TRANSMISSION DYNAMICS OF HUMAN RIFT VALLEY FEVER VIRUS IN IJARA DISTRICT, GARISSA COUNTY, KENYA

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DElarATION

This Thesis is my original work and has not been presented for a degree in any other University

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To my wife Ann and daughters Nancy, Judy, Karen and Cynthia for their endurance during the time of this study
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# TABLE OF CONTENTS

DECLARATION ...................................................................................................................... ii

DEDICATION ...................................................................................................................... iii

ACKNOWLEDGEMENT ....................................................................................................... iv

TABLE OF CONTENTS ....................................................................................................... v

LIST OF TABLES .................................................................................................................. vi

LIST OF FIGURES ............................................................................................................... x

LIST OF ANNEXES ............................................................................................................. xi

DEFINITION OF OPERATIONAL TERMS ......................................................................... xi

ABBREVIATIONS ................................................................................................................ xiii

ABSTRACT ......................................................................................................................... Error! Bookmark not defined.

CHAPTER ONE: INTRODUCTION ......................................................................................... 1
  1.1 Background Information ............................................................................................. 1
  1.2 Statement of Problem ................................................................................................ 2
  1.3 Justification ................................................................................................................ 2
  1.4 Research Questions ................................................................................................... 3
  1.5 Null Hypothesis ......................................................................................................... 4
  1.6 Objectives .................................................................................................................. 4
    1.6.1 General Objective ............................................................................................... 4
    1.6.2 Specific Objectives ............................................................................................. 4

CHAPTER TWO: LITERATURE REVIEW ............................................................................. 5
  2.1 Rift Valley Fever Virus ............................................................................................... 5
  2.2 Epidemiology of RVF ............................................................................................... 6
  2.3 Geographic distribution of RVF ................................................................................ 7
  2.4 Pathogenesis of RVF ................................................................................................ 8
    2.4.1 Pathology of RVF in humans ............................................................................. 8
2.4.2 Clinical Appearance of RVF in Human Infections................................. 9
2.4.3 Eye Involvement in RVFV Infections .................................................. 10
2.4.4 Prevention and control of RVF outbreaks .......................................... 11
2.4.5 Treatment of RVF Infections .............................................................. 13
2.5. Transmission Dynamics of RVF Virus ................................................... 13
2.5.1 Arthropod Vectors of RVF Virus ........................................................ 13
2.5.2 Mosquitoes and arboviral transmission .......................................... 18
2.5.2.1 Mosquitoes in the transmission of Rift Valley Fever Virus .............. 19
2.5.3 Non Vector Transmission of RVF virus .............................................. 20
2.5.4 Excessive Rainfall and Ecological Changes ....................................... 21
2.5.5 Occupational Related RVF Infections .............................................. 24
2.5.6 Human RVFV Epidemics ................................................................. 25
2.5.7 Association between RVF Epizootics and Epidemics .......................... 27
2.5.8 Immunoglobulins .............................................................................. 28
2.5.9 IgG ELISA in detection of anti-RVFV ............................................... 29
2.6 Mosquitoes and RVFV .......................................................................... 31
2.6.1 Distribution of mosquitoes.................................................................. 31
2.6.2 Biology of Primary and Secondary RVFV vector mosquitoes ............. 32
2.6.3 Collection of Mosquitoes ................................................................. 33
2.6.4 Molecular Identification of Mosquitoes .............................................. 34
2.6.5 The Internal Transcribed Spacer 2 (ITS2) Region of Mosquito DNA .......... 34
2.6.6 Pool Screening of Mosquitoes ............................................................ 35
2.6.7 Polymerase Chain Reaction (PCR) Amplifications ............................ 36
2.7 Geographic Positioning System .............................................................. 36
2.7.1 Geographic Information System (GIS) in RVF surveillance ............... 36
2.7.2 GIS in disease and vector spatial clustering ...................................... 38

CHAPTER THREE: MATERIALS AND METHODS ........................................ 40
3.1 Study Area ......................................................................................... 40
3.1.1 Geographical Position ...................................................................... 40
3.1.2 Climate ........................................................................................... 40
3.1.3 Administrative and Health Facilities..........................................................42
3.1.4 Study population.......................................................................................42
3.1.5 Economic activities in Ijara District ..........................................................45
3.2 Sampling Technique ....................................................................................45
3.2.1 Sample Size determination ......................................................................46
3.2.2 Study Recruitment ....................................................................................46
3.3 RVFV seropositivity by IgG ELISA ...............................................................47
3.3.1 Venous blood sample................................................................................48
3.3.2 Determination of RVFV IgG antibodies ......................................................48
3.4 Past animal and non animal exposure factors ..............................................47
3.4.1 Animal exposure factors .........................................................................48
3.4.2 Non animal exposure factors ....................................................................48
3.5 Past RVF related symptoms and eye disease examination .........................49
3.5.1 Past RVF related symptoms ....................................................................49
3.5.2 Eye examination .......................................................................................49
3.6 viral infection rates ......................................................................................49
3.6.1 Mosquito sampling ..................................................................................49
3.6.2 Mosquito morphological identification ....................................................52
3.6.3 PCR method for mosquito identification ..................................................51
3.6.4 RNA extraction method .........................................................................53
3.6.5 PCR amplification ....................................................................................55
3.6.6 PCR in pooled samples ............................................................................55
3.7 Geographical Positioning System .................................................................55
3.7.1 GIS mapping by use of Etrex GPS machine .............................................56
3.8 Ethical considerations....................................................................................55
3.9 Data Analysis................................................................................................56

CHAPTER FOUR: RESULTS ..................................................................................57
4.1 Demographic Information ..........................................................................57
4.2 Human RVFV Seropositivity .......................................................................57
4.2.1 Seropositivity of participants by age range ..............................................58
4.2.2 Seropositivity by Age, Location and Sex ..................................................................... 60
4.3 Animal and Non Animal exposure factors .................................................................. 61
  4.3.1 Animal exposure factors ......................................................................................... 61
  4.3.2 Non Animal Exposure Factors ................................................................................ 64
4.4 Past symptoms and eye disease associated with seropositivity ................................. 67
  4.4.1 Past symptoms associated with seropositivity ....................................................... 67
  4.4.2 Eye disease finding and seropositivity ..................................................................... 69
  4.4.3 Risk predictors of RVFV seropositivity by location ................................................. 71
  4.4.4 Model for risk predictors ......................................................................................... 72
4.5 Mosquito species identification ..................................................................................... 73
  4.5.1 Trapping and identification ...................................................................................... 74
  4.5.2 Viral positivity rates ................................................................................................. 74
  4.5.2.1 RVFV positivity rates on mosquito pools and MIRs ........................................... 79
4.6 Spatial distribution of RVFV seropositivity by GIS clustering analysis ....................... 79

CHAPTER FIVE .................................................................................................................. 81
5.0 DISCUSSION, CONCLUSIONS & RECOMMENDATIONS ...................................... 81
5.1 Discussion ...................................................................................................................... 81
  5.1.1 Human seropositivity .............................................................................................. 81
  5.1.2 Animal and Non-animal exposure factors ............................................................. 83
  5.1.3 Past RVFV symptoms ............................................................................................. 84
  5.1.4 Mosquito species and viral infection rates ............................................................. 87
  5.1.5 GIS spatial clustering of RVFV risk association ...................................................... 87
5.2 Conclusions and Recommendations ............................................................................. 88
  5.2.1 Human seropositivity ............................................................................................. 88
  5.2.2 Animal and Non-Animal exposure factors .......................................................... 88
  5.2.3 Past symptoms and eye disease ............................................................................. 89
  5.2.4 Mosquito identifications and infection rates ......................................................... 89
  5.2.5 GIS spatial clustering and RVF transmission ......................................................... 90
5.3 Recommendations ......................................................................................................... 91

REFERENCES .................................................................................................................... 92
LIST OF TABLES

Table 3.1: Table showing primer pairs, sequences, product size and Gen Bank accession number .................................................................................................................................................... 53

Table 4.1: Demographic data of survey participants in Gumarey and Sogan Gudud ..... 57

Table 4.2: RVFV Seropositivity by age of study population .......................................................... 59

Table 4.3: Percentage positivity by age group and location ............................................................ 59

Table 4.4: Associations of demographic factors and RVFV Seropositivity .................. 61

Table 4.5: Association of selected animal factors and RVFV Seropositivity by location 61

Table 4.6: Comparison of RVFV exposure factors by location .................................................... 63

Table 4.7: The association of non-animal contact factors with seropositivity by location 66

Table 4.8: Risk of non-animal contact factors with seropositivity ........................................... 67

Table 4.9: Percentage prevalence of selected past symptoms and RVFV ......................... 68

Table 4.10: Testing for interaction between associated predictors ........................................ 69

Table 4.11: RVFV prevalence compared with those with eye disease ......................... 70

Table 4.12: Association of eye disease with RVFV seroprevalence ........................................ 70

Table 4.13: Correlation analysis for age, sex and village with seropositivity .... 71

Table 4.14a: Testing for interactions between associated risk factors (GM) ................ 72

Table 4.14b: Testing for interactions between associated risk factors (SG) ............. 72

Table 4.15: Logistic regression analysis: .................................................................................. 73

Table 4.16: Mosquito Pool PCR Results for RVFV by Village .................................................. 77

Table 4.17: Percentage of households with positive mosquito PCR ....................... 78

Table 4.18: Integration of Household Mosquito PCR and Human IgG Elisa ............. 79
LIST OF FIGURES

Figure 2.1 A natural depression holding some water in North Eastern Kenya .......... 15
Figure 2.2 A section of a flooded village in North Eastern Kenya ............................. 16
Figure 2.3 Distribution of RVF human cases in Kenya, during 2006 – 2007 outbreak .. 18
Figure 2.4 Theoretical transmission cycle of RVF virus ......................................... 26
Figure 3.1 Map showing Ijara District and the study area ........................................ 41
Figure 3.2A and 3.2B Map showing Sogan Gudud and Gumarey villages ............... 44
Figure 4.1: A flowchart of seropositive participants by village and sex ................. 58
Figure 4.2: Proportion of RVF IgG Seropositivity by age in both villages .................. 60
Figure 4.3: Percentage of participants and selected contact factors .......................... 62
Figure 4.4: Non-animal exposure factors and RVFV seropositivity by location ......... 65
Figure 4.5: Graph showing the species distribution of total collected mosquitoes by percentage (%) .......................................................... 74
Figure 4.6 Graph showing number of mosquito tested and species distribution ......... 75
Figure 4.7 PCR agarose gel amplification .............................................................. 76
Figure 4.8 Spatial clustering of RVFV seropositivity in both villages ..................... 80
# LIST OF ANNEXES

**Annex 1** Information on adult consent to participate in the study.............................. 107  
**Annex 2** Information on child informed consent............................................................. 110  
**Annex 3** Information on General and Demographic Questions......................................... 116  
**Annex 4** Information on RVFV Exposure Factors............................................................. 117  
**Annex 5**: Information on Physical Examination of the study subjects......................... 119  
**Annex 6**: Information on Ophthalmologic Examinations.............................................. 120  
**Annex 7**: A submerged traditional hut in the study area.......................................... 121  
**Annex 8**: Sheep and Goats slaughtering................................................................. 122  
**Annex 9**: Movement of animals................................................................. 123  
**Annex 10**: Filling of demographic data................................................................. 124  
**Annex 11**: Eye examination procedure................................................................. 125  
**Annex 12**: Setting of mosquito traps................................................................. 126  
**Annex 13**: Sorting and identification of mosquitoes in the laboratory....................... 127
DEFINITION OF OPERATIONAL TERMS

<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
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<tbody>
<tr>
<td><strong>Bunyaviridae</strong></td>
<td>A family of viruses that have spherical virons measuring 90 -100 NM in diameter, a bilayered lipid envelop and three circular nucleocapsids with helical symmetry. Rift Valley Fever virus belongs to this family</td>
</tr>
<tr>
<td><strong>Dambos</strong></td>
<td>Natural depressions which hold water during flooding in East African savannah regions</td>
</tr>
<tr>
<td><strong>Enzootic Hepatitis</strong></td>
<td>Another name used in reference to Rift Valley Fever</td>
</tr>
<tr>
<td><strong>Epidemic</strong></td>
<td>Occurrence of more cases of a disease than expected in a community or region during a given time period</td>
</tr>
<tr>
<td><strong>Epizootic</strong></td>
<td>A disease outbreak affecting animals</td>
</tr>
<tr>
<td><strong>Phlebovirus</strong></td>
<td>Genus of organisms where Rift Valley Fever virus belongs</td>
</tr>
<tr>
<td><strong>Zoonosis</strong></td>
<td>A disease affecting humans but primarily a disease of animals</td>
</tr>
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</table>
# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>CCHF</td>
<td>Crimean Congo Hemorrhagic Fever</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>CWRU</td>
<td>Case Western Reserve University - USA</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DVBNTD</td>
<td>Division of Vector Borne &amp; Neglected Tropical Diseases</td>
</tr>
<tr>
<td>ESRI</td>
<td>Environmental System Research Inc</td>
</tr>
<tr>
<td>FAO</td>
<td>Food &amp; Agriculture Organization</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme Linked Immuno-Sorbent Assay</td>
</tr>
<tr>
<td>ENSO</td>
<td>El-Nino Southern Oscillation</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>ITS2</td>
<td>Internal Transcribed Spacer 2 region</td>
</tr>
<tr>
<td>JE</td>
<td>Japanese Encephalitis</td>
</tr>
<tr>
<td>GPS</td>
<td>Geographical Positioning System</td>
</tr>
<tr>
<td>GIS</td>
<td>Geographical Information System</td>
</tr>
<tr>
<td>KEMRI</td>
<td>Kenya Medical Research Institute</td>
</tr>
<tr>
<td>KWS</td>
<td>Kenya Wildlife Services</td>
</tr>
<tr>
<td>MIR</td>
<td>Minimum Infection Rates</td>
</tr>
<tr>
<td>MLE</td>
<td>Maximum Likelihood Estimates</td>
</tr>
<tr>
<td>OIE</td>
<td>Office International des Epizootics (World Organization for Animal Health)</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>PRN</td>
<td>Plaque Reduction Neutralization Test</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
</tr>
<tr>
<td>RVF</td>
<td>Rift Valley Fever</td>
</tr>
<tr>
<td>RVFV</td>
<td>Rift Valley Fever Virus</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>SST</td>
<td>Sea Surface Temperature</td>
</tr>
<tr>
<td>SOI</td>
<td>Southern Oscillation Index</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WNV</td>
<td>West Nile Virus</td>
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ABSTRACT

Rift Valley Fever (RVF) is an emerging viral disease which causes among other problems severe hemorrhagic syndrome, retinitis and meningo-encephalitis in humans. The disease was previously thought to be a disease of animals but it is increasingly developing to be a human health problem. Two human RVF epidemics have previously occurred in Kenya (1997-1997; 2006-2007) each resulting to human deaths. Most RVF outbreaks occur in rural locations where keeping of livestock is the main livelihood of the affected communities. Flooding due to excessive rainfall after a long dry spell allows the breeding of vector mosquitoes. Human beings get infected either through the bite of infected mosquitoes or exposure to infected animal material. The dominant mode of transmission of RVF from animal to human during combined epizootics and epidemics has not been clearly understood. The main objective of this study is to assess the transmission dynamics of RVF in an area where epidemics occur. The survey was done in Gumarey, a rural village and Sogan-Godud, a sub-urban village in Ijara District, Kenya where 248 study participants were randomly selected. Venous blood was then collected from the subjects for the detection of Rift Valley Fever Virus (RVFV) antibodies by IgG Enzyme Linked Immunosorbert Assay (ELISA) testing technique. Rift Valley Fever Virus (RVFV) IgG seropositivity was used as the primary measure for RVFV exposure. Animal and non- animal exposure factors were then determined against RVFV IgG seropositivity. Subjects were clinically examined for ophthalmological disorders by an Ophthalmologist. A structured questionnaire was used to collect relevant data while observations were made on the homesteads. Mosquito sampling was done by use of Centers of Disease Control (CDC) Light Traps. A total of 12252 mosquitoes were collected. The morphological identification was confirmed by Polymerase Chain Reaction (PCR). The mosquito RVF viral infection rates were also confirmed by PCR. The Geographical Positioning System (GPS) of the households under study was recorded by Etrex GPS machine. Overall anti-RVFV seropositivity by IgG was 13%. Increased IgG seropositivity was found among older male persons and evidence of interepidemic RVFV transmission was detected. In location analysis, participants who resided from Gumarey were 4 times more at risk of RVF than those residing from Sogan Gudud (P=0.001;Odds Ratio: 3.8). Gumarey village reported more animal exposures than Sogan Gudud. The results also showed significant association between eye disease and seropositivity (P=0.003) and the most common causes of eye disease in seropositive persons were visual impairments and retinal lesions. Disposal of animal fetus, birthing, skinning, butchering and milking were highly associated with seropositivity (p=0.0001). In total 22% of the mosquito pools tested by PCR were positive for RVFV. A geospatial map showing seropositive households by clusters was constructed for both villages. The study highlights the variability in exposure and anti-RVFV seropositivity between villages and also emphasizes the impact of age, gender, location and animal husbandry in RVF transmission. The results show the potential of future outbreaks in Ijara District. Public health education could be used to prevent transmission of RVF and avert possible epidemics.
CHAPTER ONE: INTRODUCTION

