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

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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.

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Signature_________________________________  Date___________________

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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.
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<th>Description</th>
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<td>ANOSIM</td>
<td>Analysis of similarity</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>APHIS</td>
<td>Animal and Plant Health Inspection Service</td>
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<td>BABV</td>
<td>Babanki virus</td>
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<td>BLAST</td>
<td>Basic Local Alignment Search Tool</td>
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<td>BOLD</td>
<td>Barcode of Life Data systems</td>
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<td>Bs</td>
<td><em>Bacillus sphaericus</em></td>
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<td>Bti</td>
<td><em>Bacillus thuringiensis israeliensis</em></td>
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<td>BUNV</td>
<td>Bunyamwera virus</td>
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<td>CHIKV</td>
<td>Chikungunya virus</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CO1</td>
<td>Cytochrome c oxidase subunit I</td>
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<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
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<tr>
<td>CPE</td>
<td>Cytopathic effect</td>
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<td>cDNA</td>
<td>Complementary deoxyribonucleic acid</td>
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<td>Cytb</td>
<td>Cytochrome b</td>
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<td>DENV</td>
<td>Dengue virus</td>
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<td>DEET</td>
<td>N,N-Diethyl-meta-toluamide</td>
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<tr>
<td>DI</td>
<td>Disseminated infection</td>
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<tr>
<td>DIR</td>
<td>Disseminated infection rate</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>dNTP</td>
<td>Deoxyribonucleotide triphosphate</td>
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<td>DPF</td>
<td>Day post flooding</td>
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<td>DTT</td>
<td>DL-Dithiothreitol</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<tr>
<td>EEEV</td>
<td>Eastern equine encephalitis virus</td>
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<tr>
<td>EFSA</td>
<td>European Food Safety Authority</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>EIP</td>
<td>Extrinsic incubation period</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<tr>
<td>EPA</td>
<td>U.S. Environmental Protection Agency</td>
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<td>FAO</td>
<td>Food and Agriculture Organization</td>
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<tr>
<td>FBS</td>
<td>Fetal bovine serum</td>
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<tr>
<td>FCCMC</td>
<td>Florida Coordinating Council on Mosquito Control</td>
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<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>GPS</td>
<td>Global positioning system</td>
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<td>IEP</td>
<td>Inter-epizootic period</td>
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<td>ILRI</td>
<td>International Livestock Research Institute</td>
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<td>IgG</td>
<td>Immunoglobulin G antibodies</td>
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<td>IGR</td>
<td>Insect growth regulators</td>
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<td>IJR</td>
<td>Ijara</td>
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<tr>
<td>IPM</td>
<td>Integrated pest management</td>
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<td>ITN</td>
<td>Insecticide treated net</td>
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<tr>
<td>KRV</td>
<td>Kamiti river virus</td>
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<td>KEMRI</td>
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<td>LAC</td>
<td>La Crosse virus</td>
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<td>LN₂</td>
<td>Liquid nitrogen</td>
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<td>MA</td>
<td>Massachusetts</td>
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<td>MEGA</td>
<td>Molecular Evolutionary Genetic Analysis</td>
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<td>MEM</td>
<td>Minimum Essential Media Eagles</td>
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<td>mtDNA</td>
<td>Mitochondrial Deoxyribonucleic acid</td>
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<td>MI</td>
<td>Mid gut infection</td>
</tr>
<tr>
<td>MIR</td>
<td>Mid gut infection rate</td>
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<tr>
<td>MPL</td>
<td>Maximum Permissible Limits</td>
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<td>Sodium bicarbonate</td>
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<td>NCBI</td>
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<td>ND</td>
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<td>NE</td>
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<td>Ngari virus</td>
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<td>NSP</td>
<td>Nonstructural protein</td>
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<td>PBS</td>
<td>Phosphate buffered saline</td>
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<td>PGAV</td>
<td>Pongola virus</td>
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<td>PH</td>
<td>Potential of hydrogen</td>
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<td>Ribonucleic protein</td>
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<td>Relative risk</td>
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<td>Ross river virus</td>
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<td>rpm</td>
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<td>RT-PCR</td>
<td>Reverse Transcription Polymerase Chain Reaction</td>
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<td>RVF</td>
<td>Rift Valley fever</td>
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<td>Rift Valley fever virus</td>
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<td>Sindbis virus</td>
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<td>TOT</td>
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<td>United States of America</td>
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<td>USAMRIID</td>
<td>United States Army Medical Research Institute of Infectious Diseases</td>
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<td>USDA</td>
<td>United States Department of Agriculture</td>
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<tr>
<td>VHF</td>
<td>Viral hemorrhagic fever</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>WNV</td>
<td>West Nile virus</td>
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<td>YFV</td>
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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, \((p=0.9)\); Lakab-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 Statement of 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 phenomenon 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 *dambos* 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 usually 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 commence 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 RVF

