastal South Africa. *South African Journal of Science,* 79**,** 61–64.

McIntoshi, B. M., Jupp, P. G., Dos Santos, I. & Barnard, B. J. H. (1980). Vector studies on Rift Valley fever virus in South Africa. *South African Medical Journal*, 58, 127–132.

Meegan, J., Le Guenno, B., Ksiazek, T., Jouan, A., Knauert, F., … Peters C. J. (1989). Rapid diagnosis of Rift Valley fever: A comparison of methods for the direct detection of viral antigen in human sera. *Research in Virology,* 140**,** 59–65.

Meegan, J. M. & Bailey, C. L. (1988). Rift Valley fever. In The arboviruses: epidemiology and ecology, Vol. IV (T.P. Monath, ed.). CRC Press, Boca Raton, Florida, 51-76.

Meegan, J. M. (1979). The Rift Valley fever epizootic in Egypt 1977–78. 1. Description of the epizzotic and virological studies. *Transactions of the Royal Society of Tropical Medicine and Hygiene,* 73**,** 618–23.

Mellor, P. S. & Leak, C. J. (2000). Climatic and geographic influences on arboviral infections and vectors. *Revue Scientifique et Technique* (Paris) *Office **International** des Epizooties (Paris).* 19, 41-54.

Metselaar, D., van Someren, E. C. C., Ouma, J. H., Koskei, H. K. & Gemert, W. (1973). Some observations on *Aedes (Aedimorphus*) *dentatus* (Theo.) (Dipt. Culicidae) in Kenya. *Bulletin of Entomological Research*, 62**,** 597-603.

Miller, B. R., Godsey, M. S., Crabtree, M. B., Savage, H. M., Al-Mazrao, Y., … Ksiazek T. G. (2002). Isolation and genetic characterization of Rift Valley fever virus from *Aedes vexans arabiensis*, Kingdom of Saudi Arabia. *Emerging Infectious Diseases*, 8, 1492-1494.

Mohamed, R. A. E. H., Abdelgadir, D. M., Hind, M. M. Bashab & H. M. M. (2013). Transovarian transmission of Rift Valley Fever Virus by two species of mosquitoes in Khartoum State (Sudan): *Aedes vexans* (Meigen) and *Culex quinquefasciatus* (Say) *Sudanese Journal of Public Health*, 8, 164-170.

Molaei, G., Oliver, J., Andreadis, T. G., Armstrong, P. M. & Howard, J. J. (2006). Molecular Identification of Blood Meal Sources in *Culiseta melanura* and *Culiseta morsitans* from an Endemic Focus of Eastern Equine Encephalitis Virus in New York. *American Journal of Tropical Medicine and Hygiene*, 75, 1140-1147.

Mondet, B., Diaïté, A., Ndione, J. A., Fall, A. G., Chevalier, V., … Ponçon, N. (2005). Rainfall patterns and population dynamics of *Aedes* (*Aedimorphus*) *vexans arabiensis*, Patton 1905 (Diptera: Culicidae), a potential vector of Rift Valley Fever virus in Senegal. *Journal of Vector Ecology* 30**,** 102-106.

Montgomery, R. E. & Stordy, R. J. Report of Veterinary Department for the 1912-1913. Annual Report, Dept. Agriculture, Kenya Colony.

Morrill, J. C. & Peters, C. J. (2003). Pathogenicity and neurovirulence of a mutagen-attenuated Rift Valley fever vaccine in rhesus monkeys. *Vaccine*, 21**,** 2994–3002.

Morrill, J. C. & McClain, D. J. (1996). Epidemiology and pathogenesis of Rift Valley fever and other phleboviruses, in: Elliott R.M. (Ed.), The Bunyaviridae, Plenum Press, New York, pp. 281–293.

Morrill, J. C., Johnson, B. K., Hyams, C., Okoth, F., Tukei, P. M., … Woody, J. (1991). Serological evidence of arboviral infections among humans of coastal Kenya. *Journal of Tropical Medicine and Hygiene*, 94,166–168.