1.1 Background

In recent years the emergence of infectious diseases has become a great concern especially in the case of vector-borne viral zoonoses that occasionally give rise to human epidemics such as Rift Valley Fever (RVF), Japanese Encephalitis (JE), Crimean Congo Hemorrhagic Fever (CCHF) and West Nile Virus (WNV) (Chevalier et al., 2004). Rift Valley Fever (RVF) is an acute fever causing viral disease that affects domestic animals and humans. Rift Valley Fever Virus (RVFV) was first isolated in 1931 from dead sheep by veterinary officers near Lake Naivasha, Kenya (Doubney et al., 1931). Reports of enzootic hepatitis resembling RVF among livestock, were reported earlier between 1910 – 1915 in western Kenya affecting European breeds of sheep and cattle (Kabete Vet Labs Annual Report, 1910). Rift Valley Fever is now one of the emergent vector-borne zoonosis caused by a virus belonging to the family Bunyaviridae, genus Phlebovirus (Murphy et al., 1995) and is a mosquito borne pathogen capable of causing explosive outbreaks of severe human and livestock disease throughout Africa and more recently (2000) on the Arabian peninsula (Sidwell & Smee, 2003; Zeller & Bouloy, 2000). Economically disastrous livestock epizootics often precede the detection of human illness and have been recorded since early 1900 (Kabete Vet Labs, 1910; Montgomery & Stordy, 1912; Stordy, 1913). Viruses previously restricted to some geographical areas are now affecting new countries and populations. The collapse of vector eradication programs in many countries as well as population increase and ecological modifications have all led to the spread of emerging viral diseases owing to the dissemination of vectors, especially mosquitoes with broad ecological ranges. In most cases the ecological
and epidemiological mechanisms at the origin of this emergence remains poorly known.

RVF is an emerging mosquito-borne zoonosis that is expanding in its range in Africa and the Middle East. RVF can have catastrophic economic impact on meat and dairy producers, causing high morbidity and mortality among affected livestock herds (WHO, 2000; Daubney *et al*., 1931), and invoking OIE-mandated international embargoes of livestock exports during epizootics. In animals RVF virus produces severe disease in sheep and cattle whereas goats are least susceptible. Lambs experience over 90% mortality, adult sheep about 25% and pregnant ewes usually abort (Gonzales-Scarano and Nathanson, 1996). The threat of expansion into other parts of Asia and Europe became real when RVF was first reported in Saudi Arabia in 2000 (WHO, 2007). But adequate information on transmission dynamics of RVF especially in East African outbreaks is needed for successful control operations both in animals and humans.

**1.2 Statement of Problem**

Little is known about the underlying factors involved in transmission of human RVF in communities living in outbreak prone areas. There is therefore need for close monitoring of RVFV seropositivity in humans during inter-epidemic period and also investigate the mosquito transmission potential in an area which has been hit by two human outbreaks

**1.3 Justification**

The dominant mode of transmission of RVF from animal to human during combined epizootics and epidemics has not been clearly established. RVFV re-emergence caused by floodwater *Aedes* mosquito is followed by widespread amplification in high risk
animal population. When epizootic conditions are right, secondary mosquito species will feed on viraemic animals and subsequently transmit the disease to humans, creating an epidemic. Humans can also become infected through exposure to infectious animal tissue or body fluids such as abortus, birthing fluids, milk or blood. Among pastoral nomads and other herders in semi arid regions of Africa, family members could be differentially exposed depending on traditional gender-specific duties thereby altering the risk modifying effects of age and gender. Specific types of animal exposure that are more risky and important non animal exposures have not yet been elucidated. Knowing which forms of exposure factors provide the greatest RVFV transmission risk may be useful in implementing the control measures in an endemic or epidemic situation or when targeting interventions such as animal vaccination. It is also important to know which secondary mosquito populations build up after the primary vector declines.

### 1.4 Research Questions

1. Which are the risk factors associated with RVFV seropositivity?

2. What level of association is there between RVFV seropositivity and eye disease?

3. Which mosquito species are associated with RVFV and what are their viral infection rates?

4. What happens during inter epidemic (between epidemics) period in RVFV transmission?
1.5 Null Hypothesis

There is no difference in risk exposure factors, mosquito viral infection rates and RVF virus seropositivity levels in rural and semi-urban communities living in Ijara district, Kenya.

1.6 Objectives

1.6.1 General Objective

To determine the transmission dynamics of human Rift Valley Fever Virus in Ijara District, Garissa county, Kenya

1.6.2 Specific Objectives

1. To determine human RVFV seropositivity in an area that has suffered repeated RVF outbreaks

2. To identify animal and non-animal exposure factors associated with human RVFV IgG seropositivity

3. To identify symptoms and eye disease findings associated with RVFV seropositivity

4. To establish the mosquito population species and their viral infection rates in the study area

5. To establish the distribution of sero-positive cases and mosquito distribution by Geographical Information System (GIS) spatial clustering
CHAPTER TWO: LITERATURE REVIEW

2.1 Rift Valley Fever virus

The Rift Valley Fever virus belongs to the family Bunyaviridae that include about 300 different viruses, 2 of them associated with severe hemorrhagic fevers in humans. These are the Rift Valley Fever virus and Crimean Congo Hemorrhagic Fever (CCHF), (Zeller & Bouloy; 2000 Sidwell & Smee, 2003). Although RVF has a more limited distribution than CCHF, the disease presents a greater potential of dissemination because of its more widespread vectors and therefore CCHF occurrence in humans is more infrequent (Lupi & Trying, 2003). Studies show low genetic diversity of RVF virus, but remarkable differences in mammalian pathogenesis among different virus isolates have been well documented (Erasmus and Coetzer, 1980; Peters and Anderson, 1981; Bird et al., 2006). This means that the design of the molecular detection techniques cannot be complex and hence effective control strategies could be put in place. However, from the historical epidemiological data RVF exhibits a wide spectrum of human disease. Rift Valley Fever virus is a single stranded ribonucleic acid (RNA) genome. The virus is a three segmented single stranded RNA virus and is grouped in Group V in the virus classification. The three segments are small (S), Medium (M), and large (L). The virus S segment encoded NSs and the M segment encoded NSm proteins are important virulence factors (Bird et al., 2008). It is an enveloped spherical virus of up to 120nm in diameter with short glycoprotein spikes projecting through a bilayered lipid envelop (Gerdes, 2004).
2.2 Epidemiology of RVF

Information regarding diseases in wildlife and especially those that cross the interface between wildlife, domestic animals and humans is increasingly becoming important in this era of emerging and re-emerging diseases. One of the missions of OIE is to disseminate information to member countries about pathogenic viruses such as RVF virus and avian influenza virus to institutions working with wildlife, domestic animals and public health (OIE, 2007). The simultaneous occurrence of numerous cases of abortions and disease in ruminants together with disease in humans following heavy and prolonged rainfall is characteristic of RVF. The first indication of an epidemic is frequently the abortion of sheep (Swanepoel, 2000). The inter-epizootic survival of RVF virus is believed to depend on trans-ovarial transmission of the virus in floodwater Aedes mosquitoes which makes the virus persist in mosquito eggs until the next period of heavy rainfall (Lupi & Trying, 2003). Epidemics are even more devastating for pastoral nomads and local herding economies through the loss of many adult animals, the devastation of the next crop of newborns, and the danger to locals who are dependent on milk and meat for survival during the epidemic. During large RVF outbreaks, significant numbers of human infections occur as well, leading to substantial healthcare challenges in resource-limited settings. Human infections or exposure to the virus is often occupational, either through handling of infected animals/products, mosquito bites and consumption of raw milk has also been documented (Gerdes, 2004).
2.3 Geographical distribution of RVF

RVF appears to be restricted to Africa but of late the trend seems to be changing after its introduction to Saudi Arabia. The ability of RVF virus to cross international boundaries is well documented. Rift Valley fever virus (RVFV), was originally described in Kenya, and is endemic to other countries of East Africa, Somalia, South Africa, and the Senegal River valley (CDC, 2002; Morrill et al., 1991; Schrire & Gear, 1956; Johnson et al., 1983). RVFV has been introduced repeatedly into Egypt since the 1970s, and most recently to the Arabian peninsula (Yemen and Saudi Arabia) in 2000 (CDC, 2000; El-Akkad, 1978; CDC, 2000). In 1979 RVF virus was identified for the first time outside continental Africa on the island of Madagascar (Morvan et al., 1992). Large outbreaks in cattle manifested by abortions have been reported in Zimbabwe and Zambia. Monitoring of sentinel cattle in Zambia indicates an annual emergence of the virus after the seasonal rains, with antibody prevalence varying from 3-20% (Davies et al., 1992). In Senegal the disease appears to be enzootic in livestock (Zeller et al., 1997). The introduction of the disease into Egypt (1977) is also thought to have been spread by livestock or infected mosquitoes from northern Sudan (Abdel Rahim et al., 1999). The potential for further introduction of RVF virus into previously unaffected countries via infected livestock, mosquito translocation, human travel or through intentional release illustrate the need for better understanding of the transmission dynamics so that effective control methods can be put in place.
2.4 Pathogenesis of RVF

2.4.1 Pathology of RVF virus in humans

There is need for better understanding of the pathogenesis of RVF virus for effective therapies of the disease. Due to its virulence and pathogenicity RFV virus has been assignment to biosafety level 3 laboratories (CDC, 1999) together with Dengue and Yellow Fever viruses for safety working measures. This leaves the option of the affected countries to do in dept studies on the epidemiology of the disease. Human illness can take four forms, uncomplicated, febrile, influenza like illness, hemorrhagic fever with liver involvement (jaundice and bleeding tendencies), encephalitis, and ocular involvement. The main sites of viral replication being the liver, spleen and often the brain (Gerdes, 2004). Among humans, RVF typically manifests as a symptomatic febrile disease with fever lasting 2 -6 days, myalgia, and malaise (Isaacson, 2001). In mild forms, the disease is characterized by a feverish syndrome with sudden flu like fever, muscle pain, joint pain and headache. These symptoms usually last from 4-7 days after which the immune response becomes detectable with the appearance of antibodies and the virus gradually disappear from the blood (WHO, 2007). Pathogenesis results from the spread of the virus from the source of introduction to the body and the initial replication sites which are the liver, spleen and the brain. No significant antigenic differences have been demonstrated between RVF isolates and laboratory strains from many countries but differences in pathogenesis have been demonstrated (Swanepoel et al.,1986). Molecular and biological variability among phleboviruses has been studied for RVF virus (Battles & Dalrymple, 1988; Anderson & Peters, 1988; Saluzzo et al., 1989). Although these studies show stability of the antigenic properties of the glycoprotein and nucleoprotein among
the RVF virus natural isolates, the Egyptian strain differed markedly in pathogenicity from the sub-Saharan strains when exposed to Wistar-Furth rats. The affected organs are directly damaged by the effects of the virus or by the immunopathological mechanisms (Swanepoel 2000). Laboratory features of patients suffering from RVF show leucytosis and then leucopenia may occur later. In severe cases there is thrombocytopenia. The bleeding time is prolonged because of the interference of the coagulation factors. There is also prolonged prothrombin time, elevated hepatic enzymes and elevated bilirubin (Swanepoel, 2000). Lumbar puncture reveals a slightly elevated protein, normal sugar, moderate pleocytosis with predominance of lymphocytes (Gear, 1988).

2.4.2 Clinical appearance of RVF in human infections

In humans RVF infections are usually in apparent or associated with a moderate to severe, nonfatal influenza like illness (Meegan, 1981). In a significant minority of cases it can lead to retinitis, encephalitis, hemorrhagic fever, and death, with ~ 1% overall mortality rate (CDC, 2002; Laughlin et al., 1979). Symptoms of the hemorrhagic form of the disease appear 2-4 days after the onset of the illness and begins with evidence of severe liver impairment with jaundice. Signs of hemorrhage such as vomiting blood, passing blood in feces, purpuric rash, bleeding of the nose or gums and bleeding from venipuncture sites (WHO, 2008). Retinitis and retinal vasculitis have also been reported from patients infected with RVF (Sherif et al., 1997) in more recent studies. Encephalitis may leave the patient with a residual brain damage (Isaacson, 2001). RVF is a biosafety level III agent and can cause serious human infections in laboratory workers. Laboratory
staff should be either be vaccinated, work under biosafety laboratory level III conditions, or wear respiratory protection clothes (OIE, 2000) and particular care is needed especially when working with infected animals. There is no established course of treatment for patients infected with Rift Valley Fever virus, but early diagnosis and supportive care can be lifesaving for most patients. Patients with RVF should be nursed in mosquito protected premises (Isaacson, 2001) to diminish chances of further transmission. An antiviral drug by the name ribavirin has shown promise in experimental animals but it has not been approved for human use. Additional studies have suggested that interferons, immune modulators and convalescent-phase plasma may also help in the treatment of RVF (CDC 2004). The mechanism of death in RVF hemorrhagic fever is shock (insufficient flow of blood into the body tissues) and multi-organ failure.

2.4.3 Eye involvement in RVFV infections

Earlier reports of eye involvement in RVF infections present a spectrum of diseases ranging from a blurred vision syndrome to macular exudate-like lesions, retinal detachment and retinitis (Schrire, 1951; Joubert et al., 1951). Although RVF infections have not been reported outside Africa and Arabian Peninsula, RVF retinitis have been reported in North America and Europe (Canada Diseases Weekly Report, 1979; Deutman and Klomp, 1981). In late course of the illness or early in the convalescence, patients may complain of defective vision associated with retinitis with typical “cotton wool” exudates on the macula. When only one eye is involved, as in most cases, the effect is not too disabling for most individuals can do well with the vision of one eye. When both eyes are affected the patient is severely handicapped by the loss of central vision. However most
patients have gradually resolved with the return of normal vision but in some the defect is permanent (Gear, 1998).

### 2.4.4 Prevention and control of RVF outbreaks

The transmission of RVF occurs during the course of clinical disease when the virus is present in the blood as well as other body fluids of the diseased animal (Abd El Rahim et al., 1999; Sall et al., 2002; OIE, 1996). Mosquito control activities are made difficult in the affected areas due to flooding and many roads are inaccessible (WHO, 2007). Thus RVF control programs should be directed towards avoiding the occurrence or reducing the frequency of clinical disease. In view of this, possible control strategies should include vaccination, restriction of animal movements and stamping out of the entire affected herd. Vaccines have been the principal mean used to control RVF. Efforts to prevent RVF virus infection via vaccination began shortly after the isolation of the virus in 1931 (Findlay & Daubney, 1931) and the earliest one developed (Mackenzie, 1935) relied on formalin inactivated live wild type virus. Two types of vaccines have been described for use against RVF infections. These are the inactivated and live attenuated types of vaccines. The inactivated RVF vaccine type has been used to immunize animals, laboratory workers, veterinarians and other people at risk of RVF exposure. However, its limitation is the high cost which is unaffordable by the affected communities. For the attenuated types, two vaccines are available, the Smithburn neurotropic strain and the MP12 strain (Smithburn et al., 1949; Caplen et al., 1985). The Smithburn is the only widely used vaccine for veterinary use but it has a disadvantage of causing abortions in young ewes. MP12 has a low probability of reversion (Saluzzo and Smith 1990; Vialat et
al., 1997) and has been shown to be safe in humans (Peters, 1997). Though promising in animals (Morrill et al., 1987; Morrill and McClain, 1996), trials in laboratory animals have revealed teratogenic effects (abortions) during the first trimester of pregnancy. Immunization of susceptible animal hosts remains one of the most effective means for control of RVF (Meegan and Bailey, 1989). Control of RVFV therefore implies better identification of factors involved in the emergence and its maintenance in nature. It is also necessary to understand the circulation and evolution of RVFV in the African region. Increased surveillance and awareness (Sall et al., 1998) of RVFV still remains an important option in containing the disease.