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 suspicion 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 (Swanpoel, 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 (Swanpoel, 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 (Línhicium et al., 1985a). The virus has been isolated from a large number of species during and between epidemics (Línhicium 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 & Cornell, 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 (Línhicium 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 (Linthicium *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, Kęcki & 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 occurrence 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 wildlife 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 1,517) 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 behavior (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. quasiumvittatus*, *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, *Mansonina* 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](image1.png)  
*Plate 2.1* Flooded dambo in Sangailu, Ijara District, Garissa County, following heavy rains

![Plate 2.2](image2.png)  
*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).
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 hemorrhagic 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/ km² (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 km² with ≈31 people/ km² (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) $n=Z^2(1-\alpha/2)P(1-P)/d^2$ 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;
- $P = \text{the expected prevalence or proportion, taken as 3\% (Nguku et al., 2012)}$.
- $d = \text{precision in proportion of one, taken to be half of 3\% since the expected prevalence/proportion was below 10\% (d=0.015)}$.

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](image)
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™ 35 (Eutech Instruments/ Oakton) (Plate 3.2).

Plate 3.2 The PCTestr™ 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 NaHCO₃ (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% CO₂ 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](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

<table>
<thead>
<tr>
<th>Virus or genus</th>
<th>Gene Target</th>
<th>Primer Sequence</th>
<th>Position</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RVFV</td>
<td>M Segment</td>
<td>F - RVF1: (5'-GAC TAC CAG TCA GCT CAT TAC C-3')</td>
<td>777-798</td>
<td>Kuno et al., (1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R - RVF2: (5'-TGT GAA CAA TAG GCA TTG G-3')</td>
<td>1309-1327</td>
<td></td>
</tr>
<tr>
<td>2. Flavivirus</td>
<td>NS5</td>
<td>F - FU 1; (5'-TAC AAC ATG ATG GGA AAG AGA GAG AA-3')</td>
<td>9007-9032</td>
<td>Bryant et al., (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R - CFD2; (5'-GTG TCC CAG CCG GCG GTG TCA TCA GC-3')</td>
<td>9308-9283</td>
<td></td>
</tr>
<tr>
<td>3. Bunyavirus</td>
<td>nucleocapsid protein</td>
<td>F - BCS82C; (5'-ATG ACT GAG TTG GAG TTT CAT GAT GTC GC-3')</td>
<td>86-114</td>
<td>Kuno et al., (1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R - BCS332V; (5'-TGT TCC TGT TGC CAG GAA AAT-3')</td>
<td>309-329</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>R - VIR 2052R; (5'-TAC GAT GTT GTC GTC GCC GAT GAA-3')</td>
<td>7086–7109</td>
<td></td>
</tr>
<tr>
<td>5. Ndumu</td>
<td>envelope (E1) gene</td>
<td>F - ND 124F; (5'-CAC CCT AAA AGT GAC GAC GTT-3')</td>
<td>124-141</td>
<td>Bryant et al., (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R - ND 615R; (5'-ATT GCA GAT GGG ATA CCG-3')</td>
<td>615-632</td>
<td></td>
</tr>
<tr>
<td>6. Babanki</td>
<td>E1 envelope glycoprotein</td>
<td>F - Bab 3368F; (5'-CAG CAG ATT GCG CGA CTG ACC-3')</td>
<td>3368-3388</td>
<td>Bryant et al., (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R - Bab 4203R; (5'-GCT CAC GAT ATG GTC AGC AGG-3')</td>
<td>4184-4203</td>
<td></td>
</tr>
<tr>
<td>7. Sindbis</td>
<td>Non Structural Protein</td>
<td>F - SINV1; (5'-TTT AGC GGA TCG GAC AAT TC-3')</td>
<td>5194-5213</td>
<td>Bryant et al., (2005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>R - SINV2; (5'-GCG GTG ACG AAC TCA GTA G-3')</td>
<td>6482-6500</td>
<td></td>
</tr>
</tbody>
</table>
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 1 min 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’-TGTTAAACGACGGCCAGTTCTCAACCAACCACAARGAYATYGG-3’) and VR1d_dt reverse (5’-CAGGAAACAGCTATGACTAGACTTCTGCGTGCTGCCCRAARA AYCA-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’-CCATCCACATCTCAGCATGATGAAA-3’) and H151494 Reverse (5’-GCCCCTCAGAATGATATTGTGTCTCTCA-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 COI 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
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-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 arboviruses 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](http://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 ≤ 50 mm Relative humidity remained fairly constant throughout (Fig. 4.1).
Figure 4.1 Daily rainfalls, relative humidity and temperature for September to December 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>
<thead>
<tr>
<th>Species</th>
<th>Days post-flooding in April 2013</th>
<th>Relative abundance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td><em>Ae. tricholabis</em></td>
<td>7,850</td>
<td>5,231</td>
</tr>
<tr>
<td><em>Ae. ochraceus</em></td>
<td>596</td>
<td>775</td>
</tr>
<tr>
<td><em>Ae. mcintoshi</em></td>
<td>341</td>
<td>285</td>
</tr>
<tr>
<td><em>Cx. pipiens</em></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. sudanensis</em></td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td><em>Anopheles</em> spp.</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>8,794</td>
<td>6,309</td>
</tr>
</tbody>
</table>