Mukabana, W. R., Takken, W., Seda, P., Killeen, G. F., Hawley, W. A. & Knols, B. G. J. (2002). Extent of digestion affects the success of amplifying human DNA from blood meals of Anopheles gambiae (Diptera: Culicidae). *Bulletin of Entomological Research*, 92**,** 233–239,

Muller, R., Saluzzo, J. F., Lopez, N., Dreier, T., Turell, M., … Bouloy, M. (1995). Characterization of clone 13, a naturally attenuated avirulent isolate of Rift Valley fever virus, which is altered in the small segment. *American Journal of Tropical Medicine and Hygiene*, 53, 405–411.

Murithi, M. F., Mulinge, M. W., Wandera, O. E. & Maingi, M. P. (2007). Livestock Survey in the Arid Land Districts of Kenya. For Arid Lands Resource Management Project (ALRMP-II) Office of the President, P. O. Box 53547, Nairobi, Jan, 2007.

Murphy, F. A., Fauquet, C. M., Bishop, D. H. L., Ghabrial, S. A., Jarvis, A. W., … Summers M. D. (eds) (1995). Virus taxonomy: classification and nomenclature of viruses. Sixth Report of the International Committee on Taxonomy of Viruses. Springer-Verlag, Vienna and New York, 586 pp.

Mutero, C. M., Nga'ang'a, P. N., Wekoyela, P., Githure, J. & Konradsen, F. (2004). Ammoniumsulphate fertilizer increases mosquitoes in rate field. *Acta Trop*ica, 89**,** 187-192.

Nguku, M. P, Sharif, S. K., Mutonga, D., Amwayi, S., Omolo, J., … Breiman E. R. (2010). An Investigation of a Major Outbreak of Rift Valley Fever in Kenya: 2006-2007. *American Journal of Tropical Medicine and Hygiene*, 83, 5-13.

Niklasson B., Grandien M., Peters C. J. and Gargan T. P. II. (1983). Detection of Rift Valley fever virus antigen by enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* **17:** 1026–1031.

Ochieng, C., Lutomiah, J., Makio, A., Koka, H., Chepkorir, E., … Sang R. (2013). Mosquito-borne arbovirus surveillance at selected sites in diverse ecological zones of Kenya; 2007–2012. *Virology Journal* 10, 140. http://www.virologyj.com/content/10/1/140.

Olive, M. M., Goodman, S. M. & Reynes, J. M. (2012). The role of wild mammals in the maintenance of Rift Valley fever virus. *Journal of Wildlife Disease**s***, 48, 241-66.

Olson, J. K. & Meek, L. C. (1977). Soil moisture conditions that are most attractive to ovipositing females of Psorophora columbiae in Texas ricelands. *Mosquito News*, 37, 19-26.

O'Malley, C. M. (1990). Aedes vexans (Meigen): an old foe. *Proceedings of New Jersey Mosquito Control Association*, 90-95.

Pelizza, S. A., Lopez–Lastra, C. C., Becne, J. J., Bisara, V. & Garcia, J. J. (2007). Effects of temperature, pH and Salinity on the infection of Leptolegnia Capmany Seymour (Peronosporomycetes) in mosquito larvae. *Journal of invertebrate Pathology*, 96, 133-137.

Pepin, M., Bouloy, M., Bird, B. H., Kemp, A. & Paweska, J. (2010). Rift Valley fever virus (*Bunyaviridae: Phlebovirus*): an update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Veterinary Research,* 41, 61. DOI: 10.1051/vetres/2010033.

Peters, C. J. (1997). Emergence of Rift Valley fever. In: Saluzzo J. F., Dodet B, editors. Factors in the emergence of arbovirus diseases. Paris, France: *Elsevier*, 253–262.

Peters, C. J. & Linthicum, K. J. (1994). Rift Valley fever. In Handbook of Zoonoses.Second Edition. Section B: Viral. G.B. Beran (Ed.), CRC Press, Inc. *Boca Raton*, Fl.

Petersen, J. J. & Rees, D. M. (1966). Selective oviposition response of Aedes dorsalis and Aedes nigromaculis soil salinity. *Mosquito News*, 26, 168-174.

Peyrefitte, C. N., Boubis, L., Coudrier, D., Bouloy, M., Grandadam, M., … Plumet, S. (2008). Real-time reverse-transcription loop-mediated isothermal amplification for rapid detection of Rift Valley fever virus. *Journal of Clinical Microbiol*ogy, 46, 3653–3659.