RVF Virus and other pathogens of social economic and human health importance are present in wild animals. These pathogens can be transmitted between wild animals, domestic animals and humans. Each of these groups can serve as reservoir and a source of infection for others. Many pathogens of OIE concern infect wild animals as well and this makes control thus increasing the duration of trade sanctions (OIE, 2007). It is however scientifically and ecologically clear that control and management of these pathogens can only be carried out successfully if all the three host groups; humans, domestic animals and wildlife are involved. In order to control human infections, RVF outbreaks must be contained in the animal population. The restriction of livestock movement is one of the successful methods of control because animals can carry very high levels of viraemia and therefore serve to amplify transmission. The development of safe effective RVF vaccines is also critical for future control strategies for both in animals and humans (Bird et al., 2008). The implementation of vector control in mosquitoes by way of larviciding helps in reducing mosquito populations. Prevention of
human exposure to infected animal tissues and abortus through educational campaigns contributes immensely in the prevention of non vector transmission (CDC, 2000). Also the prevention of exposure to mosquito bites by way of protective clothing, insect repellents and avoidance of outdoor activities during peak of vector activity may help in disease reduction.

2.4.5 Treatment of RVF Infections

There is no established course of treatment for patients infected with RVF virus. Treatment is supportive and may require intensive care (CDC, 1998). Early diagnosis and supportive care can be lifesaving for most patients with RVF. The cornerstone of RVF supportive therapy is judicious fluid electrolyte management. Patients with RVF should be nursed in mosquito protected premises (Isaacson, 2001). Studies in monkeys and other animals have shown promise for ribavirin an antiviral drug for future use in humans. Additional studies suggest that, interferons, immune modulators, and convalescent-phase plasma may also help in treatment of RVF patients.

2.5 Transmission Dynamics of RVF

2.5.1 Arthropod Vectors of RVF Virus

RVFV is embedded in endemic ecosystems by means of vertical transmission in certain floodwater Aedes mosquito species (WHO, 2000). Eggs of the ground pool breeding Aedes species are attached to vegetation at the edge of depressions commonly known as dambos (Figure 2.1) and when there are floods, large numbers of floodwater breeding mosquitoes emerge (Davies et al., 1985). The virus has also been isolated in 23 mosquito
species belonging to 5 genera and also from pools of *Culicoides, Simulium* and *Rhipicephalus* ticks but by far mosquitoes are the most important arthropod vectors being major vectors to livestock and humans (WHO, 2003). As a result, RVF outbreaks are strongly linked to excessive rainfall and local flooding events (Figure 2.2) which make favorable breeding conditions for various mosquitoes. RVFV transmission is therefore associated with a wide range of mosquitoes, *Aedes* species being the primary vector. Direct animal to animal contact transmission of RVF has been difficult to demonstrate. Low specificity of the RVF virus has been observed by Horsfall & Novak, (1991), who found that the virus can be transmitted to many vertebrate hosts. The ability of RVFV to move out of its range (traditional areas in East and South Africa) or even out of the continent lies on the fact that there is a large range of mosquito vectors capable of transmitting the virus from viremic ruminants to humans (Gerdes, 2004). Different viruses belonging to 30 different groups which included RVF and WNV have also been isolated from the mosquito *Aedes vexans*. There is no evidence from the field that this transmission route is significant in epizootics. Movement of infected sheep and susceptible sheep away from mosquito challenge results in complete disappearance of RVF within days (Davies & Martin, 2003). RVF is therefore a vector propagated virus disease in animals.
**Figure 2.1** A natural depression commonly known as “dambo” found in North Eastern Kenya where *Aedes* mosquitoes deposit their eggs.
Figure 2.2 A section of a flooded village in North Eastern Kenya during a period of excessive rainfall.
However studies show that infected insects are usually rare, even during peak transmission periods (Reiter, 1988) and so emphasis need to be placed on adequate sample size, even if this means large collections. The latest Kenya Rift Valley fever outbreak occurred in association with excessive rains between November 2006- April 2007 (WHO, 2007; CDC, 2007); (Figure 2.3). The previous and largest RVF outbreak in Kenya took place in a similar El Niño Southern Oscillation(ENSO)-related flooding period in 1997-1998 (Woods, 2002). Even within different climate zones, RVFV transmission may vary significantly as a function of fine-scale differences in local environment. Studies done in West Africa by Favier et al., (2006) showed that vertical transmission can sustain the virus without the involvement of wild animals. Moreover RVFV infections were confirmed among cattle in Southern Mauritania a few months before the 1987 epidemic (Saluzzo et al., 1987).

2.5.2 Mosquitoes and arboviral transmission

Because arthropod-borne viruses, or arboviruses, have the ability to spread across great distances via competent mosquito vectors, they pose significant risk to non-endemic regions (Gubler, 2002). Zoonotic arboviruses circulate in the wild in sylvatic cycles and in peridomestic cycles involving wild animals and nearby humans, often remaining undetected to human observers (LaBeaud et al., 2007; Sanders et al., 1996). Kenya has suffered multiple arbovirus outbreaks in the last two decades resulting in economic and
public health distress, including yellow fever in 1992 (Okello et al., 1993; Sanders et al., 1998), Chikungunya in 2004 (Sergon et al., 2008), and Rift Valley fever virus in 1997 (Woods, 2002) and 2006 (CDC, 2007). Though the financial and health impact of these diseases can be disastrous for affected communities, much remains unknown about the true prevalence of arboviruses in Kenya and the mosquito vectors responsible for maintenance and transmission. This study investigates the local abundance of Kenyan
mosquito vectors infected with Rift Valley Fever virus an important arboviruses in the region.

2.5.2.1 Mosquitoes in the transmission of Rift Valley Fever Virus

At least 40 different mosquito species of the genus Aedes and Culex found in East Africa have been shown to harbor RVFV, although their ability to transmit the virus varies from species to species (Turell et al., 2008); (Sang & Dunster, 2001); (Turell et al., 2007); (Wilson et al., 1994); (Worth, 1960). When selected Anopheles, Aedes and Culex mosquitoes species of North America were evaluated for potential vectors of RVF, field populations of Aedes canadiensis, Aedes cantator, Aedes excrucians, Aedes taeniorhynchus, Aedes triseriatus, Aedes bradleyi, Culex salinaris Culex tarsalis and Culex territans readily became infected after being experimentally exposed to RVF virus (Gargan et al., 1988; Turrel et al., 1988). This suggests that if RVF were to be introduced to North America, several mosquito species could be capable of transmitting the virus. In East Africa the endemic cycle involves the vertical transmission of RVFV in Aedes species (Linthicum et al., 1985). In Egypt there is no mosquito capable of transovarial transmission and the major vector is a Culex species a highly anthropophilic mosquito which breeds in polluted waters (Mellor & Leake, 2000). In East Africa the three known RVF vectors are Aedes cumminsii, Aedes circumluteolus and Aedes mcintoshi (Meegan and Bailey, 1988) which are also found in West Africa but their role in transmission has not been demonstrated (Foutenille et al., 1998). This suggests that these are the geographical vectors in Eastern and Southern Africa. During large epidemics and epizootics, the high numbers of infected individuals can greatly strain the
capacity of public health and veterinary officers in the affected countries with ill equipped control strategies. The modes of circulation for RVF virus may be grouped into two ways. One is by distant spread from one region to another and the other is by localized circulation in an enzootic/endemic area.

2.5.3 Non Vector Transmission of RVF virus

Unlike in humans, non vector transmission is not considered important factor in livestock. The risk of human to human transmission through direct contact appears also to be very low. However, in human infections mosquito transmission and contact with body fluids of infected animals through contact with abraded skin, wounds or mucus membranes or inhalation of aerosols are important modes of transmission (FAO, 2000). Thus, slaughtering of infected animals, necropsy procedures and laboratory manipulations of animal tissues carry a high risks of disease transmission. Low concentrations of RVF virus are found in milk of infected animals and a connection has been made between human infections and consumption of raw milk (Swanepoel, 2000). The milk transmission factor is significant in pastoral communities where milk is major component of their diet. There is also a high risk of infection during vaccination of animals if same needle is used in more than one animal, given that there is a high concentration of RVF virus in the blood of affected animals. Because RVF outbreaks typically occur in remote locations under extreme weather conditions, relatively little is known about the underlying health status of at-risk communities. Likewise, debate continues regarding the likely dominant mode of animal-to-human transmission during combined epizootics and epidemics.
2.5.4 Excessive Rainfall and Ecological Changes

Water (including excessive rainfall) and ecological changes have been mentioned by Wilson (1994) and Peters (1997) as two major factors triggering RVF epidemics. Water and excessive rainfall has been blamed for the Egypt, Mauritania and the East African outbreaks while the Madagascar one has been blamed due to ecological changes. Water is usually involved either through dams or irrigation for agriculture development as illustrated in Egypt (1977) and Mauritania (1987) or under excessive rainfall and flooding as observed in East Africa (1997-1998). The ecological changes in Madagascar was strongly supported by factors such as deforestation and changing agricultural practices (Peters & Linthicum, 1994). Although the two key ecological factors have been clearly identified and characterized, the circulation of the viral strains in these geographical areas are not fully understood. Presence or absence of rainfall is an important factor in the establishment of breeding sites for mosquitoes. Above average and sustained rainfall creates conditions for an outbreak of RVFV. Persistent rainfall raises the water table and floods grasslands and shallow depressions which characteristically dots plateau regions of some African states. Eggs of the ground pool breeding *Aedes* are attached to vegetation at the edge of these depressions and when they flood large numbers of floodwater mosquitoes emerge (Davies *et al.*, 1985). In addition to flooding, changing land use is another major factor in emergence of RVF outbreaks and this is reflected in the building of dams in Senegal (1987) and Egypt (1977) (Gerdes, 2004). Many authors have connected weather factors in the description of arboviral outbreaks. In an attempt to explain the severe epidemic of St Louis Encephalitis (SLE) in the USA in 1933, the US Department of Agriculture noted that the winter of 1932-1933 was the second warmest
on record at the time and the rains were twice normal (US Department of
Agriculture, 1933). RVF outbreaks are shown to follow periods of widespread and
persistent rainfall in dry land areas (Davies et al., 1985). The primary vector *Aedes*
mosquitoes breeds on temporary flood water pools found in East Africa savannah
regions. Flooding is dependent upon rainfall patterns and occurs frequently and
infrequently in the arid and semi arid zones (Davies and Martin, 2003). Correlation
between increased vector activity and heavy rainfall predictions from weather satellite
data has been investigated and used successfully in a number of important human and
animal disease studies (Bayliss et al., 1999). Predictions have been made for RVF by
assessing information about El Nino to predict a wet season. Retrospective studies made
following 1997-1998 Kenya RVF outbreak showed that rainfall measurements taken in
the catchment areas away from the floodplains by use of satellite data models could have
been used to predict excess rainfall and the amplitude of the expected river flow
(Linthicum et al., 1987). The predictive models give at least three months lead time
(Linthicum et al., 1987; 1990; 1991; 1999). These models can be used to monitor RVF
virus activity in a certain area and get prepared for combating outbreaks. Pope et al.,
(1992) extended Linthicum’s work by using Landsat TM to identify potential breeding
sites. This was done by high resolution multipolarization and the X-band data was
evaluated by discriminating between flooded and non flooded breeding sites. A system
has been developed a risk mapping system using normalized difference vegetation index
(NDVI) derived from the advanced very high resolution radiometer (AVHRR) instrument
on polar orbiting national oceanographic and administration satellites to map areas with
potential of RVF outbreaks (Anyamba et al., 2002). NDVI measures the greenness and
brownness of the vegetation and the green leaf biomass or photosynthesis capacity of a region as indicator of the amount of moisture in the soil. It offers an opportunity to identify eco climate conditions associated with disease outbreaks over large areas (Linthicum et al., 1999; USA DoD-GEIS, 2007). This system is potentially an important tool for local, national and international organizations involved in the control of RVF in animals and humans. It permits focused and timely implementation of an outbreak. Other types of remote sensing satellite warning systems are the Sea Surface Temperature (SST) of the Indian and Pacific oceans and Southern Oscillation Index (SOI) Gerdes, 2004). The SST conditions are neutral if the SST remains at 0.5°C above or below the normal of a given area of ocean. In the El-nino year of 1997-1998 the SST was 5°C above the norm and severe flooding occurred in the horn of Africa. Southern Oscillation Index (SOI) measures the difference in atmospheric pressure between Tahiti (East Pacific) and Darwin (West Pacific). Heavy rainfall is associated with negative SOI. In Africa major epidemics of RVF occur at irregular intervals of 3-15 years or longer, the frequency depending on ecological characteristics of the country or part of the country. For an epidemic to occur three factors must be present, there must be a pre existing (inter epidemic) virus circulation in the area, presence of susceptible ruminants and environmental conditions that encourage a massive build up in mosquito vector population (Geering et al., 2003). Global warming modifies temperature, rainfall, wind, and sea levels and therefore alters vector, vertebrate and virus interactions. A study done in the United States on the effects of global warming on mosquito borne arboviruses revealed movement of two mosquito species northwards to slightly cooler areas to escape escalating temperatures (Reeves et al., 1994). The potential health effects of climate
change on a dozen human illnesses were evaluated and malaria scored three, dengue two and other viral diseases scored one (WHO, 1990). This suggests a strong association between climate change and vector borne diseases. Studies done on *Aedes* mosquitoes the primary vectors of RVF in West Africa show that they have low host specificity (Fontenille *et al.*, 1998). This means they will bite different hosts which come on their way.

### 2.5.5 Occupational Related RVF Infections

Laboratory acquired infections probably due to aerosols, have long been recognized (Gonzales and Nathanson, 1996). Laboratory acquired infections with RVF through inhalation of infective aerosols have been recorded (Harding and Byer 2006) and there have been 103 laboratory acquired RVF infections resulting into four deaths published in scientific literature (Paragas and Endy 2006). Based on the above evidence the potential for spread of RVF virus in an area could appropriately be studied through the use of two types of societal contacts i.e. the animal (veterinary) work related activity and health care worker contacts. However mosquito transmission of the disease must also be considered. Among pastoral nomads and other herders in the semi-arid regions of Africa, family members could be differentially exposed depending on cultural assignment of tasks, and thereby alter the risk modifying effects of age or gender. The specifics of what types of animal exposure are most risky, and what non-animal exposures are important, have not yet been elucidated. Urban consumers of animal products are not at risk of being infected by the disease as the virus does not survive below pH 6 and the pH of meat generally falls below 6 during processing (Swanepoel & Coetzer, 1994). This is why there has been no
cases of RVF in the urban setup in Kenya and the cases found are traced back to 
transporting animals or working in slaughterhouses. Knowing which forms of exposure 
provide the greatest RVFV transmission risk may be useful for endemic or epidemic 
public health education, and for targeting interventions such as animal vaccination that 
can decrease infection or morbidity during an epidemic.