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

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

![Cumulative abundance of mosquito species collected as larvae per dip for each day sampled in Marey.](image)

*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 6\textsuperscript{th} (6 DPF) to November 18\textsuperscript{th} 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.](image)

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 7\textsuperscript{th} (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).
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

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

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).
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).
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

<table>
<thead>
<tr>
<th>Co-ordinates</th>
<th>Dambo</th>
<th>Surface area (m²)</th>
<th>Depth (cm)</th>
<th>Daily mean temp (°C)</th>
<th>pH</th>
<th>Vegetation cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>S01°14'46.8&quot; E040°39'30.4&quot;</td>
<td>1</td>
<td>24</td>
<td>10</td>
<td>36.6</td>
<td>10.3</td>
<td>Open, no canopy, no vegetation cover</td>
</tr>
<tr>
<td>S01°14’45.5&quot; E040°39'29.6&quot;</td>
<td>2</td>
<td>36</td>
<td>20</td>
<td>37.4</td>
<td>9.8</td>
<td>Grass and water plants</td>
</tr>
<tr>
<td>S01°14’46.2&quot; E040°39'27.6&quot;</td>
<td>3</td>
<td>20</td>
<td>20</td>
<td>35.3</td>
<td>10</td>
<td>Open with grass on edges</td>
</tr>
<tr>
<td>S01°14’47.0&quot; E040°39'27.7&quot;</td>
<td>4</td>
<td>9</td>
<td>10</td>
<td>36.2</td>
<td>10.8</td>
<td>Grass and twigs on edges</td>
</tr>
<tr>
<td>S01°14’47.4&quot; E040°39'27.5&quot;</td>
<td>5</td>
<td>56</td>
<td>15</td>
<td>35.4</td>
<td>10.7</td>
<td>Open, no plant canopy, no vegetation cover</td>
</tr>
<tr>
<td>S01°14’44.5&quot; E040°39'05.7&quot;</td>
<td>6</td>
<td>64</td>
<td>18</td>
<td>32.6</td>
<td>9.7</td>
<td>Canopy present, grass on edges and center</td>
</tr>
<tr>
<td>S01°14’45.9&quot; E040°39'05.3&quot;</td>
<td>7</td>
<td>40</td>
<td>10</td>
<td>36.1</td>
<td>8.7</td>
<td>Open with grass on the edges</td>
</tr>
<tr>
<td>S01°14’38.4&quot; E040°39'04.0&quot;</td>
<td>8</td>
<td>20</td>
<td>22</td>
<td>35</td>
<td>9.1</td>
<td>Open with grass on the edges only</td>
</tr>
<tr>
<td>S01°14’46.3&quot; E040°39'31.6&quot;</td>
<td>9</td>
<td>15</td>
<td>20</td>
<td>36.9</td>
<td>10.1</td>
<td>Open, no plant canopy, no vegetation cover</td>
</tr>
<tr>
<td>S01°14’47.6&quot; E040°39'05.0&quot;</td>
<td>10</td>
<td>100</td>
<td>17</td>
<td>32.3</td>
<td>9.9</td>
<td>Plant canopy, with grass all over</td>
</tr>
</tbody>
</table>