Pittman, P. R., Liu, C. T., Cannon, T. L., Makuch, R. S., Mangiafico, J. A., … Peters, C. J. (1999). Immunogenicity of an inactivated Rift Valley fever vaccine in humans: a 12-year experience. *Vaccine,* 18**,** 181–189.

Pourrut, X., Nkoghe, D., Souris, M., Paupy, C., Paweska, J., … Leroy, E. M. (2010). Rift Valley fever virus seroprevalence in human rural populations of Gabon. *PLoS Neglected Tropical Diseases,* 4, e763.

Pritchard, G. & Scholefield, J. E. (1983). Survival of Aedes larvae in constant area ponds in Southern Alberta (Diptera: Culicidae). *The Canadian Entomologist,* 115**,** 183-188.

Randolph, S. E. & Rogers, D. J. (2010). The arrival, establishment and spread of exotic diseases: Patterns and predictions. *Nature Reviews Microbiology*, 8**,** 361–371.

Ratnasingham, S. & Hebert, P. D. N. (2007). BOLD: The barcode of life data system (www.barcodinglife.org). *Molecular Ecology Notes*, 7, 355–364.

Reiskind, M. H. & Zarrabi, A. (2012). Water Surface Area and Depth Determine Oviposition Choice in *Aedes albopictus* (Diptera: Culicidae). *Journal of Medical Entomology*, 49, 71-76.

Rich, M. K. & Wanyoike, F. (2010). An Assessment of the Regional and National Socio-Economic Impacts of the 2007 Rift Valley Fever Outbreak in Kenya. *American Journal of Tropical Medicine and Hygiene,* 83, 52–57.

Roberts, N. (1988). Dambos in development: management of a fragile ecological resource. *Journal of Biogeography,* 15**,** 141-148.

Romoser, W. S., Oviedo, M. N., Lerdthusnee, K., Patrican, L. A., Turell, M. J., … Bailey C. L. (2011). Rift Valley fever virus-infected mosquito ova and associated pathology: possible implications for endemic maintenance. *Research and Reports in Tropical Medicine,* 2, 121 – 127.

Rydzanicz, K., Kącki, Z. & Jawień, P. (2011). Environmental factors associated with the distribution of floodwater mosquito eggs in irrigated fields in Wrocław, Poland. *Journal of Vector Ecolology,* 36**,** 332-342.

Sall, A.A., Macondo, E.A., Se`ne, O. K., Diagne, M., Sylla, R., … Mathiot C. (2002). Use of reverse transcriptase PCR in early diagnosis of Rift Valley fever. *Clinical and Diagnostic Laboratory Immunology,* 9, 713–715.

Sanford, M. R. (2005). Effects of Inorganic Nitrogen Enrichment on Mosquitoes (Diptera: Culicidae) and associated aquatic Community in a Constructed Treatment Wetland. *Journal of Medical Entomology*, 42, 766-776.

Sang, R., Kioko, E., Lutomiah, J., Warigia, M., Ochieng, C., … Richardson J. (2010). Rift Valley Fever Virus Epidemic in Kenya, 2006/2007: The Entomologic Investigations. *American Journal of Tropical Medicine and Hygiene,* 83**,** 28–37.

Sanger, F., Nicklen, S., & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors (DNA polymerase/nucleotide sequences/bacteriophage 4X174). Proceedings of the National Academy of Science, USA, 74, 5463-5467.

Sarkar, I. N. & Trizna, M. (2011). The Barcode of Life Data Portal: bringing the biodiversity informatics divide for DNA barcoding. (C. Ouzounis, Ed.) Retrieved from: *PloS one*, 6, e14689. doi:10.1371/journal.pone.0014689.

Schäfer, M. L. & Lundström, O. J. (2006). Different responses of two floodwater mosquito species, *Aedes vexans* and *Aedes sticticus* (Diptera: Culicidae), to larval habitat drying. *Journal of Vector Ecology,* 31, 123-128.

Schmaljohn, C. S. (1996). Bunyaviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, Chanock RM, Melnick JL, Monath TP, et al. Fields virology. 3rd ed. Philadelphia: Lippincott-Raven Publishers; p. 1447–71.