2.5.6 Human RVFV Epidemics

There are two types of secondary cycles by which RVF Virus is transmitted to humans; 
the sylvatic cycle and the urban peridomestic cycle (Figure 2.4). The sylvatic cycle 
affects people working in livestock industry and humans become infected through contact 
with animals to whom the virus has been transmitted by zoophilic mosquitoes 
(Swanepoel & Coetzer, 1994). This type of cycle is the one which triggered the 1997- 
1998 RVF epidemic in Kenya. The second type of secondary cycle is the urban 
peridomestic cycle in which humans become infected with RVF through the bites of 
anthropophilic mosquitoes. The large number of human cases in Egypt (1977) occurred 
largely as a result of this type of transmission (Gerdes, 2004). This epidemic was 
responsible for 598 deaths in Egypt. Prior to 1977, RVF was a veterinary problem and 
previous human infections have been identified in laboratory workers and people who 
live close to affected animals (Gerdes, 2004). Apart from East and South Africa, two 
other epidemics occurred in other regions following dam construction in subsequent 
major ecological modification of the environment.
In Egypt the epidemic occurred in 1977 six years after the completion of the Aswan dam (Meegan, 1979; Meegan et al., 1979) and in Mauritania, the outbreak occurred in 1987 after the completion of Diama dam (Diogoutte & Peters, 1989; Jouan et al., 1989; Lancelot et al., 1999).
2.5.7 **Association between RVF Epizootics and Epidemics**

RVF virus is one of the 15 pathogens listed by OIE as a disease with high potential for rapid spread, serious economic or public health consequences and significant impact on international trade of animals and animal products. Currently OIE regulations require surveillance and absence of RVF virus activity for 2 years following an outbreak before the resumption of disease free status and subsequent easing of import/export trade restrictions (OIE, 2007; RVF Working Group, 2004). The epidemiology of RVF consists of both epizootic and inter epizootic cycles (Meegan & Beiley 1989). Epizootics of RVF in Africa often occurs when there is unusually heavy rains. During epizootics, the virus circulates among infected arthropod vectors and mammalian hosts, particularly cattle and sheep which represents the most significant livestock amplifiers of RVFV. The inter epizootic survival of RVFV is believed to depend on transovarial transmission of the virus in floodwater *Aedes* mosquitoes (Linthicum *et al.*, 1985). The virus can persist in mosquito eggs until the next period of heavy rainfall when they hatch and yield RVFV infected mosquitoes. Depending on factors such as availability of sufficient numbers of competent mosquito vectors, presence of susceptible vertebrates, appropriate environmental conditions, infected mosquitoes have the potential to infect a relatively small number of vertebrate hosts or to initiate a widespread RVF epizootic. Statistical analysis of data collected from sentinel animals in Jazan, Saudi Arabia demonstrated a strong and positive association between RVF and a dense mosquito population (OR=4.2) (Elfadil *et al.*, 2006). Epizootics of RVF are cyclical in nature and characterized by long inter-epizootic periods. These cycles may be short (5-15years) in wetter areas or much longer (15-30years) in drier areas. During inter epizootic period the virus may be present
in forest edge habitats in an endemic cycle between Aedine mosquitoes and unknown vertebrate hosts. Alternatively there could be low level transmission in livestock associated with Aedine mosquitoes that breed in low level depressions (dambos) in savannah regions (Gerdes, 2004). RVF virus re-emergence via floodwater mosquitoes is followed by significant amplification in high-risk animal populations, with progressively greater animal prevalence. When epizootic conditions are right, additional mosquito species will feed on viremic animals and subsequently pass RVF virus to humans, potentially creating an epidemic. In human infections RVF can also become an occupational infection through exposure to infectious animal tissues or bodily fluids such as abortus, birthing fluids, milk, or blood. During epizootics when there are many infected and dying animals, contact transmission may be more important source of human infection than mosquito transmission. In outbreaks mosquito transmitted infections of humans are occasionally reported.

2.5.8 Immunoglobulins

These are specialized class of serum proteins which occur naturally in serum and are usually produced following exposure to antigens. Called also antibodies, immunoglobulins combine with only the antigen related to it or that elicited its production. The five classes of immunoglobulins are IgG, IgM, IgA IgD and IgE. Only IgG, IgM and IgA are found in all species of domestic animals and humans. IgM is the first antibody produced in the primary immune response and represents about 20% of the serum antibodies. IgG antibodies are produced in the secondary immune response. IgG and IgM are the two classes of immunoglobulins that serve as specific anti toxins against
micro-organisms and play a major role in defense against most infectious diseases (McGraw, 2002).

IgG is an immunoglobulin built of two heavy chains and two light chains. It is the most abundant immunoglobulin and is approximately equally distributed in blood and tissue liquids. It is the only immunoglobulin which can pass through the placenta, thereby providing protection to the fetus in the first few weeks of life before its immune system is developed. IgG antibodies can bind to many kinds of pathogens including viruses, bacteria and fungi and protects the body against them by complement fixation, phagocytosis and neutralization of their toxins. Therefore IgG antibodies are considered to be long term or memory antibodies. For example, once we have been exposed to the measles virus our body fights off the original infection over time and further re infection is prevented because the B lymphocytes now have a memory of the original measles virus antigen. From then on your bloodstream contains traces of anti IgG antibodies which serves as a protection against re infection. IgG antibodies do not themselves kill invaders but they attach or “tag” the virus so as to give notice to other circulating cells of the immune system known as the macrophages (Ago, 1998). In RVF surveillance in domestic animals and humans IgG ELISA is used.

2.5.9 IgG ELISA in detection of anti-RVFV

In Rift Valley Fever infections, IgM and IgG antibodies are detectable in serum by means of Enzyme Linked Immunosorbent Assay (ELISA) from about day 6 of the infection (Burt et al., 1994; Drosten et al., 2002). IgM antibodies becomes detectable for up to 4 months while IgG antibodies decline but continues to be detectable for up to 5 years
(Burt et al., 1993). But RVFV has a lifelong immunoglobin G (IgG) and neutralization antibody response in humans (RVF Working Group, 2004). IgG RVF ELISA therefore detects past infection and is used as a diagnostic tool in serological surveys. Compared with virus neutralization and hemagglutination inhibition tests, the IgG sandwich ELISA was found to be more sensitive in detection of the earliest immunological response to infection or vaccination with RVF virus. Its sensitivity and specificity derived from field data sets ranged in different ruminant species from 99.5% to 100% and 99.1% to 99.9% respectively (CDC, 1988). Evidence of prior RVFV infection can be tested via ELISA for the presence of anti-RVFV IgG (Niklasson et al., 1983; 1984). Earlier studies have shown that RVF seroprevalence in Kenyan populations can be as high as 32% in high-risk areas during epidemics (Woods, 2002). During interepidemic periods, observed community anti-RVFV seroprevalence rates can range from 1% - 19% among different settings within Kenya (LaBeaud et al., 2007). In animals, IgG and IgM sandwich ELISA have been found to be useful in serological surveillance. Poweska et al., (2003) reported the sensitivity of capture ELISA for detecting IgM antibodies in domestic ruminants against RVF virus to be 100% from 5-42 days after infection. Neutralization Tests are highly specific and picks the positive cases at the earliest response but they have one disadvantage in that live viruses are used for neutralization and so not recommended for use outside endemic areas (Gerdes, 2004). The principle underlying IgG ELISA is explained by the following steps. Diluted patients serum is added to the wells coated with purified RVF virus antigen. IgG specific antibody if present binds to the antigen. All unbound materials are washed away and the enzyme conjugate is added to bind the antibody-antigen complex. Excess conjugate is washed away and substrate is added. The
plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount the IgG specific antibody present in the samples.

2.6 Mosquitoes and RVFV

2.6.1 Distribution of mosquitoes

Mosquitoes are found in almost all over the world except in oceans and some few islands. Expanding of international trade has increased the chances of introducing new mosquito species in other areas. About 3500 mosquitoes belong to the family Culicidae and the most important genera are Anopheles, Culex, Mansonia and Aedes. Mosquitoes were the first insects to be associated with transmission of diseases when in 1877, Patrick Manson linked them to the transmission of Bancroftian filariasis. Since then they have been known to be vectors of viral and bacterial diseases but are mostly known for malaria transmission the most important tropical disease (Goddard, 2003). However, with the emerging of RVF virus and other arboviral infections as important human pathogens, the role of mosquitoes is becoming increasingly important. Adult of the genus Anopheles rest their body parts (head, proboscis, thorax and abdomen) in a straight line and the wing veins are covered with a characteristic pattern of dark and pale scales. Adult Culex rests their bodies nearly parallel to the surface and the sternites and tergites are densely covered with scales. For detailed morphological identification, dichotomous keys have been developed for various geographical regions. In East Africa mosquito keys for the sub Saharan region, formerly known as the Ethiopian region are used. The distribution of
mosquitoes in RVF virus epidemic prone areas is important in assessing the morbidity of the disease.

2.6.2 Biology of primary and secondary RVFV vector mosquitoes

In RVFV and other arboviral transmissions, the primary vector species are considered to be the main factor driving the outbreaks before the secondary vector (bridge vector) takes over. Secondary vectors do not become infected by the virus before the infection rate has reached high levels in the population of primary vector species. Understanding life cycles of target mosquito species is a key step in developing an effective control strategy. Information on flight patterns and periods of peak mosquito activity helps in the control of adult mosquitoes by knowing when or where to spray with an insecticide. Knowing the larval biology also helps in encountering the mosquitoes in their breeding sites and observing their developmental stages which can guide in larviciding activities.

Mosquitoes can be divided into three major breeding groups; permanent water breeders, floodwater breeders and artificial container/tree hole breeders. *Anopheles* and *Culex* mosquitoes select permanent water bodies such as swamps, ponds, lakes and ditches that do not usually dry up (Goddard, 2003). Floodwater mosquitoes lay eggs on the ground in low areas so that hatching takes place in times of flooding. The *Aedes* group of mosquitoes belong to both floodwater and artificial container/tree hole breeders. *Aedes vexans* is an example of a floodwater mosquito while *Aedes aegypti* and *Aedes albopictus* are both examples of artificial container/tree hole breeders. When *Aedes* eggs are first laid they may die if they become too dry, but if the embryo develops then the eggs can withstand dry conditions for long periods of time. This phenomenon has allowed *Aedes*
mosquitoes to use temporary water bodies for breeding such as artificial containers, tree holes or periodically flooded marshes. In this way, *Aedes* mosquito has been taken to many parts of the world as dry eggs in tires, water cans or other artificial containers. The eggs hatch into larvae which are air breathers and must therefore come up to the surface periodically for oxygen. Mosquito larvae undergo three molts (shedding of outer skin). During each molt the size of the head increases by about 50%. The period between molts is called an instar. After the fourth instar the larvae will molt into a pupae which further develops to an adult. Floodwater areas are temporary water sites and therefore do not contain natural predators. These sites will breed several species of floodwater mosquitoes if water remains for a week or longer. In RVF virus transmission *Aedes* mosquito is considered to be the primary vector in the transmission cycle while *Culex* and *Anopheles* are the secondary species.

2.6.3 Collection of mosquitoes

The CDC light trap developed by Centers of Disease Control is the most widely used portable trap. Light trap runs on a six volt lantern battery and a smaller versions runs on a “D” cell battery. The principle behind light traps as a sampling device is that mosquitoes are attracted by the small light at the top of the trap and are then sucked into a net at the bottom the trap by a fan. The traps are usually set out and turned on at dusk and picked up at dawn. Only selected species of mosquitoes are attracted by light traps (Goddard, 2003). All the four genera of mosquitoes (*Anopheles, Culex, Mansonia, Aedes*) can be collected by use of light traps and are associated with RVFV transmission. In this study CDC light traps were used for trapping mosquitoes.
2.6.4 Molecular identification of mosquitoes

Information about viral infection in mosquitoes is essential in understanding the disease epidemics especially when knowledge of the range of wild reservoirs is limited. Information of wild caught mosquitoes can furnish critical details about the disease epidemiology. To prepare mosquito samples for RT PCR amplification a suitable RNA extraction procedure is required to isolate viral RNA from pools of mosquitoes without degradation of the RNA or inhibition of PCR amplification. In this study the RNA extraction from the mosquito legs was used performed by Spin Technology (Qiagen).

2.6.5 Internal Transcribed Spacer 2(ITS2) region of mosquito DNA

In the wake of re emergence of mosquito diseases, studies at the molecular level have gained momentum during the last decade (Amit et al., 2007). In the past, mosquito taxonomy has been achieved mostly by using morphologic characteristics. However molecular methods for species identification have received great attention in recent years. Behavioral variations and vectorial capacity of various mosquito species groups or complexes constitute the major reasons for need of accurate and precise identification. Two internal transcribed spacers (ITS) occur in eukaryotic organisms namely ITS1and ITS2. ITS1 is flanked by the 18s gene and 5.8s gene while ITS2 is flanked by 5.8s and 28s genes (Perry, 1976).The ITS2 region is considered more conservative than the ITS1 owing to its numerous presence of tandem repeats. The ITS2 region is now one of the most widely sequenced DNA region in Anopheles, Culex and Aedes mosquitoes (Wesson et al., 1992). It is among the highly considered molecular markers for mosquito taxonomy (Marrelli et al., 2006) In this study the internal transcribed spacer 2 (ITS2) was used as a confirmation test in the identification of mosquitoes which included Anopheles, Culex,
Mansonia and *Aedes*. A study by Severini *et al.*, (1996) on two closely related species *Culex pipiens* and *C. quinquefasciatus* by ITS2 showed a 97% identity. ITS2 marker has been extensively used for the taxonomy of *Anopheles maculipennis* and *A. quadrimaculatus* complexes (Cornel *et al.*, 1996). Thus, the ITS2 assay makes use of nucleotide difference in Internal Transcribed Spacer 2 rDNA sequences to generate PCR products of specific length for each of the species.

### 2.6.6 Pool Screening of mosquitoes

After identification is complete, the next step is to know whether the mosquitoes are infected with RVFV or not. The goal of pool screening is to estimate the proportion of infected mosquitoes more efficiently by reducing the number of tests required compared to individual screening. Pool screening is a common procedure for estimating the level of mosquito infections by grouping mosquito samples into pools for viral testing using ELISA or DNA based screening technique. Minimum Infection Rates (MIRs) is the method used to measure levels of mosquito infections. MIR is calculated as the ratio of number of positive pools to the total number of tested mosquitoes assuming that there is only one infected individual in each positive pool (Gu and Novak, 2004). The test gets its popularity due to the fact it is assumed that the arboviral transmission is generally low, usually less than 0.1%. In this study mosquito legs DNA extract were pooled according to site of collection and species.
2.6.7 Polymerase Chain Reaction (PCR) amplifications

RVF is a mosquito borne disease and is an RNA virus. When the target nucleic acid is RNA it must first be converted into complementary DNA (cDNA) by enzyme reverse transcriptase (RT) before subsequent amplifications. But these two steps can be combined into one single tube reaction (Harris et al., 1998). Once amplification is complete the products must be detected and visualized. There are many ways to achieve this objective, from simple gel electrophoresis to more complicated product capture technique using hybridization strategies or labeled primers coupled with colorimetric detection systems. Commonly used is the agarose gel electrophoresis and visualization of the ethidium bromide stained DNA to detect the presence or absence of expected fragment and the size of the product (Harris et al., 1998). In this study Sybr Gold was used to stain the 3% agarose gel.

2.7 Geographic Positioning System

2.7.1 Geographic Information System (GIS) in RVF surveillance

The idea that place and location can influence health is a very old and familiar concept in medicine and dates back at the time of Hippocrates (460-370BC) when he observed that certain diseases seem to occur in some places but not in others. Since 1990 GIS is increasingly being used in various public health settings (Kaiser et al., 2003). The dramatic increase in international travel and trade in recent years has provided opportunities for diseases to spread across geographical and international boundaries at exponential rates. The past few years have seen a rapid growth in the number and capabilities of earth observation satellites being used for environmental monitoring and
hazard assessment (Wedge, 1994). However, despite the increasing availability of such images the products of remote sensing remains largely underutilized particularly in developing countries. Lower spatial resolution images from orbiting satellites have been used in detecting breeding sites of Rift Valley Fever vectors (Linthicum et al., 1990). Satellite image data must be given in a way that it can be used in decision making and this can be achieved by Geographical Information System (GIS) which is able to read, process, analyze and present spatially related data for use in environment and resource management purposes (Connor et al., 1998). Remote sensing imagery when combined with GIS spatial analysis techniques can play an important role in existing vector surveillance and control programs at local and regional levels (Washino and Wood, 2009). These combined techniques can provide scientists with new tools to influence the patterns of RVFV and other vector-borne diseases. Emerging diseases are therefore threatening people worldwide and this highlights the need for active surveillance system. Uncertainty is a major feature of human and animal health decision making and in recent years there is increasing attention to methods that detect measures to reduce uncertainty (Eastman, 2001). This is more so in developing tropical countries where there are ill equipped technologies. Recent advances in GIS and remote sensing has been applied in wide range of studies on the spatial distribution of tropical diseases and the factors that influence disease pattern. GIS has been used to improve disease control and surveillance activities (Clement et al., 2006). A successful disease surveillance will require appropriate tools, accurate synthesis of data and most important timely dissemination of resulting information to health workers for implementation. By use of GIS, virtually any data can be placed on a digital map then visualized, compared, measured and analyzed.
GIS can give a diversity of information types that can be analyzed ranging from population demographics to health statistics, epidemiology, transport network, flood protection zones, animal migration routes, crime patterns, historical battlefields, disaster destruction areas and many more (Davies, 2003). GIS has emerged as a powerful tool in enhancing decision making at all levels of disease surveillance. Incorporating GIS in any disease surveillance system enhances and provides comprehensive information system that is more efficient and less cumbersome (ESRI, 2005). Therefore to bring disease surveillance system to the next level, public health providers must take advantage of such advances in information technology. These systems must be managed in a way that it disseminates the GIS information regarding RVF virus morbidity and mortality.