*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**

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

_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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Marey</th>
<th>Wakab-Harey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>s.e.</td>
</tr>
<tr>
<td>Surface area (m²)</td>
<td>38.40</td>
<td>8.86</td>
</tr>
<tr>
<td>Depth (cm)</td>
<td>16.20</td>
<td>1.48</td>
</tr>
<tr>
<td>Daily mean temp. (°C)</td>
<td>35.38</td>
<td>0.54</td>
</tr>
<tr>
<td>pH</td>
<td>9.91</td>
<td>0.21</td>
</tr>
</tbody>
</table>

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 positive, 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.

<table>
<thead>
<tr>
<th>Species</th>
<th>RR (95% CI)</th>
<th>Site</th>
<th>Surface area (m²)</th>
<th>Depth (cm)</th>
<th>Daily mean temperature</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.46 (0.39-0.55)</td>
<td>1.01 (1.01-1.01)</td>
<td>1.06 (1.05-1.06)</td>
<td>1.01 (0.99-1.04)</td>
<td>0.73 (0.7-0.77)</td>
<td></td>
</tr>
<tr>
<td>Cx. pipiens</td>
<td>0.21 (0.16-0.27)</td>
<td>1.02 (1.02-1.02)</td>
<td>1.07 (1.06-1.07)</td>
<td>0.93 (0.91-0.95)</td>
<td>0.73 (0.68-0.78)</td>
<td></td>
</tr>
<tr>
<td>Cx. univittatus</td>
<td>1.98 (1.26-3.12)</td>
<td>1.00 (0.99-1.00)</td>
<td>1.00 (0.98-1.01)</td>
<td>1.89 (1.68-2.14)</td>
<td>1.17 (1.01-1.35)</td>
<td></td>
</tr>
<tr>
<td>Ae. ochraceus</td>
<td>0.52 (0.06-3.93)</td>
<td>1.01 (0.97-1.04)</td>
<td>0.93 (0.85-1.01)</td>
<td>1.45 (1-2.30)</td>
<td>0.61 (0.33-1.11)</td>
<td></td>
</tr>
<tr>
<td>Cx. vansomereni</td>
<td>0.002 (0.00-0.04)</td>
<td>1.05 (1.02-1.09)</td>
<td>1.12 (1.05-1.22)</td>
<td>1.10 (0.91-1.33)</td>
<td>1.02 (0.58-1.9)</td>
<td></td>
</tr>
<tr>
<td>Culex spp.</td>
<td>0.001(0.00-0.03)</td>
<td>1.00 (0.98-1.02)</td>
<td>0.86 (0.71-0.98)</td>
<td>39.15 (7.55-512.99)</td>
<td>3.55 (1.32-11.46)</td>
<td></td>
</tr>
<tr>
<td>Ae. sudanensis</td>
<td>1.45 (0.07-28.41)</td>
<td>0.99 (0.93-1.03)</td>
<td>0.97 (0.85-1.07)</td>
<td>1.28 (0.79-2.43)</td>
<td>0.63 (0.24-1.55)</td>
<td></td>
</tr>
<tr>
<td>Ae. mcintoshi</td>
<td>1.05 (1.01-1.12)</td>
<td>0.93 (0.73-1.07)</td>
<td>0.73 (0.35-1.52)</td>
<td>1.4 (0.74-2.76)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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 ≤ 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

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

*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.