Schmude, K. L., Balcer, M. D. & Lima, A. R. (1998). Effects of the mosquito control agents *Bti* (*Bacillus thuringiensis israelensis*) and methoprene on non-target acroinvertebrates in wetlands in Wright County, Minnesota (1997). Final report. Metropolitan Mosquito Control District, St. Paul, MN, 28 pp. + appendices.

Sérandour, J., Willson, J., Thuiller, W., Ravanel, P., Lempériére, P. & Raveto, M. (2010). Environmental drivers for *Coquillettidia* mosquito habitat selection: a method to highlight key field factors. *Hydrobiology*, 652, 377–388

Sergon, K, Njuguna, C, Kalani, R, Ofula, V, **Onyango, C., … Breiman, R. F. (2008).** Seroprevalence of chikungunya virus (CHIKV) infection on Lamu Island, Kenya, October 2004. *American Journal of Tropical Medicine and Hygiene*, 78, 333–337.

Sergon, K., Yahaya, A. A., Brown, J., Bedja, S. A., Mlindasse, M., … Breiman, R. F. (2007). Seroprevalence of chikungunya virus infection on Grande Comore Island, Union of the Comoros, 2005. *American Journal of Tropical Medicine and Hygiene*, 76**,** 1189–1193.

Simmons, J. S., St. John, J. H., Holt, R. L. & Reynolds, F. H. K. (1931). The possible transfer of dengue virus from infected to normal mosquitoes during copulation. *American Journal of Tropical Medicine and Hygiene,* 11, 199-216.

Slater, J. D. & Pritchard, G. (1979). A stepwise computer program for estimating development time and survival of *Aedes vexans* (Diptera: Culicidae) larvae and pupae in field populations in Southern Alberta. *The Canadian Entomologist,* 111, 1241-1253.

Smithburn, K. C. (1949). Rift Valley fever: the neurotropic adaptation of the virus and the experimental use of this modified virus as a vaccine. *British Journal of Experimental Pathology*, 30, 1–16.

Statoids, (2007). *Administrative Subdivisions of Countries*. Retrieved from: http://www.statoids.com/yke.html.

Stordy, R. J. (1913). Mortality among lambs. Annual Report Department of Agriculture, British East Africa 1912-1913.

Strickman, D. (1982). Stimuli affecting selection of oviposition sites by Aedes vexans (Diptera: Culicidae): Light. *Journal of Medical Entomology,* 19, 181-184.

Strickman, D. (1980a). Stimuli affecting selection of oviposition sites by *Aedes vexans*: Conditioning of the soil. *Mosquito News*, 40, 413-417.

Strickman, D. (1980b). Stimuli affecting selection of oviposition sites by *Aedes vexans*: Moisture. *Mosquito News*, 40, 236-245.

Sumaye, R. D., Geubbels, E., Mbeyela, E. & Berkvens, D. (2013). Inter-epidemic Transmission of Rift Valley Fever in Livestock in the Kilombero River Valley, Tanzania: A Cross-Sectional Survey. *PLoS Neglected Tropical Diseases,* 7, e2356. doi:10.1371/journal.pntd.0002356.

Swanepoel, R. & Coetzer, J. A. (2004). Rift Valley fever, in: Coetzer J.A., Tustin R.C. (Eds.), Infectious diseases of livestock, Oxford University Press Southern Africa, Cape Town, pp. 1037–1070.

Swanepoel, R. & Coetzer, J. A. W. (1994). Rift Valley fever. In Infectious diseases of livestock with special reference to southern Africa, Vol II 0. A.W. Coetzer, G.R.

Swanepoel, R., Struthers, J. K. & Erasmus, M. J. (1986). Comparison of techniques for demonstrating antibodies to Rift Valley fever virus. *Journal of Hygiene,* 97, 317–329.

Swanepoel, R., Manning, B. & Watt, J. A. (1979). Fatal Rift Valley fever of man in Rhodesia. *Central African Journal of Medicine,* 25, 1–8.

Tempelis, C. H. (1975). Host-feeding patterns of mosquitoes, with a review of advances in in analysis of blood meals by Serology. *Journal of Medical Entomology,* 6, 635-653.