2.7.2 GIS in disease and vector spatial clustering

Mapping and spatial analysis, reveal trends, dependencies and inter relationships that would not be put on a tabular format. And because preventing disease is the ultimate goal of disease surveillance, GIS can be used in mapping locations such as in disease occurrence, vectors and hosts (Wiafe and Davenhall, 2005). The use of aerial photography and manual interpretation technique to identify and map disease vector habitats dates from 1949 when Andy published a study in distribution of *Scrub Typhus* in South Eastern Asia. He determined the association between hyperendemicity and a vegetation type. Mosquito vectors require an aquatic environment for larval development. The aquatic environments are frequently described in terms of vegetation and water. In many cases it is possible to reduce the prevalence of a vector borne disease by locating and treating the larval habitat (Ross, 1911). In 1973 NASA scientists used visual
interpretation of color and color-infrared photography to map vegetation types associated with *Aedes sollicitans* breeding habitats near New Orleans, Louisiana and in 1979, Wagner produced a detailed map of *Aedes* breeding sites in a newly formed mosquito abatement district in Michigan (USA). But unfortunately current techniques for locating larval habitats and monitoring mosquito populations are labour intensive, time consuming and impractical over large areas. The use of GIS mapping in RVF monitoring of vector bionomics can help in containing RVF outbreaks. This can be done by use of GIS spatial clustering statistics for identifying hot spots of RVF transmission. Clustering quantifies clusters around individual locations that comprise the study geography (Ord and Getis 1995). Jeffrey *et al* (2002) used spatial statistics to determine the pattern of mosquito vectors of the Ross River virus and Barmah Forest virus on Russel island, Australia. They found significant clustering on the Southern end of Russel Island. A positive spatial correlation indicates a map pattern where geographical features of similar value tend to cluster on a map, whereas a negative spatial correlation indicates a pattern in which geographical units of similar values scatter throughout the map,
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Area

3.1.1 Geographical position

The study was carried out in Gumarey and Sogan Godud villages of Masalani location in Ijara District (Figure 3.1) of North Eastern Kenya. The district borders Garissa to the North, Tana River District to the West, Lamu District to the South and Republic of Somalia to the East.

About one quarter of the district is covered by the Boni Forest which is an indigenous open canopy forest. A section of the forest known as the Boni National Reserve is managed by Kenya Wildlife Services (KWS) as a protected conservation area. Topographically Ijara District is situated along the Tana River plain and the ethnic community are the Somalis. The district is prone to flooding during times of seasonal or heavy upland rainfall due to its topographic location.

3.1.2 Climate

Rainfall occurs in a bimodal pattern with two peaks in March-May and October-December. Temperature ranges from 15°C to 38°C. The number of settled markets is dispersed throughout the district and the rural population is principally composed of nomadic herdsmen. There are three economic livelihood zones namely the pastoral zone (predominant), agro-pastoral (Southern part) and the casual labour in Sogan Godud area. Over 80% of the land is under livestock production and it contributes to 60% of the household income. Annual rainfall varies from 250mm to 500mm but varies considerably from year to year.
Figure 3.1: Map showing Ijara District and the study area
3.1.3 Administrative and health facilities

Administratively, the district is divided into four divisions namely Masalani with a population of 13358, Ijara with 17236, Hulugho with 20831 and Sangailu with 11217 people. The district is served by a District Hospital, 2 Health Centres and 6 Dispensaries. The district has an area of 11332km² and a population of 62,642 people according to the 1999 census (Kenya Population Census 1999). Ijara district was an active area of RVF in 1997-1998 and also in 2006-2007 outbreaks. The 2006-2007 short rains were above normal across the district and led to flooding especially along the Tana River. The road network was severely damaged and the district was cut off from the rest of the country for almost three months. In December 2006 RVF hit the district affecting livestock and humans and consequently livestock markets were closed and ban imposed on animal slaughter (GOK, 2007).

3.1.4 Study population

Our study is based on a location-stratified household-based cluster sampling of the human populations residing in two areas near Masalani town, Ijara District situated in a semi-arid region of Northeastern Province, Kenya. The study was performed in March and April of 2006, approximately 8.5 years after the last RVF outbreak of 1997-1998, and well before the flooding events of fall 2006, that were associated with the most recent RVF epizootic/epidemic. Due to the absence of mosquitoes during the dry season, sampling was done in the month of December 2006 and January 2007 when heavy rains were on. Based on the objectives of our study, the balanced sampling frame for selection of the planned 248 participants was divided between a rural village area, Gumarey
(centered at 1°, 40’, 12” S, 40°, 10’, 48”E), and a town area, Sogan-Godud (centered at 1°, 41’, 24” S, 40°, 10’, 12”E). Both are sub-locations defined within the Kenya Census (1999) and are located within 500 m of each other and within 10 km of the Tana River, which is prone to flooding (Annex 7) during periods of excessive rainfall. Flatness of the local terrain, combined with poor drainage makes the area a prime environment for RVFV transmission during flooding events, as evidenced by ongoing RVF outbreaks. Sogan-Godud (Figure 3.2a) is a semi-urban centre with more permanent tin-roofed dwellings and stores. Gumarey (Figure 3.2b) has a largely semi-nomadic pastoralist population and local homes consist of traditional grass huts. The study population consisted of a locally representative ethnic mix of >99% Somali or Bantu and <1% Indian or other Asian.

Adults recruited in the study signed the informed consent form before joining the study. Parents of participating children below the age of 7 years signed the assent form for their children. Children below 1 year and those who had resided in the area for less than 2 years were excluded from the study. Households not willing to participate in the study were excluded.
Figure 3.2A & 3.2B: Sogan Gudud village (above) and Gumarey (below) during the dry season (2006)
3.1.5 Economic activities in Ijara District

Livestock production contributes directly or indirectly to the livelihood of over 90% of the population of the district. The livestock kept are mainly Boran cattle, Somali black head sheep and crosses of the Galla goat. Camels are not commonly kept in Ijara district due to tsetse flies especially around the Boni forest area. Livestock diseases such as RVF, Foot and Mouth and poor marketing infrastructure for livestock markets and its products are some of the main challenges facing this subsector (GOK: Ministry of Livestock Development, 2008). Slaughtering of animals (Annex 8) for local meat consumption is done in abattoirs owned by the local county council. In most of the times sheep and goats are preferred for slaughtering. The movement of animals (Annex 9) is a common phenomenon especially during the dry season among herdsmen. The herdsmen move the animals in search of greener pastures to the southern side of the district. However during the wet season animal movement is not an important feature.

3.2 Sampling Technique

A total of 248 survey participants were selected by randomized cluster sampling of households in the two designated subsections of Masalani area. Participating households were sampled using a probability proportionate to size (PPS) approach. Non-participating households were substituted for by using additional, randomly-selected households. Children aged <1 year and those residing in the area < 2 years were excluded. All adult participants provided informed consent (Annex 1). Parents provided informed consent (Annex 2) for participating children and children >7 years provided individual assent.
3.2.1 Sample size determination

The sample size was determined in accordance with WHO (2001) thus:

\[ n = Z^2 (p) (100-p) \times \text{DEFF/d}^2 \]

Where

- \( n \) = Sample size
- \( Z \) = standard normal deviate (1.96) which corresponds to 95% confidence interval.
- \( p \) = Proportion of target population estimated to have particular characteristics.
- \( d \) = Acceptable difference of estimate from the true value
- \( \text{DEFF} \) = Design effect = 1

The proportion of Rift Valley Fever IgG antibody prevalence in the general population in Kenya is estimated to be from 20-30%. In this study, a prevalence of 20 % was assumed. Thus \( n=1.96^2 \times 20 \times 80 \times 1/5^2 = 245 \), and therefore the minimum sample size required was 245 study subjects.

3.2.2 Study recruitment

Study recruitment was begun after consultation and approval by local administrators, health officials and religious leaders. A demographic census (Annex 10) to determine the current local population and its distribution was done in the two study villages. A total of 66 households in both locations were recruited. In each homestead the GIS co-ordinates were taken. These included 30 households from Gumarey and 36 households from Sogan Gudud.
3.3 RVFV Seropositivity by IgG ELISA Method

3.3.1 Venous blood sample

Blood collection was done in the laboratory. Study participants aged ≥5 years gave 5 ml of blood and those aged <5 years gave 1ml of blood. The blood samples were allowed to clot at room temperature and serum was separated and transferred into 1.5ml vials. The serums were then refrigerated at 2-4 °C in the field laboratory before transporting them to Nairobi for testing.

3.3.2 Determination of RVFV IgG antibodies

The primary measure of RVF virus exposure was seropositivity measured by anti-RVFV IgG detection via enzyme linked immunosorbent assay (ELISA) of subject serum. Specimens were screened for the presence of anti-RVF IgG via ELISA using lysates of VERO cells infected with MP-12 strain (vaccine strain) of RVFV as the test antigen, and lysates of mock infected cells as the internal control antigen. This Elisa has been established and validated in previous studies by Niklasson et al., (1983) and LaBeaud et al., 2007). Serum samples diluted 1:100 were read at 405nm and those scoring an optical density (OD) value greater than the mean of the negative controls were deemed positive. Pooled RVF positive sera was used as the positive control and pooled negative North American sera was used as the negative control. Serological screening was done at the Division of Vector-Borne Diseases in Nairobi and confirmed at the Case Western Reserve University with excellent correlation of results. Confirmatory plaque reduction neutralization (PRN) test was performed at the University of Texas Medical School to rule out any false positive results which may occur due to Elisa cross reaction with related viruses. All positive samples (N=33) and the negative samples (N=33) matched
with the plaque reduction neutralization (PRN) as previously described by Meadors et al., (1986). All positive ELISA samples had plaque reduction neutralization (PRN) titers of 1:80 and above, the majority having titers of 1:320.

3.4 Past Animal and Non-animal Exposure Factors

3.4.1 Animal exposure factors

Study participants were subjected to a structured interview (Annex 3 & 4) regarding housing and animal exposure. Participants gave information on whether they had come into contact with sheep, cow, goat or camel. They were also asked whether they had sheltered, killed, butchered, skinned, cooked, or milked the animals they had come into contact with. They also gave information on whether they had drunk raw milk from them, helped in birthing or disposed off an animal fetus.

3.4.2 Non animal exposure factors

Participants were asked to give information on the status of their homes during times of excessive flooding, mosquito net use, fire use or any other mosquito control method they may have been using. History of recent mosquito bites, personal illness, an ill family member or contact with dead human body was also recorded. Participants gave the dates of the contacts or the exposure.
3.5 Past RVFV Related Symptoms and Eye Disease Examination

3.5.1 Past RVFV related symptoms

Information on visual function and recent or remote RVF-related symptoms (Annex 5 and Annex 6) was filled in the structured questionnaire. Participants gave information on common RVF related symptoms which included fever, malaise, myalgia, chills, backache, eye pain, headache, photophobia, nausea, vomiting, meningismus, poor vision, hematemesis, confusion, stupor and coma. The questionnaire process was facilitated by the accompanying parents who served as proxies for children participants.

3.5.2 Eye examination

A complete physical examination was performed and with the help of an ophthalmologist, a vision test and indirect ophthalmoscope examination (Annex 11) to check for signs of current or previous retinal inflammation. Information on ophthalmologic examination included visual acuity, retinal hemorrhage, retinal scarring, optic atrophy, retinal vasculitis and retinal maculopathy.

3.6 Viral Infection Rates

3.6.1 Mosquito sampling

To evaluate the temporal profile of vector mosquitoes in Ijara, North Eastern Kenya, trapping was performed during the long rains (December 2006-January 2007). Mosquitoes collected between December 2006 and January 2007 were trapped during an epizootic/epidemic of Rift Valley Fever. Households in the regions were randomly selected from previously prepared census lists, although restricted to only those homes
where cows, goats, and/or sheep were housed alongside human habitats. CDC light traps (Annex 12) were located next to these animal structures and were set for 12 hours between 6pm and 6am.

Mosquito sampling was conducted in Gumarey and Sogan Godud villages of Ijara District, where human surveillance had taken place eight months prior to the RVF outbreak. Traps were located in the rural village of Gumarey (centered at 1° 40´12´´S, 40°10´48´´E) and the town of Sogan-Godud (centered at 1°41´24´´S, 40°10´12´´E). The human population residing in Gumarey consists of semi-nomadic herders who maintain close proximity to their livestock and live in traditional grass huts. Sogan-Godud is a more urban village with a marketplace and contains a greater proportion of tin-roofed permanent dwellings. The centroids of these two locations are 5 km apart and the borders are located within 500 m of each other. Both locations are within 10 km of the Tana River, which is prone to flooding during periods of excessive rainfall.

3.6.2 Mosquito morphological identifications

All mosquitoes trapped in the light traps were killed by freezing for one hour and then put on labeled petri-dishes. They were sorted out (Annex 13) under a dissecting microscope and identified to species by use of morphological keys by Edwards (1941) for culicines and Gillies & Coetzee (1987) for anophelines. Mosquitoes were identified to species level if possible but in cases where minor details of morphologically similar species group were not easily visible, then the specimens were only identified to genus. For confirmation of morphological identifications, PCR method was applied on mosquito leg
which was removed by means of a forceps and immediately placed into labeled vials (1.5ul) containing RNA later (Ambion) solution (250ul) for preservation of RNA.

3.6.3 Polymerase Chain Reaction (PCR) Method for mosquito identifications

After the initial mosquito trapping was done a minimum sample size of 920 single leg specimens were preserved in RNAlater (Ambion, Austin, TX) were transported to Case Western Reserve University for processing. DNA and RNA were extracted from mosquito legs using the RNeasy column purification kit (Qiagen, Valencia, CA) with the following modifications. Briefly, each mosquito leg was placed into a microcentrifuge tube containing 150μl of RLT buffer and finely ground with a disposable RNase/DNase free pestle. After homogenization, samples were processed according to established protocol through either individual Qiagen Columns or RNeasy 96 well plates, washed and eluted in RNase-free water. The DNase step was omitted so that both DNA and RNA could be collected from samples. Ten microliters of each individual RNA sample was combined in pools less than or equal to 12 mosquitoes (median 10 based on household location for DNA synthesis and PCR or quantitative RT-PCR testing).

3.6.4 RNA extraction method

Mosquito legs were removed from the RNA later (Ambion) solution by use of a fine forceps and placed into labeled vials containing RLT buffer (150μl). Between each tube the forceps was dipped in bleach and then dried with ethanol swabs. The legs were grinded using a pestle to disrupt the cells. For each tube a new pestle was used to avoid contamination. RNA was extracted from the mosquito legs by use of RNeasy extraction
kit (Spin Technology) using the animal cell extraction method (Qiagen, Valencia, California). PCR was then performed on the eluted RNA.

3.6.5 Polymerase Chain Reaction (PCR) amplification

For each plate a 10xPCR Buffer (300µl), 2.5 mM dNTPs (240µl), Mac taq enzyme (72µl) and sterile DH2O (2244µl) was used. Amplification was performed by use of a single set of genus specific ITS2 primers, ITS2A (5’-TGTGAACTGCAGGACACAT-3’) and ITS2B (5’-TATGGCTTAAATTTCAGGGGT-3’). Amplification reactions were performed in the PCR mixtures with PTC – 225 Thermal Cycler (MJ Research, Watertown, Mass). A reaction volume of 25ul was used in each well of the plate. Two step PCR amplification conditions were 95 ºc for 2 min, 95 ºC for 30 sec (35 cycles), 55 ºC for 30 sec (35 cycles), 72 ºC for 1min (35 cycles) and 72 ºC for 4 min. To evaluate the overall amplification efficiency, PCR products were electrophoresed on 2% Agarose gel (Amresco, Solon, OH). The gels were stained for 30 minutes, with SYBR Gold (Molecular probes, Eugene, OR) diluted 1:10000 in 1xTBE buffer and the DNA products were visualized on a STORM 860 fluorescence scanner with image Quant (Version 5.2) software (Molecular Dynamics, Sunnyvale, California).