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

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>No.</th>
<th>Vertebrate host</th>
<th>No. of blood meals</th>
<th>RVFV +ve blood meals</th>
<th>MIR (%)</th>
<th>RVFV +ve heads</th>
<th>DIR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ae. ochraceus</em></td>
<td>452</td>
<td>Human</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cattle</td>
<td>79</td>
<td>1</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sheep</td>
<td>21</td>
<td>2</td>
<td>9.5</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Goat</td>
<td>185</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unidentified</td>
<td>69</td>
<td>1</td>
<td>1.4</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>71</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. mcintoshi</em></td>
<td>124</td>
<td>Donkey</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Goat</td>
<td>47</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Camel</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>68</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. sudanensis</em></td>
<td>1</td>
<td>Sheep</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>577</td>
<td></td>
<td>577</td>
<td>5</td>
<td>0.9</td>
<td>3</td>
<td>60</td>
</tr>
</tbody>
</table>

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 mosquitoes collected from El-Humow, Garissa County, in relation to their vertebrate hosts

<table>
<thead>
<tr>
<th>Mosquito species</th>
<th>No.</th>
<th>Vertebrate host</th>
<th>No. of blood meals</th>
<th>NDUV +ve blood meals</th>
<th>MIR (%)</th>
<th>NDUV +ve heads</th>
<th>DIR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ae. ochraceus</em></td>
<td>452</td>
<td>Human</td>
<td>27</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cattle</td>
<td>79</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sheep</td>
<td>21</td>
<td>1</td>
<td>4.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Goat</td>
<td>185</td>
<td>10</td>
<td>5.4</td>
<td>3</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unidentified</td>
<td>69</td>
<td>2</td>
<td>2.9</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>71</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. mcintoshi</em></td>
<td>124</td>
<td>Donkey</td>
<td>6</td>
<td>1</td>
<td>16.7</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Goat</td>
<td>47</td>
<td>3</td>
<td>6.4</td>
<td>2</td>
<td>66.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Camel</td>
<td>3</td>
<td>1</td>
<td>33</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Others</td>
<td>68</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Ae. sudanensis</em></td>
<td>1</td>
<td>Sheep</td>
<td>1</td>
<td>1</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>577</td>
<td>577</td>
<td>19</td>
<td>3.3</td>
<td>8</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Sites</th>
<th>Virus isolation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sangailu</td>
<td>Marigat</td>
<td>Total</td>
</tr>
<tr>
<td><em>Ae. tricholabis</em></td>
<td>350</td>
<td>0</td>
<td></td>
<td>350</td>
</tr>
<tr>
<td><em>Ae. ochraceus</em></td>
<td>185</td>
<td>2</td>
<td></td>
<td>187</td>
</tr>
<tr>
<td><em>Ae. mcintoshi</em></td>
<td>7</td>
<td>2</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td><em>Ma. uniformis</em></td>
<td>0</td>
<td>95</td>
<td></td>
<td>95</td>
</tr>
<tr>
<td><em>Ma. africana</em></td>
<td>0</td>
<td>129</td>
<td></td>
<td>129</td>
</tr>
<tr>
<td><em>An. ocustani</em></td>
<td>0</td>
<td>13</td>
<td></td>
<td>13</td>
</tr>
<tr>
<td><em>An. gambiae</em></td>
<td>0</td>
<td>23</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td><em>Cx. poicilipes</em></td>
<td>0</td>
<td>6</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td><em>Cx. bitaeniorhynchus</em></td>
<td>0</td>
<td>2</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>Cx. pipiens</em></td>
<td>0</td>
<td>7</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td><em>Cx. univittatus</em></td>
<td>0</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>542</td>
<td>280</td>
<td></td>
<td>822</td>
</tr>
</tbody>
</table>
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 semi-arid 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 up to 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 RVF 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 RVF 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 magnitude 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.
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*...
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. mcintoshii*, *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. mcintoshii*. However, unengorged members of these two mosquito spp. collected during the same period comprised mostly of *Ae. mcintoshii* relative to *Ae. ochraceus* (Sang *et al*., 2010). Assuming that blood seeking *Ae. ochraceus* and *Ae. mcintoshii* are equally attracted to the CDC light traps, these results may suggest that bloodfed *Ae. ochraceus* survived for longer compared to *Ae. mcintoshii*, 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/km², 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 CO₂-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 *Mansonina* 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 develop 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 targeting 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


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


APPENDICES

Appendix I: Scientific Steering Committee Approval letter.