Tesh, R. B. (1984). In: Current Topics In Vector Research. Vol. 2. P.57-76. Edited by Kerry F. Harris.

Tesh, R. B. (1982). Arthritides caused by mosquito-borne viruses. *Annual Review of Medicine*, 33, 31-40.

Tesh, R. B. & Cornet, M. (1981). The location of San Angelo virus in the developing ovaries of transovarially infected *Aedes albopictus* mosquitoes as revealed by fluorescence antibody technique. *American Journal of Tropical Medicine and Hygiene,* 30, 212-218.

Theiler, M. (1957). Action of sodium desoxycholate on arthropod-borne viruses. *Proceedings of the Society of Experimental and Biological Medicine,* 96, 380–382.

Thiemann, T. C. & Reisen, W. K. (2012). Evaluating sam-pling method bias in Culex tarsalis And Culex quinquefasciatus (Diptera: Culicidae) bloodmeal identification studies. *Journal of Medical Entomoloygy*, 49, 143–149.

Tiimub, B. M., Adu, B. K. & Obiri-Danso, K. (2012). Physico-chemical Assessment of Mosquito Breeding Sites from Selected Mining Communities at the Obuasi Municipality in Ghana. Journal of Environment and Earth Science Retrieved from: www.iiste.org ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online), 2, No.10, 2012.

Tolou, H., Plumet, S., Leparc-Goffart, I. & Couissinier-Paris, P. (2009). Le virus de la fie`vre de la valle´e du Rift: e´volution en cours, Me´d. Trop. 69, 215–220.

Torres-Velez, F. & Brown, C. (2004). Emerging infections in animals—potential new zoonoses? *Clinical and Laboratory Medicine*, 24, 825–838.

Townzen, J. S., Brower, A. V. Z. & Judd, D. D. (2008). Identification of mosquito bloodmeals using mitochondrial cytochrome oxidase subunit I and cytochrome b gene sequences. *Medical and Veterinary Entomology*, 22**,** 386-393.

Travis, B. V. (1953). Laboratory studies on the hatching of marsh-mosquito eggs. *Mosquito News*, 13**,** 190-198.

Turell, J. M., Linthicum, K. J., Patrican, A. L., Davies, F. G., Kairo, A. & Bailey, C. L. (2008). Vector competence of selected African mosquito (Diptera: Culicidae) species for Rift Valley Fever Virus. *Journal of Medical Entomology*, 45, 102-108.

Turell, M. J., Morrill, J. C., Rossi, C. A., Gad, A. M., Cope, S. E., Clements, T. L., … Presley, S. M. (2002). Isolation of West Nile and Sindbis Viruses from Mosquitoes Collected in the Nile Valley of Egypt During an Outbreak of Rift Valley Fever. *Journal of Medical Entomology*, 39, 248-250.

Turell, M. J., Bailey, C. L. & Beaman, J. R. (1988). Vector competence of a Houston, Texas strain of *Aedes albopictus* for Rift Valley fever virus. *Journal of American Mosquito Control Association*, 4, 94-96.

Turell, M. J. & Bailey, C. L. (1987). Transmission studies in mosquitoes (Diptera: Culicidae) with disseminated Rift Valley fever virus infections. *Journal of Medical Entomology*, 24**,** 11-18.

Wanja, E., Parker, Z., Rowland, T., Turell, J. M., Clark, W. J., … Sang R. (2011). Field Evaluation of a Wicking Assay for the Rapid Detection of Rift Valley Fever Viral Antigens in Mosquitoes. *Journal of the American Mosquito Control Association*, 27**,** 370-375.

Washino, R. K. & Tempelis, C. H. (1983). Mosquito host bloodmeal identification: methodology and data analysis. *Annual Review of Entomology*, 28, 179–201.

Watts, S. L., Fitzpatrick, D. M. & Maruniak, J. E. (2009). Blood Meal Identification From Florida Mosquitoes (Diptera: Culicidae). *Florida Entomologist*, 92, 619-622).

Waugh, J. (2007). DNA barcoding in animal species: progress, potential and pitfalls.

Wharton, R. H. (1962). The biology of Mansonia mosquitoes in relation to the transmission of filariasis in Malaya. *Bulletin - Institute for Medical Research*, 11, 114.