To verify the quality of the RNA and the integrity of the DNA products following reverse transcription, mosquito 18S ribosomal RNA primers were designed to amplify within a region conserved among many Culicidae species (Hoffmann, 2004). These mosquito primers were designed against the 18S rRNA gene sequences for both Aedes (GenBank: AB085210) and Culex (GenBank: U48385) mosquitoes to amplify an optimally-sized
product (124bp) for qRT-PCR. RVFV primers, which amplify a conserved region of the L segment (90bp), were employed as described by Bird et al., (2007).

RVFV standard was generated by amplifying RVFV vaccine strain rMP-12 in Vero E6 cells for 72 hours, and then extracting viral RNA from supernatant and cell lysate using the PureLink Total RNA Purification System (Invitrogen, Carlsbad, CA). RVFV positive controls were generated using the primers listed in Table 3.15 and cloned using the pCR®8/GW/TOPO® TA kit (Invitrogen, Carlsbad, CA). All inserts were verified by sequencing of the plasmids.

**Table 3.1:** Primer pairs, sequences, product size, and GenBank accession number

<table>
<thead>
<tr>
<th>Target</th>
<th>Forward</th>
<th>Reverse</th>
<th>Product size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mosquito 18s</td>
<td>GATCAAGTGAGGGCAAGTC</td>
<td>AAGGAGTAGCACCCGTGTTG</td>
<td>124</td>
</tr>
<tr>
<td>RVFV</td>
<td>TGAAAATTCTCTGAGACACATGG</td>
<td>ACTTCTTGCATCATCTGTG</td>
<td>90</td>
</tr>
</tbody>
</table>

### 3.6.6 PCR on pooled mosquito samples

Two-step reverse transcription RT-PCR was performed on all pooled samples. First-step total DNA synthesis was performed on RNA extracted from mosquito leg tissue using random hexamers. Given the minute quantity of sample, the maximum volume of each RNA pool was used in this reaction (10μl), and combined with 1μl random hexamers, 1μl 10mM dNTP mix and 2μl sterile nuclease-free water. The reaction was incubated at 65°C for 5 minutes, chilled on ice, and combined with 4μl 5X First-Strand Buffer, 1 μl 0.1M
DTT, 1μl RNase inhibitor and 0.5μl SuperScript III Reverse Transcriptase (Invitrogen, Carlsbad, CA). The final reaction mix was held at 25°C for 10 minutes, incubated at 50°C for 50 minutes, and heat-inactivated at 70°C for 15 minutes.

After DNA synthesis, 1μl of total DNA was added to the RT-PCR mixture containing 0.25μl forward primer and 0.25μl reverse primer (18S), 12ul FastStart Universal SYBR Green Master mix (Roche, Indianapolis, IN), and 12μl sterile, nuclease-free water. The RT-PCR was carried out in an Applied Biosystems 7300 instrument with a heating cycle of 50°C for 2 minutes and 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds, 60°C for 1 minute, 95°C for 15 seconds and an additional dissociation step of 60°C for 1 minute. All samples which registered a positive cycling threshold (C_T) value and had a lower C_T value than negative controls, consistent with the standards, were considered positive samples for their respective targets. All pools were further PCR tested for RVFV using 2ul DNA, 0.5ul each forward/reverse RVFV primers, 10.5ul sterile, nuclease free water and 12.5ul JumpStart ReadyMix Taq (Sigma-Aldrich, St. Louis, MO). PCR cycling parameters were as follows: 94°C for 5 minutes, with 30 cycles of 95°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute, and a ten minute 72°C extension. PCR products were run on a 2% agarose gel with SYBR Safe (Invitrogen, Carlsbad, CA) for band visualization. An initial sampling of RVFV PCR products were cloned using previously described methods and sent for confirmatory sequencing (Agencourt Bioscience, Beverly, MA).
3.7 Geographical Positioning System (GPS)

3.7.1 GIS Mapping by use of Etrex GPS machine

The GIS coordinates for the 66 households under study in Gumarey and Sogan Godud were recorded by use of GPS machine. In this study the Geographical Positioning System (GPS) coordinates were taken by use of Etrex (Garmin) device and the information was analyzed by GIS program from Environment and Social Research Institute (ESRI) (Redland, California). Each household was identified by a longitude and latitude coordinates which could be reached by Geographical Information System (GIS) navigation system. To that effect GIS software Arcview was used to define around each point in order to determine a geographical area within which the virus could be circulating.

3.8 Ethical Consideration

This study was carried out under a human research protocol approved by the Ethical Review Committee of Kenya Medical Research Institute (KEMRI), then registered as a Clinical Trial NCT00287014 and available from www.clinicaltrial.gov.website. It was also approved by the Human Investigation Review Board of University Hospitals of Cleveland, USA. The purpose and benefits of the study were explained to the community through public barazas and they were given an opportunity to ask questions. A village wide consent was obtained and the selected households were approached through trained field workers. Since the study was done in a non English speaking community, consent forms were translated in to Kiswahili.
3.9 Data Analysis

Following initial bivariate analysis of RVF seropositivity outcomes, based on chi-square or Fisher Exact test, predictor variables were further tested for significance with the use of multivariable logistic regression. Beginning with 200 randomly selected subjects, the most promising predictors were used to create a set of nested models via stepwise procedure that resulted in models with improved predictive value, as determined by the Aikake Information Criterion (AIC). Generalized logistic models were also run for each location subset that is Gumarey for rural and Sogan-Gudud for the sub-urban area. All models were validated using the subset of subjects not included in initial model development and parameter estimation. All bivariate analysis and logistic modeling was performed using R software, version 2.3.1. Univariate analysis was performed using the Odds Ratio (OR) statistic to determine the strength of epidemiological association between RVF and each risk factor (Martin et al., 1987).

Mosquitoes were pooled into laboratory constructed pools and minimum infection rates (MIR) were estimated by maximum likelihood estimates (MLE) calculated using the PoolScreen 2.0 program (University of Alabama, Birmingham) (Gu et al., 2004; Katholi et al., 2006). The Pool Screen 2.0 program was used to read the MIRs on PCR results entered in Microsoft Excel data file. GIS data was initially entered in to an Excel spreadsheet and analysis was done by Arc GIS Geo-spatial analysis using Getis G-statistic for local clustering (Getis and Ord, 1992).
CHAPTER FOUR: RESULTS

4.1 Demographic Information

A total of 270 study subjects were selected by randomized cluster sampling of 66 households in two designated administrative sub locations of Masalani division in Ijara District. The sub locations were namely Gumarey (GM) representing the rural population and Sogan Godud (SG) representing the urban population. Out of the 270 selected participants 248 (91.9%) completed all study sampling procedures including serum testing. The age range of participants was 2-81 years in Gumarey and 1-86 years in Sogan Godud. The sex distribution among study participants was similar with Gumarey having 73 (60%) and 82 (65%) females (Table 4.1).

Table 4.1: Demographic data of survey participants in Gumarey and Sogan Godud

<table>
<thead>
<tr>
<th>Demographic variable</th>
<th>Gumarey (GM)</th>
<th>Sogan Godud (SG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Area Setting</td>
<td>Rural</td>
<td>Urban</td>
</tr>
<tr>
<td>Number of participating</td>
<td>30</td>
<td>36</td>
</tr>
<tr>
<td>Households</td>
<td>Study participants</td>
<td>122 (49%)</td>
</tr>
<tr>
<td>Female participants</td>
<td>73 (60%)</td>
<td>82 (65%)</td>
</tr>
<tr>
<td>Male participants</td>
<td>49 (40%)</td>
<td>44 (35%)</td>
</tr>
</tbody>
</table>

4.2 Human RVFV Seropositivity

Overall seropositivity for RVFV among the total study population of 248 subjects was 13.3% (33/248). There was significant difference in seropositivity for RVFV by village. Out of 122 study subjects from Gumarey, 25 (20%) were positive for RVFV by IgG assay compared to 8 (6%) of the 126 subjects from Sogan Godud. Female seropositivity
for RVFV in Gumarey was 11 (15%) and 3 (3.7%) for Sogan Godud. Male participants RVFV seropositivity in Gumarey village was higher 14 (28%) compared to 5 (11%) in Sogan Godud (Figure 4.1).

Figure 4.1: A flowchart of seropositive participants by village and sex

4.2.1 Seropositivity of participants by age range

Of the study participants who were positive for RVFV, their age ranged between 12-81 years in Gumarey and 4-71 years in Sogan Godud. One hundred and thirty (52%) were 15 years and above while 118 (48%) were below 15 years. Only 4 (3%) out of a total of 118 children were positive for RVFV and the youngest was 4 years old. (Table 4.3). The seropositivity for RVFV increased by age with the lowest level recorded in age group 0-4 years. Among 130 residents of 15 years and above in the sampled cohort, 29 (22.3%)
turned positive for anti RVFV by IgG results and the oldest subject was 81 years (Table 4.2).

Table 4.2: RVFV seropositivity by age of study population

<table>
<thead>
<tr>
<th>Age group of subjects</th>
<th>Number of subjects</th>
<th>Age Range (Years)</th>
<th>RVFV IgG ELISA Seropositivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;15yrs</td>
<td>118 (48%)</td>
<td>4-14</td>
<td>4 (3%)</td>
</tr>
<tr>
<td>≥15yrs</td>
<td>130 (52%)</td>
<td>16-81</td>
<td>29 (22%)</td>
</tr>
</tbody>
</table>

By age, majority of seropositives were 25 years of age and seropositivity peaked in age group 45-54 years in Gumarey and 65-74 years in Sogan Gudud. Overall the highest level of seropositivity for RVFV was recorded among those above 65 years. Interestingly, for those below 5 years residing in Gumarey, the prevalence was 0% compared to Sogan Godud with 5.5%. Overall, the seropositivity increased with age in both villages with children from Sogan Godud having significantly lower prevalence than adults (Table 4.3; Figure 4.2).

Table 4.3: Percentage seropositivity of RVFV by Age group and location

<table>
<thead>
<tr>
<th>Age Group (years)</th>
<th>Gumarey n=122</th>
<th>Sogan Godud n=126</th>
<th>Total n=248</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>0%</td>
<td>5.5%</td>
<td>2.5%</td>
</tr>
<tr>
<td>5-14</td>
<td>7.5%</td>
<td>0%</td>
<td>3.896%</td>
</tr>
<tr>
<td>15-24</td>
<td>20%</td>
<td>5.55%</td>
<td>9.09%</td>
</tr>
<tr>
<td>25-34</td>
<td>25%</td>
<td>0%</td>
<td>12.5%</td>
</tr>
<tr>
<td>35-44</td>
<td>62.5%</td>
<td>7.142%</td>
<td>25%</td>
</tr>
<tr>
<td>45-54</td>
<td>44.44%</td>
<td>0%</td>
<td>25%</td>
</tr>
<tr>
<td>55-64</td>
<td>30%</td>
<td>14.285%</td>
<td>23.52%</td>
</tr>
<tr>
<td>65-74</td>
<td>33.3%</td>
<td>80%</td>
<td>54.54%</td>
</tr>
<tr>
<td>74+</td>
<td>100%</td>
<td>50%</td>
<td>50%</td>
</tr>
</tbody>
</table>
In Figure 4.2, the prevalence of RVFV seropositivity differed persistently even at village level with children from Sogan Godud showing significantly lower prevalence than adults. Gumarey had higher prevalence in almost all age groups.

**Figure 4.2:** Proportion of RVFV Seropositivity by IgG for various age groups and location

### 4.2.2 Seropositivity by age, location and sex

Univariate analysis for association of RVFV seropositivity by age, location and sex varied significantly. Participants aged >15 years were found to be more at risk of seropositivity ($p=0.0001; \chi^2=18.8$) and by gender, more male participants were found positive for RVFV than female participants ($p=0.011; \chi^2=6.55$). Further, those
participants who resided from Gumarey had nearly 4 times higher association with RVFV than those residing in Sogan Godud with an Odds Ratio (OR) of 3.8. (Table 4.4).

Table 4.4 Association of demographic factors and RVFV seropositivity

<table>
<thead>
<tr>
<th>Demographic variable</th>
<th>Chi-Square test (χ²)</th>
<th>P-value</th>
<th>Odds Ratio (OR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>18.772</td>
<td>p=0.0001</td>
<td>8.03 (&lt;15/&gt;15yrs)</td>
</tr>
<tr>
<td>Location</td>
<td>10.747</td>
<td>P=0.001</td>
<td>3.80 (GM/SG)</td>
</tr>
<tr>
<td>Sex</td>
<td>6.546</td>
<td>P=0.011</td>
<td>2.59 (Male/Female)</td>
</tr>
</tbody>
</table>

4.3 Animal and Non Animal Exposure Factors

4.3.1 Animal exposure factors

Several animal exposure factors were tested for association with RVFV seropositivity. These included the sheltering of livestock, butchering, skinning of animals, boiling animal meat, milking animals, assisting in birthing of animals, and disposal of animal fetus. The information on exposure to sheltered livestock included the information on sheltering of sheep, cow, goat and camel (Figure 4.3).
Figure 4.3: Percentage of participants associated with selected livestock contact factors for RVFV exposure

The study participants who were involved in sheltering or keeping of young livestock (sheep and goats) in their living house overnight had greater odds of RVFV seropositivity than those who did not shelter livestock ($\chi^2=8.623; p=0.005$). Similarly, those who reported that they were involved in butchering of livestock had greater odds of RVFV seropositivity than those who did not butcher ($\chi^2=23.81; p=0.0001$). Skinning of livestock was also significantly associated with RVFV seropositivity ($\chi^2=15.24; p=0.0001$). Cooking of animal meat before consumption was preventive against RVFV ($\chi^2=7.87; p=0.005$), whereas milking of animals showed that the activity increase the risk of RVFV...
exposure ($\chi^2=16.64; p=0.05$). Assisting livestock to give birth to newborns was positively associated with RVFV exposure ($\chi^2=32.44; p=0.001$) and those who disposed off aborted animal fetuses were highly significantly associated with RVFV seropositivity as shown in Table 4.5.

**Table 4.5 Association of Selected Animal Exposure Factors and RVFV Seropositivity**

<table>
<thead>
<tr>
<th>Animal contact factor</th>
<th>Chi – square Test</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camel contact</td>
<td>4.75</td>
<td>0.029</td>
</tr>
<tr>
<td>Cattle contact</td>
<td>6.28</td>
<td>0.012</td>
</tr>
<tr>
<td>Shelter animals in living house</td>
<td>8.62</td>
<td>0.003</td>
</tr>
<tr>
<td>Butchering livestock</td>
<td>23.81</td>
<td>0.001</td>
</tr>
<tr>
<td>Skinning of animals</td>
<td>15.23</td>
<td>0.001</td>
</tr>
<tr>
<td>Cooking animal meat</td>
<td>7.87</td>
<td>0.005</td>
</tr>
<tr>
<td>Milking livestock</td>
<td>16.64</td>
<td>0.001</td>
</tr>
<tr>
<td>Birthing livestock</td>
<td>32.44</td>
<td>0.001</td>
</tr>
<tr>
<td>Disposal of animal fetus</td>
<td>28.30</td>
<td>0.001</td>
</tr>
<tr>
<td>Sheep contact</td>
<td>2.22</td>
<td>0.136</td>
</tr>
<tr>
<td>Goat contact</td>
<td>2.39</td>
<td>0.122</td>
</tr>
</tbody>
</table>

The majority of participants in Gumarey and in Sogan Godud (>80%) reported contact with sheep. Similarly cow contact and drinking of raw milk in Gumarey were reported by over 90% of study respondents compared to 77% and 79% respectively for respondents in Sogan Godud. The practice of keeping young livestock (sheep and goats) in the living house was more common in Gumarey (83%) compared to 64% in Sogan Godud.
Analysis by location, indicate that participants from Gumarey were more associated with animal exposure factors. Comparison of exposure levels for risks between the two villages showed that there were significant differences; goat contact (OR=2.64; \( p=0.046 \)), cattle contact (OR= 4.74; \( p=0.0001 \)), consumption of raw milk (OR=4.12; \( p=0.0001 \)), sheltering livestock (OR=2.62; \( p=0.002 \)), butchered livestock (OR=1.50; \( p=0.0001 \)), birthed livestock in the home (OR=2.14; \( p=0.005 \)), disposal of a livestock fetus (OR=1.74; \( p=0.042 \)) (Table 4.6)

**Table 4.6:** Comparison of RVFV animal exposure factors by location

<table>
<thead>
<tr>
<th>Animal contact factor</th>
<th>Odds ratio (Gumarey: Sogan Godud)</th>
<th>( p - \text{value} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goat contact</td>
<td>2.64</td>
<td>0.046</td>
</tr>
<tr>
<td>Cattle contact</td>
<td>4.74</td>
<td>0.0001</td>
</tr>
<tr>
<td>Camel contact</td>
<td>0.50</td>
<td>0.034</td>
</tr>
<tr>
<td>Sheltering of animals</td>
<td>2.62</td>
<td>0.002</td>
</tr>
<tr>
<td>Butchering of animal meat</td>
<td>1.50</td>
<td>0.0001</td>
</tr>
<tr>
<td>Birthing livestock</td>
<td>2.14</td>
<td>0.005</td>
</tr>
<tr>
<td>Disposal of aborted animal fetus</td>
<td>1.74</td>
<td>0.042</td>
</tr>
<tr>
<td>Drinking raw animal milk</td>
<td>4.12</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

**4.3.2 Non animal exposure factors**

Several non animal exposure factors were evaluated to determine their association with RVFV positivity. These factors included flooding, use of mosquito nets, personal illness
and contact with dead human body. Home flooding was reported by 81% of participants in Sogan Godud compared with 77% in Gumarey. Similar results were reported for displacement by floods in both villages. Sleeping under mosquito nets, use of firewood and other mosquito control methods were reported more frequently applied in Sogan Godud with an urban setting than in Gumarey with a rural setting. Gumarey participants reported 25% to have had contact with dead human body compared with 14% from Sogan Godud (Figure 4.4).