KENYA MEDICAL RESEARCH INSTITUTE
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ESACIPAC/ESC/9686  29th August, 2011

Joel Lutomiah

Thro’

Director, CVR
NAIROBI

REF: SSC No.2121 (Revised) –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

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
Appendix II: Ethical Review Committee Approval letter.

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54628-00200, NAIROBI, Kenya
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KEMRI/RES/7/3/1

TO: JOEL LUTOMIAH (PRINCIPAL INVESTIGATOR)

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

Dear Sir,


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 the 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

26 OCT 2011
Appendix III: First Publication in Peer Reviewed Journals, arising from this work


Joel Lutomiah,1 David Omondi,2,4 Daniel Maringa,2 Collins Muta1, Paul O. Mirej1, Juliette Cngus,1 Ken J. Linticum,2 and Rosemary Sang3

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 (16.5%), 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 hosts (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, M. 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. Nduvu 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—Nduvu 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 Linton 1994). RVFV is caused by the RVF virus (RVFV), (Bunyaviridae: Phlebovirinae: (Torres-Velázquez and Brown 2004). Although first described by Daubs, Hudson, and Gammohan 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; Storrs 1912–1913). The largest RVF outbreaks reported in Kenya occurred during the
Appendix IV: Second Publication in Peer Reviewed Journals, arising from this work

**Short Communication**

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

**JOEL LUTOMIAH, JULIETTE NGUSI, KENNETH J. LINTHICUM, and ROSEMARY SANG**


**ABSTRACT** Ndmuu virus (NDVU) 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 known about the transmission dynamics or vertebrate reservoirs of this virus. NDVU was isolated from two pools of female *Culex pipiens* mosquitoes, JF037 (n = 18) and JF075 (n = 3), which were collected as larvae on 15 April 2001 from two dambos near the village of Maney, Ijara District, Garissa County, Kenya, and reared to adults and identified to species. These results represent the first field evidence of vertical transmission of NDVU among mosquitoes.

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

Ndmuu virus (NDVU) is a member of the family Togaviridae and genus Alphavirus, which comprises >31 viruses (Forrester et al. 2012). NDVU was first isolated in South Africa from *Monomorium minuculosum* (Thobald) in 1958 (Kokernut 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 (Crabb et al. 2009; Ochieng et al. 2011).

In general, infection with alphaviruses such as chikungunya, Ross River, Mararo, and Sindbis causes clinical manifestations characterized by fever, rash, and arthralgia, although severe conversion to the absence of clinical disease is uncommon with all alphaviruses (Schmaljohn and McClain 1996). Although there is experimental evidence of infection in NDVU, there is no report of vertical transmission in mosquito vectors. This is because of the lack of affordable diagnostic kits to test fetuses infected with NDVU or the absence of studies lacking evidence that NDVU causes disease in humans. There are no studies that have been conducted specifically to determine whether the virus can be transmitted by vertical transmission in mosquito vectors. Even this study did not target NDVU in particular but was primarily designed to explore vertical transmission of Rift Valley fever virus (RVFV) and other alphaviruses 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 NDVU.

**Materials and Methods**

**Study Sites.** Mosquitoes were sampled as larvae from dambos in the villages of Maney and Wahaba-Harey sublocations in Sangaru 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 epidemic in Kenya.

**Larval Sampling, Rearing, and Identification.** Larval sampling was conducted using standard larval dipper (350 ml) (Buersip Inc, Compton, CA), from the beginning to the end of the rainy season. Larvae collected from the dambos were transferred to 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 1-liter plastic cages. The emerging adults were immersed in 99.5% triethylamine (Sigma-Aldrich, St. Louis, MO) and identified to species using the keys of Edwards (1941), Gillies and Coetzee (1967), and Jupp et al. (1976) and pooled up to 25 mosquitoes per pool. Pooled mosquitoes were preserved in liquid nitrogen for transport 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 NaHCO₃ (Sigma-Aldrich, 0022-2593 (c) 1993-1999 by the Entomological Society of America.