Whitley, R. J. (1990). Viral encephalitis. *New England Journal of Medicine*, 323, 242–250.

Whitlow, R. (1984). Some morphological characteristics of dambo features in Zimbabwe, *Transactions of the Zimbabwe Scientific Association*, 62, 1–15.

Wilson, G. R. & Horsfall, R. W. (1970). Eggs of floodwater mosquitoes. XII. Installment hatching of Aedes vexans. *Annals of the Entomological Society of America*, 63, 1644-1647.

World Health Organization. (2010). Rift Valley fever, Fact sheet No. 207.

World Health Organization. (1989). Geographical distribution of arthropod-borne diseases and their principal vectors. *WHO/VBC/89.967*.

World Health Organization. (1982). Manual on environmental management for mosquito control. Geneva: *World Health Organization Offset publication*. 1982: 66.

Woods, C. W, Karpati, M. A., Grein, T., McCarthy, N., Gaturuku, P., … the World Health Organization Hemorrhagic Fever Task Force. (2002). An outbreak of Rift Valley fever in northeastern Kenya, 1997–1998. *Emerging Infectious Diseases*, 8, 138–144.

Young, A. (1976). Tropical soils and soil survey, Cambridge University Press, Cambridge.

APPENDICES

Appendix I: Scientific Steering Committee Approval letter.

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

ESACIPAC/SSC/9686 **29th August, 2011**

Joel Lutomiah

Thro'
Director, CVR
NAIROBI

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P. O. Box 54628
NAIROBI

REF: SSC No.2121 (Revised) –The ecology of Rift Valley fever virus vectors and the role of host-vector interation in the transmission and maintenance of the virus during and between epidemics in Baringo and Garissa counties

Thank you for your letter dated 23rd August, 2011 responding to the comments raised by the KEMRI SSC.

I am pleased to inform you that your protocol now has formal scientific approval from SSC.

The SSC however, advises that work on the proposed study can only start after ERC approval

Christine Wasunna, PhD
FOR: SECRETARY, SSC

CENTRE FOR VIRUS RESEARCH
30 AUG 2011
P.O. Box 54628 - NAIROBI

Appendix II: Ethical Review Committee Approval letter.

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **October 25, 2011**

TO: **JOEL LUTOMIAH (PRINCIPAL INVESTIGATOR)**

THROUGH: **DR. F. OKOTH**
DIRECTOR, CVR,
NAIROBI

DIRECTOR
CENTRE FOR VIRUS RESEARCH
P. O. Box 54628
NAIROBI

Dear Sir,

RE: **SSC PROTOCOL No. 2121 – 3RD REVISION (*RE-SUBMISSION*): THE ECOLOGY OF RIFT VALLEY FEVER VIRUS VECTORS AND THE ROLE OF HOST-VECTOR INTERACTION IN THE TRANSMISSION AND MAINTENANCE OF THE VIRUS DURING AND BETWEEN EPIDEMICS IN BARINGO AND GARISSA COUNTIES**

Reference is made to your letter dated October 25, 2011. Thank you for your prompt response to the ERC letter dated October 20, 2011.

We acknowledge receipt of the revised proposal. This is to inform you that the Ethics Review Committee (ERC) finds that the issues raised at the 193rd meeting have been adequately addressed. Consequently, the study is granted approval for implementation effective this **25th day of October 2011**.

Please note that authorization to conduct this study will automatically expire on **October 23, 2012**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **September 11, 2012**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the SSC and ERC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Sincerely,

CHRISTINE WASUNNA,
FOR: SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

CENTRE FOR VIRUS RESEARCH
26 OCT 2011
P.O. Box 54628 - NAIROBI

Appendix III: First Publication in Peer Reviewed Journals, arising from this work

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume 14, Number 9, 2014

DOI: 10.1089/vbz.2013.1564

Blood Meal Analysis and Virus Detection in Blood-Fed Mosquitoes Collected During the 2006–2007 Rift Valley Fever Outbreak in Kenya

Joel Lutomiah,[1] David Omondi,[2,4] Daniel Masiga,[2] Collins Mutai,[1] Paul O. Mireji,[4] Juliette Ongus,[5] Ken J. Linthicum,[3] and Rosemary Sang[1]