**Figure 4.4:** Non-animal exposure factors and RVFV seropositivity by location

Among the non animal exposure factors significantly associated with seropositivity by locations were, dead human contact ($\chi^2=36.97; p=0.0001$) and home flooding ($\chi^2=5.15; p=0.024$). Displacement by floods was also associated with RVFV seropositivity in Gumarey but not in Sogan Godud (Table 4.7).
Table 4.7: Association of non animal contact factors and seropositivity by location

<table>
<thead>
<tr>
<th>Non-animal contact</th>
<th>Chi – square test ($\chi^2$)</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home flooding</td>
<td>5.15</td>
<td>0.024</td>
</tr>
<tr>
<td>Contact with dead human body</td>
<td>36.97</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mosquito Coil use</td>
<td>3.6</td>
<td>0.058</td>
</tr>
<tr>
<td>Recent Mosquito Bite</td>
<td>0.038</td>
<td>0.538</td>
</tr>
<tr>
<td>Recent Illness</td>
<td>3.045</td>
<td>0.081</td>
</tr>
<tr>
<td>Ill family member</td>
<td>0.154</td>
<td>0.43</td>
</tr>
<tr>
<td>Displacement by floods</td>
<td>2.696</td>
<td>0.101</td>
</tr>
<tr>
<td>Use of fire</td>
<td>0.038</td>
<td>0.864</td>
</tr>
</tbody>
</table>

Participants from Gumarey who had reported contact with human remains were more than 2 times likely to be seropositive compared to their counterparts in Sogan Godud (OR 2.1). Other reported significant non animal risk factors were use of mosquito nets (OR 0.194; p=0.0001), use of firewood (OR 0.472; p=0.0001), and use of mosquito coils (OR 0.122; p=0.0001) (Table 4.8) which indicate that they were more preventive of being seropositive for RVFV. Having an ill family member was marginally associated with positive results of RVFV infection.
Table 4.8: Risk of non animal contact factors with seropositivity by location

<table>
<thead>
<tr>
<th>Non-animal contact</th>
<th>Odds ratio</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Home flooding</td>
<td>0.79</td>
<td>0.45</td>
</tr>
<tr>
<td>Displacement by flood</td>
<td>0.876</td>
<td>0.66</td>
</tr>
<tr>
<td>Contact with dead human body</td>
<td>2.09</td>
<td>0.026</td>
</tr>
<tr>
<td>Use mosquito nets</td>
<td>0.194</td>
<td>0.0001</td>
</tr>
<tr>
<td>Use Fire</td>
<td>0.472</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mosquito Coils</td>
<td>0.122</td>
<td>0.0001</td>
</tr>
<tr>
<td>Recent Mosquito Bite</td>
<td>0.802</td>
<td>0.61</td>
</tr>
<tr>
<td>Recent Illness</td>
<td>1.075</td>
<td>0.776</td>
</tr>
<tr>
<td>Ill family member</td>
<td>0.354</td>
<td>0.072</td>
</tr>
</tbody>
</table>

4.4 Past Symptoms and Eye disease associated with RVFV seropositivity

4.4.1 Past symptoms associated with seropositivity

Among the past symptoms and signs reported among seropositive cases in the survey questionnaire included myalgia, backache, red eyes, eye pain, poor vision, meningismus, fever, malaise, chills, headache, poor appetite, nausea, vomiting (57%), hematemesis, flushing, and coma. Out of all the IgG RVFV positive participants, over 80% reported myalgia (muscle pain) and backache 76% as past symptoms while over 40% responded to meningismus and poor vision (Table 4.9).
Table 4.9: Percentage prevalence of selected past symptoms and RVFV seropositivity

<table>
<thead>
<tr>
<th>Past symptom</th>
<th>Number of IgG RVFV positive</th>
<th>Percentage of past symptom against the overall RVFV seropositivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myalgia</td>
<td>33</td>
<td>(27) 82%</td>
</tr>
<tr>
<td>Backache</td>
<td>33</td>
<td>(25) 76%</td>
</tr>
<tr>
<td>Eye pain</td>
<td>33</td>
<td>(19) 56%</td>
</tr>
<tr>
<td>Red eyes</td>
<td>33</td>
<td>(18) 58%</td>
</tr>
<tr>
<td>Meningismus</td>
<td>33</td>
<td>(16) 48%</td>
</tr>
<tr>
<td>Poor vision</td>
<td>33</td>
<td>(15) 45%</td>
</tr>
</tbody>
</table>

Of the 33 IgG RVFV seropositive cases, those reporting past symptom of muscle pain (myalgia) were significantly more frequent (p=0.0001). Other reported past symptoms which were significantly associated with seropositivity included backache (p=0.003), eye pain (p=0.034), red eyes (p=0.008), neck stiffness (meningismus) (p=0.004), and poor vision (p=0.008) (Table 4.10). The rest of the past symptoms which included headache, fever, malaise, chills, nausea, poor appetite and hematemesis were not significantly associated with RVFV seropositivity.
Table 4.10: Testing the associations of past symptoms with RVFV seropositivity

<table>
<thead>
<tr>
<th>Past symptom</th>
<th>Chi – square Test $\chi^2$</th>
<th>Odds Ratio</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myalgia</td>
<td>12.97</td>
<td>6.03</td>
<td>0.0001</td>
</tr>
<tr>
<td>Backache</td>
<td>9.059</td>
<td>3.86</td>
<td>0.003</td>
</tr>
<tr>
<td>Eye Pain</td>
<td>4.511</td>
<td>2.27</td>
<td>0.034</td>
</tr>
<tr>
<td>Red Eyes</td>
<td>7.036</td>
<td>2.75</td>
<td>0.008</td>
</tr>
<tr>
<td>Meningismus</td>
<td>8.24</td>
<td>2.97</td>
<td>0.004</td>
</tr>
<tr>
<td>Poor vision</td>
<td>6.985</td>
<td>2.74</td>
<td>0.008</td>
</tr>
</tbody>
</table>

4.4.2 Eye disease findings and RVFV seropositivity

The prevalence of eye disease was 18/248 (7%) Out of overall 33 RVFV seropositive cases identified in the study, 7/33 (21%) of them had eye disease while 26/33 (79%) had no eye disease. There were 18 eye disease subjects identified in the overall study. Of the 18 identified cases of substantial retinal disease 7/18 (39%) were seropositive compared with 11/18 (61%) who were seronegative (Table 4.11). All participants with eye disease were more than 20 years of age and all seropositive participants with eye disease were more than 49 years of age. Among the 7 seropositive participants, their eye disease profile included optic atrophy (3), retinal hemorrhage (1) and retinal scarring (3). The eye disease profile of the 11 seronegative participants were uveitis (1), vasculitis (1), maculopathy (3), peripallitis (1), retinal scarring (1), optic scarring (2), retinal atrophy (1) and retinal degeneration (1).
Table 4.1: RVFV Seroprevalence of participants compared with those with eye disease

<table>
<thead>
<tr>
<th></th>
<th>Eye disease</th>
<th>No eye disease</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RVFV seropositive</td>
<td>7</td>
<td>26</td>
<td>33</td>
</tr>
<tr>
<td>RVFV seronegative</td>
<td>11</td>
<td>204</td>
<td>215</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td>230</td>
<td>248</td>
</tr>
</tbody>
</table>

Table 4.12 shows the association between presence of eye disease and seropositivity ($\chi^2 = 11.0; p=0.003$). The data provides sufficient evidence of an association between eye diseases and RVFV seropositivity.

Table 4.12: The association of eye disease and RVFV seropositivity

<table>
<thead>
<tr>
<th>Variable</th>
<th>Chi square</th>
<th>P = value</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye disease</td>
<td>$\chi^2 = 11.011$</td>
<td>P=0.003</td>
<td>4.99</td>
</tr>
</tbody>
</table>

In an attempt to further determine the association of various factors and seropositivity for RVFV, correlation and multiple logistic regression analysis were performed. The correlation analysis showed that there is no significant correlation between age and village (p=0.873) while for age and sex (p=0.886) and there is no significant difference between the means of the two exposures and therefore no association exists. From the statistic in Table 4.13 Age and disposal of aborted animal fetus: there is significant difference (p=0.0001) between the means of the two exposures and there is significant
difference between the means of the two exposures and therefore an association exists between age and disposal of aborted fetus.

From the correlation statistic in Table 4.13 on village and disposal of aborted animal fetus there is significant difference (p=0.042) and therefore an association exists between the two factors.

**Table 4.13:** Correlation analysis for age, sex, village and disposal of aborted animal fetus

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>Village</th>
<th>Sex</th>
<th>Disposal of Aborted Animal Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Village</td>
<td>0.161</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(p=0.873)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>0.151</td>
<td>0.727</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(p=0.886)</td>
<td>(p=0.394)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disposal of Aborted Animal Fetus</td>
<td>0.102</td>
<td>0.145</td>
<td>0.278</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>(p=0.0001)</td>
<td>(p=0.042)</td>
<td>(p=0.598)</td>
<td></td>
</tr>
</tbody>
</table>

4.4.3 Risk predictors in RVFV seropositivity by location

Subgroup analysis by village showed significant predictors of RVFV seropositivity to be an ill family member, disposal of an animal abortus and gender. Male participants were more than 3 times likely to be seropositive as compared with female participants (adjusted OR 3.45). Disposal of aborted animal fetus (OR 15.12) and presence of an ill family member (OR 18.0) were also associated with seropositivity (**Table 4.14a**). In Sogan Godud the logistic model to predict seropositivity included age such that the odds of seropositivity increased every one year increase in age (OR 1.05).
Table 4.14a Testing for interaction between associated risk predictors and seropositivity

Location= Gumarey

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Point Estimate (Odds Ratio)</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>3.454</td>
<td>P=0.025</td>
</tr>
<tr>
<td>Discarded aborted animal fetus</td>
<td>15.12</td>
<td>P=0.0001</td>
</tr>
<tr>
<td>Ill family member</td>
<td>18</td>
<td>P=0.029</td>
</tr>
<tr>
<td>Constant</td>
<td>0.029</td>
<td>P=0.0001</td>
</tr>
</tbody>
</table>

The adjusted OR for seropositivity (calculated from the overall logistic model) was 1.05 per year of age (Table 4.14b). This difference presented at the sub-location level with those children in Sogan Godud still with significantly lower risks than adults.

Table 4.14b: Location= Sogan-Godud

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Point Estimate (Odds Ratio)</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.054</td>
<td>P=0.0001</td>
</tr>
<tr>
<td>Constant</td>
<td>0.01</td>
<td>P=0.0001</td>
</tr>
</tbody>
</table>

4.4.4 Model for risk predictors

The final logistic model to predict RVFV seropositivity included age, location, gender and disposal of an aborted animal fetus (Table 4.15). In multivariable logistic regression models used to predict adjusted odds (OR) of RVFV seropositivity, location was significant when age and gender were controlled for; those residing in Gumarey were at 4
times the risk of those at Sogan Godud (OR 4.5). Seropositivity also varied by gender when age and location were controlled for; male participants had >3 times the risk of women participants (20% vs. 9% adjusted OR 2.78 for male participants vs. female participants), but this difference did not remain in sub location analysis. After age gender and location were controlled for; those who had disposed of an aborted animal fetus were 3 times more likely to be seropositive (72.7% vs. 35.7%, adjusted OR 2.78). Age and location but not gender were associated with disposal of an aborted animal fetus, such that those who were older or from Gumarey were more likely to dispose an abortus (Table 4.15).

**Table 4.15; Logistic Regression Analysis**

<table>
<thead>
<tr>
<th>Predictor Variables</th>
<th>Odds Ratio (OR) of seropositivity</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.039</td>
<td>0.001</td>
</tr>
<tr>
<td>Location (Sogan-Godud vs. Gumarey)</td>
<td>0.241</td>
<td>0.004</td>
</tr>
<tr>
<td>Sex (Male vs. Female)</td>
<td>2.782</td>
<td>0.020</td>
</tr>
<tr>
<td>Disposal of Aborted Fetus</td>
<td>2.779</td>
<td>0.044</td>
</tr>
<tr>
<td>Constant</td>
<td>0.119</td>
<td>0.005</td>
</tr>
</tbody>
</table>

### 4.5 Mosquito Species Identification

#### 4.5.1 Trapping and identification

Out of 74 trapping events, 38 occurred in different household locations in the study villages. Overall 12,080 mosquitoes were collected and out of these, 9,701 were collected
during the seven trapping nights in 2006 and 2,379 were collected during the six trapping nights in 2007.

**Figure 4.5:** A graph showing the species distribution of the total mosquitoes collected during the study by % (N=12080)

\[ \text{Culex mosquitoes were the most abundant species observed in the traps. During the entire trapping period 7,853 Culex (7,845 Culex quinquefasciatus and 8 Culex tigripes), 3,488 Anopheles, 682 Mansonia and 57 Aedes species were trapped and morphologically identified. Each trap had an average of 199 mosquitoes with an average of 141 Culex species per trap (Figure 4.5).} \]

**4.5.2 Viral positivity rates by PCR**

The minimum sample size of 920 mosquitoes sampled from the total collections in the field were pooled in the laboratory (average 1-12 mosquitoes each) based on trapping
location to comprise the 105 pools for testing. The majority of tested species were morphologically identified as *Culex quinquefasciatus* (N=654, 71%). The remaining mosquitoes were identified as follows: *Anopheles sp.* (N=107, 12%), *Mansonia sp.* (N=101, 11%), and *Aedes sp.* (N=58, 6%) (Figure 4.6).

**Figure 4.6:** Graph showing number of mosquitoes tested and species distribution.

Five hundred fifty-two mosquitoes (552) trapped in 23 different Gumarey households were divided into 65 total pools in the laboratory (1-12 individual mosquito legs per pool). A total of 368 mosquitoes were trapped at seven different Sogan-Godud households, and divided into 40 individual pools for testing (1-11 individual mosquito legs per pool). Synthesis of total DNA was successful with 99% of samples amplifying ITS2 (Figure 4.7).
4.5.2.1 RVFV Positivity rates on mosquito pools and Minimum Infection Rates

Of the 105 laboratory constructed pools demonstrated 22% positivity for RVFV in mosquito samples (Table 4.16). Of the 65 pools from Gumarey, 30% tested positive for RVFV. The 40 pools from Sogan-Godud demonstrated only 10% positivity for RVFV. A comparison of RVFV positivity in mosquito pools across villages was significant (p=0.0279). Minimum infection rate (MIR) is the number of positive pools divided by the
total number of mosquitoes tested multiplied by 1000. MIR assumes that there is only one infected individual in each pool and the accepted arboviral infection threshold is 0.1%. Minimum infection rates (MIR) for the total number of mosquitoes tested was 2.7%. At location level, MIR for Gumarey village was 3.9% compared with 1.1% for Sogan Godud (Table 4.16).