Abstract

Background: Rift Valley fever (RVF) is a zoonosis of domestic ruminants in Africa. Blood-fed mosquitoes collected during the 2006–2007 RVF outbreak in Kenya were analyzed to determine the virus infection status and animal source of the blood meals.
Materials and Methods: Blood meals from individual mosquito abdomens were screened for viruses using Vero cells and RT-PCR. DNA was also extracted and the cytochrome *c* oxidase 1 (*CO1*) and cytochrome *b* (*cytb*) genes amplified by PCR. Purified amplicons were sequenced and queried in GenBank and Barcode of Life Database (BOLD) to identify the putative blood meal sources.
Results: The predominant species in Garissa were *Aedes ochraceus,* ($n = 561$, 76%) and *Ae. mcintoshi,* ($n = 176$, 24%), and *Mansonia uniformis,* ($n = 24$, 72.7%) in Baringo. *Ae. ochraceus* fed on goats (37.6%), cattle (16.4%), donkeys (10.7%), sheep (5.9%), and humans (5.3%). *Ae. mcintoshi* fed on the same animals in almost equal proportions. RVFV was isolated from *Ae. ochraceus* that had fed on sheep (4), goats (3), human (1), cattle (1), and unidentified host (1), with infection and dissemination rates of 1.8% (10/561) and 50% (5/10), respectively, and 0.56% (1/176) and 100% (1/1) in *Ae. mcintoshi.* In Baringo, *Ma. uniformis* fed on sheep (38%), frogs (13%), duikers (8%), cattle (4%), goats (4%), and unidentified hosts (29%), with infection and dissemination rates of 25% (6/24) and 83.3% (5/6), respectively. Ndumu virus (NDUV) was also isolated from *Ae. ochraceus* with infection and dissemination rates of 2.3% (13/561) and 76.9% (10/13), and *Ae. mcintoshi,* 2.8% (5/176) and 80% (4/5), respectively. Ten of the infected *Ae. ochraceus* had fed on goats, sheep (1), and unidentified hosts (2), and *Ae. mcintoshi* on goats (3), camel (1), and donkey (1).
Conclusion: This study has demonstrated that RVFV and NDUV were concurrently circulating during the outbreak, and sheep and goats were the main amplifiers of these viruses respectively.

Key Words: Rift Valley fever virus—Ndumu virus—Mosquito vectors—*Aedes ochraceus*—*Mansonia uniformis*—Blood meal identification—Host animals—Bunyaviridae—Alphavirus.

Introduction

RIFT VALLEY FEVER (RVF) is a mosquito-borne zoonosis of domestic ruminants that causes epizootics/epidemics in Africa (Peters and Linthicum 1994). RVF is caused by the RVF virus (RVFV), (Bunyaviridae: *Phlebovirus*) (Torres-Velez and Brown 2004). Although first described by Daubney, Hudson, and Garnham during a 1930 outbreak in sheep, it is clear that periodic RVF epizootics were first observed in Kenya as early as 1912 among livestock in the Rift Valley province (Montgomery 1912, Stordy 1912–1913). The largest RVF outbreaks reported in Kenya occurred during the

[1]Kenya Medical Research Institute (KEMRI), Nairobi, Kenya.
[2]International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya.
[3]USDA/ARS Center for Medical, Agricultural and Veterinary Entomology, Gainesville Florida.
[4]Egerton University, Dept. of Biochemistry and Molecular Biology, Njoro, Kenya.
[5]Jomo Kenyatta University of Agriculture and Technology, Ruiru, Kenya.

Appendix IV: Second Publication in Peer Reviewed Journals, arising from this work

SHORT COMMUNICATION

Natural Vertical Transmission of Ndumu Virus in *Culex pipiens* (Diptera: Culicidae) Mosquitoes Collected as Larvae

JOEL LUTOMIAH,[1,2] JULIETTE ONGUS,[3] KENNETH J. LINTHICUM,[4] AND ROSEMARY SANG[1]

J. Med. Entomol. 51(5): 1091–1095 (2014); DOI: http://dx.doi.org/10.1603/ME14064

ABSTRACT Ndumu virus (NDUV) is a member of the family Togaviridae and genus *Alphavirus*. In Kenya, the virus has been isolated from a range of mosquito species but has not been associated with human or animal morbidity. Little is know about the transmission dynamics or vertebrate reservoirs of this virus. NDUV was isolated from two pools of female *Culex pipiens* mosquitoes, IJR37 ($n = 18$) and IJR73 ($n = 3$), which were collected as larvae on 15 April 2013 from two *dambos* near the village of Marey, Ijara District, Garissa County, Kenya, and reared to adults and identified to species. These results represent the first field evidence of vertical transmission of NDUV among mosquitoes.

KEY WORDS *Culex pipiens*, Ijara, Ndumu virus, vertical transmission

Ndumu virus (NDUV) is a member of the family Torgaviridae and genus *Alphavirus*, which comprises >31 viruses (Forrester et al. 2012). NDUV was first isolated in South Africa from *Mansonia uniformis* (Theobald) in 1959 (Kokernot et al. 1961) and has since been described as widespread in most of Africa. It was recently documented in Kenya for the first time circulating among mosquitoes (Crabtree et al. 2009; Ochieng et al. 2013).

In general, infection with alphaviruses such as chikungunya, Ross River, Mayaro, and Sindbis causes clinical manifestations characterized by fever, rash, and arthralgia, although seroconversion in the absence of clinical disease is uncommon with all alphaviruses (Schmaljohn and McClain 1996). Although mice experimentally infected with NDUV do not survive the infection (Kokernot et al. 1961), NDUV has not been associated with human or animal morbidity. This is because of lack of affordable diagnostic kits to test febrile patients for infection with this virus and the lack of studies looking for evidence that NDUV causes diseases in humans.

There are no studies that have been conducted specifically to determine whether the virus can be maintained by vertical transmission in mosquito vectors. Even this study did not target NDUV in particular but was primarily designed to explore vertical transmission of Rift Valley fever virus (RVFV), and other arboivruses occurring in mosquito species in natural habitats. There is little evidence to suggest that vertical transmission of alphaviruses does occur (Tesh 1984). Therefore, the finding of vertical transmission here provides important information regarding the natural maintenance of NDUV.

Materials and Methods

Study Sites. Mosquitoes were sampled as larvae from *dambos* in the villages of Marey and Wakab-Harey sublocations in Sangailu Division, Ijara District, Garissa County, Kenya (Fig. 1), during the long rainy season of April–May and short rainy season of November–December in 2013. This site was selected based on prior RVFV activity during the 2006–2007 RVF epizootic in Kenya.

Larval Sampling, Rearing, and Identification. Larval sampling was conducted using standard larval dippers (350 ml) (BioQuip, Inc., Compton, CA), from the beginning to the end of the rainy season. Larvae collected from the *dambos* were transferred, in well labeled (date of collection and number collected) Whirl-Pak bags (Universal Medical, Norwood, MA), to a field laboratory where they were reared to adults in 4-liter capacity plastic cages. The emerging adults were immobilized using 99.5% triethylamine (Sigma-Aldrich, St. Louis, MO) and identified to species using the keys of Edwards (1941), Gillies and Coetzee (1987), and Jupp et al. (1976) and pooled up to 25 mosquitoes per pool. Pooled mosquitoes were preserved in liquid nitrogen for transportation to the KEMRI laboratories in Nairobi for virus isolation attempts.

Sample Preparation and Virus Isolation in Cell Culture. Mosquito pools were homogenized using copper beads in 1 ml of homogenizing media comprising Minimum Essential Medium Eagle (MEM), with Earle's salts and reduced $NaHCO_3$ (Sigma-Aldrich,

This paper was published with the permission from the Director, Kenya Medical Research Institute, KEMRI.

[1] Centre for Virus Research, Kenya Medical Research Institute, PO Box 54628, Nairobi, Kenya.
[2] Corresponding author, e-mail: joel.lutomiah@usamru-k.org.
[3] Department of Human Pathology and Laboratory Medicine, Jomo Kenyatta University of Agriculture and Technology, PO Box 62000, 00200-Nairobi, Kenya.
[4] USDA/ARS Center for Medical, Agricultural and Veterinary Entomology, 1600/1700 SW 23rd Dr., Gainesville, FL 32608.