Table 4.16: Mosquito Pool PCR Results for RVFV by Village

<table>
<thead>
<tr>
<th></th>
<th>Number of mosquito pools</th>
<th>Number positive pools by PCR</th>
<th>Minimum Infection Rates (MIR) Estimate (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total RVFV Testing Pools</td>
<td>105</td>
<td>23 (22%)</td>
<td>2.7% (1.7-4.2%)</td>
</tr>
<tr>
<td>Gumarey RVFV Testing Pools</td>
<td>65</td>
<td>19 (30%)</td>
<td>3.9% (2.2-6.2%)</td>
</tr>
<tr>
<td>Sogan Godud RVFV Testing Pools</td>
<td>40</td>
<td>4 (10%)</td>
<td>1.1% (0.29-2.9%)</td>
</tr>
</tbody>
</table>

When analyzed by household, 26% of all tested mosquitoes tested positive for RVFV. RVFV positivity of households was not significantly different between the two villages (p=1.000). RVFV household positivity rates were similar between the two village locations with 52% of households in Gumarey testing positive versus 43% in Sogan-Godud (Table 4.17).
Table 4.1: Percentage of households with positive mosquito for RVFV by PCR results

<table>
<thead>
<tr>
<th></th>
<th>Number of HH Sampled</th>
<th>Number of HH Positive by PCR</th>
<th>Minimum Infection Rate (MIR) Estimate (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Number of Households (HH)</td>
<td>30</td>
<td>15 (50%)</td>
<td>4.6% (2.2-8.9%)</td>
</tr>
<tr>
<td>Gumarey Households (HH)</td>
<td>23</td>
<td>12 (52%)</td>
<td>5.2% (2.3-10%)</td>
</tr>
<tr>
<td>Sogan Godud Households (HH)</td>
<td>7</td>
<td>3 (43%)</td>
<td>2.5% (0.38-14%)</td>
</tr>
</tbody>
</table>

Entomological surveillance took place among households that had been enrolled in an RVFV human surveillance project nine months earlier (LaBeaud, 2008). Fewer households (30) underwent mosquito trapping for RVFV testing than households where testing was performed on human samples (63). One RVFV positive mosquito pool was documented from a household with seronegative humans (Table 4.18). Both positive and negative mosquito pools were found in households containing RVFV seropositive humans. Ten positive mosquito pools had no human IgG results 7 negative mosquito PCR pools did not have IgG results (Table 4.18).
Table 4.1: Integration of Household Mosquito PCR Results and Human IgG Elisa test

<table>
<thead>
<tr>
<th></th>
<th>Mosquito RVFV Positive pools by PCR</th>
<th>Mosquito RVFV Negative pools by PCR</th>
<th>Total number of households</th>
</tr>
</thead>
<tbody>
<tr>
<td>Households with Positive human IgG RVFV</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Households with Negative human IgG RVFV</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Households with no Human IgG RVFV Result</td>
<td>10</td>
<td>7</td>
<td>17</td>
</tr>
<tr>
<td>Total Number of Households (HH)</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

4.6 Spatial distribution of RVFV seropositives by GIS clustering analysis

When spatial distribution of the RVFV seropositive cases was analyzed by Arc GIS cluster analysis, the results showed cluster distribution of high and low concentration. Both villages showed significant clusters but varied according to village with Gumarey showing higher spatial significance than Sogan Godud. Gumarey village shows clusters of high value significance of RVFV seropositivity while Sogan Godud shows clusters of low value significance (Figure 4.8). Interestingly similar results were recorded in the IgG Elisa with Gumarey and Sogan Godud villages sero prevalence rate being 20% and 6% respectively. In total the GIS co-ordinates of 66 households were taken in both locations which comprised of 30 from Gumarey and 36 from Sogan Godud.

In comparison, rural location had more RVFV seropositive cases than in urban setting. These observations were made by satellite mapping of all the households in the study.
area by help of GPS machine. The clustering analysis suggests Gumarey to be an area of high concentration RVFV activity than Sogan Godud.

**Figure 4.8;** Spatial clustering of ant-RVFV seropositivity across village households
CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS & RECOMMENDATIONS

5.1 Discussion

5.1.1 Human Seropositivity

This study highlights the variability in RVFV seroprevalence in high risk settings. In Ijara district North-Eastern Kenya, older age, rural village location, male sex, disposal of an aborted fetus, and eye disease were significantly associated with anti-RVFV seropositivity. RVF seropositivity was relatively high in our sample population in the Ijara study area, particularly in the village area (Gumarey), where seropositivity rates were five times higher than the sub-urban area (Sogan-Godud) separated by a distance of only 500m. Clues to the reasons for this discrepancy in sero-prevalence were identified in our study. Those from Gumarey were significantly more likely to have greater mosquito and animal exposures than those from Sogan-Godud. These risk factors coupled with the most important predictors of rural sero-positivity, male and disposal of an aborted animal fetus, yield evidence for disparate risks for RVF in different communities.

RVF sero-prevalence varies significantly across Kenya (LaBeaud et al., 2007). This study shows that large sero-prevalence discrepancies can also occur over very short distances.

RVF IgG ELISA antibodies are believed to last decades after infection and so provide a reliable index of prior RVF exposure. In contrast, though less well studied, it appears that IgM is lost in 50% of patients by day 45, and is absent in 100% by 4 months after infection (Madani et al., 2003). IgM testing was not performed in this study although it might have yielded useful additional information about acute RVFV infection. Plaque
Reduction Neutralization test (PRN) was further used to detect any cross reactions which could have occurred in the IgG ELISA. However, the use of live viruses in PRN puts the test at a disadvantage due to risks of infection especially in low level bio-safety laboratories. The use of confirmatory PRN testing of ELISA positives is able to greatly improve viral specificity (Tesh et al., 1982). However, new ELISA methods being developed may prove to be even more sensitive and specific than PRN tests in the future (Paweska et al., 2005).

For each year of life, the odds of being RVF seropositive increased by 5%. Males were nearly three times more likely to be seropositive than females in this study, a risk that was noted in the 1997 RVF outbreak investigation (Woods et al., 2002). The difference in sero-positivity between genders is not explained on the basis of reported animal or non-animal exposures which were comparable and not statistically different between genders. The increased sero-positivity among males may have an important biologic basis, as infectivity, replicating capacity, and resultant immune response to other viruses have been linked to sex differences (Hannah et al., 2007). Even in the 2006-2007 outbreak in Kenya the majority of cases were among herdsmen aged between 20-30 years of age (WHO, 2007).

There was evidence of interepidemic human transmission of RVFV, which has not been previously shown. Our validation of seropositive young children, born after the last documented outbreak in 1997-1998, indicate that low-level interepidemic transmission to humans is continuing in the Masalani area, and likely in other areas in Kenya (LaBeaud et al., 2007).
The natural reservoir for RVFV and the mechanism by which humans become infected during interepidemic periods are unknown. Recent evidence suggests that wild animals are infected with RVFV but further studies must determine whether these animals play a role in RVFV maintenance between outbreaks (Evans et al., 2007).

5.1.2 Animal and non-animal exposure factors

Disposal of an aborted animal fetus was associated with a three-times increased risk of RVF sero-positivity. This finding may indicate the importance of RVF transmission by aerosolization of blood and amniotic fluid during animal birthing in Kenya. It is unknown whether aerosol or vector-borne transmission is the dominant form of transmission during inter-epidemic and epidemic periods. Our analysis indicates that disposal of an aborted animal fetus was an important associated risk factor at both the composite and sub-location level. Other animal exposure factors which were found important in RVFV transmission due to their association with seropositivity were animal sheltering, butchering of animal meat, skinning of animals, birthing of animals and milking of animals. Since RVF is a zoonosis, different animal exposure factors could be key mode of transmission from animals to humans. However in location analysis, there was more disposal of animal fetus in Gumarey than in Sogan Godud suggesting that Gumarey community were living closer to animals than their counterparts in Sogan Godud. For non exposure factors, flooding and contact with dead human body were found to be associated with seropositivity. Flooding has for long been included in the list of climate and environmental changes which trigger RVFV outbreaks (Geering et al., 2003). At location level, non exposure factors which were associated more with Gumarey than
Sogan Godud were, use of mosquito nets, mosquito coils which suggested that people living in rural set up like Gumarey could be exposed to more mosquito bites than their counterparts in semi-urban set up.

5.1.3 Past RVFV symptoms and eye disease
There were important differences between the seropositivity rates of those with and without eye disease. Those with chronic retinal disease were 5 times more likely to be RVF seropositive. Although there were no ocular findings that were suggestive for a prior RVF infection, this finding supports evidence from previous studies on the oculopathogenesis of RVFV (Al-Hazmi et al., 2005). No specific non-ocular exam finding was associated with RVFV seropositivity, but there were several reported symptoms that were statistically more common in those who were RVFV seropositive. The majority of these symptoms were severe neurologic manifestations of disease, such as neck stiffness, confusion, and coma. It is known that RVF can cause encephalitis (WHO, 2000), and this type of inflammation may explain the higher prevalence of these reported symptoms among seropositive subjects. Myalgia and backache may be present in the majority of non-severe RVF cases, and are not specific to RVFV infection. Poor vision may be an indicator for RVFV retinitis, a common sequel of RVFV infection (Al-Hazmi et al., 2005, Siam et al., 1980).

5.1.4 Mosquito species and viral infection rates
A very large proportion of the mosquito population collected within our study area during the RVF outbreak in Ijara District, Northeast Province, consisted of potential RVFV
vectors. *Culex quinquefasciatus* was the predominant species trapped during this study, although *Aedes, Anopheles* and *Mansonia* species were also recovered during nocturnal light trapping. Interestingly, all *Aedes* mosquitoes collected were trapped in December, but absent in the traps in January. The presence of RVFV in mosquitoes collected within the area was quite high. The isolation of RVFV from these samples confirms that the virus was disseminated within the bodies of the mosquito species tested. The results also confirm that single mosquito leg samples are sufficient for PCR/RT-PCR detection of RVFV. Positive results from testing of the mosquito leg diminish any concern of false positives that could result from testing whole mosquitoes, which might contain recent blood meals with significant viral content. Our study confirms that RVFV disseminates to the legs of wild *C. quinquefasciatus* mosquitoes and suggests that this species, a promiscuous feeder, could play a role in the maintenance or transmission of RVFV in endemic regions (Turell, 2007; Turell, 2008). Additional studies are required to determine the vector competence (the ability both to disseminate and transmit arboviruses) of *C. quinquefasciatus* and of the other positive mosquito species. Improved field diagnostics are necessary for rapid accurate diagnosis of circulating arboviral threats and expedient translation into preventative public health practices. The pooling scheme allowed for greater ease and faster testing of samples, without diluting virus beyond the scope of RT-PCR/PCR technology. The pools varied by size (due to location parameters); however, the ability of RT-PCR to identify at least one positive mosquito leg amidst a group of between 1 and 11 legs shows the effectiveness of our methods and corroborates other studies that have used similar methods to identify one positive mosquito in pool sizes up to 600 uninfected mosquitoes (Jupp et al., 2000). The
choice of screening pools for arboviruses offers many benefits, especially during an outbreak situation. The potentially limiting factors of cost and time are avoided, while the identification of mosquito positivity is accurately achieved (Gu et al., 2008; Katholi et al., 2006). It is possible that during an arbovirus outbreak, other viruses may be circulating concomitantly without recognition, and serve as competing causes of undiagnosed fever. Additional arthropod surveillance studies during RFVF outbreaks in Kenya have highlighted the presence of arboviruses in mosquito species, including flaviviruses and alphaviruses that can result in febrile human illness (Crabtree et al., 2009). Because human and animal diseases from arboviral infections can be non-specific, it is necessary, even during large outbreaks, to document the true cause of disease with detailed testing. Cases of other arboviral infections could be missed if suspected cases are attributed to the epidemic arbovirus without accurate diagnosis.

Although mosquito infection rates (MIRs) for RVFV were similar in the two studied villages, human seroprevalence studies found those living in Gumarey at 4 times the odds of being RVFV seropositive than those in Sogan-Godud. Individuals residing in Gumarey were more likely to report greater contact with both animals and mosquitoes. Although RVFV can be transmitted to humans by the bite of an infected mosquito, our data suggests that alternative forms of human transmission such as aerosol and direct contact may be more important for transmission during epidemics. More research must be done to elucidate the most common routes of RVFV human transmission during epidemic and interepidemic periods. Mosquito sampling was conducted only at homes where specific animal species, those known to be reservoirs of RVFV, were housed closely with humans. This type of targeted sampling, however, can provide earlier detection of
arboviruses and provide greater understanding of transmission and maintenance factors of these viruses in the wild (Gu et al., 2008). While only 920 mosquitoes were tested for RVFV, a fraction of the total mosquito population collected, it has previously been shown that testing of mosquito pools versus testing of all samples can yield suitable results, thereby conserving time and resources (Gu et al., 2008).

5.1.5 GIS spatial clustering of RVFV seropositivity

The temporal distribution of these mosquitoes correlates with previous studies that have found *Aedes* mosquitoes to predominate in the initial weeks following significant dambo flooding and then curtail after the first month of flooding, at which time *Culicine* and *Anopheline* mosquitoes emerge as the predominant species (Linthicum et al., 1985; Bird et al., 2009). It has been suggested that the dramatic proliferation of transovarially infected *Aedes* mosquitoes immediately following flooding re-introduces virus into an epizootic/epidemic cycle, after which *Culex* mosquitoes then propagate the virus in an epizootic/endemic cycle amongst humans and animal species (Linthicum et al., 1985). Spatial risk assessments of animal RVF in Senegal have been developed using seasonal rainfall, land surface temperature, distance to perennial water bodies, and time of year (Clements et al., 2007). Designing such risk maps with inclusion of human risk factor data may allow for improved surveillance systems and better prediction of the spatial distribution of RVF. This information gathered with satellite imagery (Linthicum et al., 1999), and large scale cluster analysis (Clements et al., 2007) can be used not only to predict large outbreaks, but also to identify local hot spots of RVFV transmission to optimize RVF control in resource limited settings.
5.2 Conclusions and Recommendations

5.2.1 Human seropositivity

1. This study highlights the large-scale variability in exposure and anti-RVFV seropositivity between Kenyan villages and emphasizes the impact of age, gender, location, and animal husbandry in RVFV transmission. The study also established the interepidemic transmission in the area.

2. This information is important for local public health agencies so that they can target protective interventions according to risk factors in different populations.

3. Rift Valley Fever virus transmission is known to be ongoing in livestock in RVFV endemic areas during inter epidemic periods and the study also shows that this extends to humans.

5.2.2 Animal and non animal exposure factors

1. Disposable of dead animal fetus was found to be an important animal exposure factor in this study as far as RVFV transmission is concerned due to its strong association with RVFV seropositivity for both composite and at location level.

2. Butchering of animal meat, skinning of animals, birthing, milking and sheltering of animals are other animal exposure factors which were found to be associated with RVFV seropositivity. This means that these activities happen in both locations.

3. Non- animal exposure factors were not as important as the animal exposure factors in terms of the association with seropositivity except in flooding which was reported to be an important factor in both locations.
5.2.3 Past symptoms and eye disease

1. There were important differences between seropositivity of those with and without eye disease with those with chronic retinal disease being 5 times more likely to be RVF seropositive.

2. Those with past symptom of poor vision were also associated with seropositivity and therefore conclude that there is a relationship between RVFV and eye disease.

5.2.4 Mosquito identifications and viral infection rates

1. On mosquitoes the study shows that *Culex quiquefasciatus* and *Anopheles gambiae sl* as the most predominant species collected during the study. The *Aedes* mosquitoes considered to be primary vectors of RVF were few in numbers and this could be due to the fact that they are day biters and these collections were done during the night.

2. The most common identified species of *Aedes* found in the traps was *Aedes scatophagoides*. The vector study outcome confirms conclusions made by Reiter, (1988) that understanding of arthropod portion is worth pursuing because of its potential contribution to epidemic forecasting and improving of our insight to vector control responses.

3. Some mosquito vectors collected during the 2006 -2007 human RVF outbreak in North Eastern Kenya were found to be potentially efficient, with high MIRs for RVFV. MIR between villages did not differ, though human seroprevalence (as measured in a previous household-based study) did.
5.2.5 GIS spatial clustering and RVFV transmission

1. Efforts to predict hot spots of infection should be intensified on both small and large scales so that at-risk communities are able to use the information to target mosquito control or initiate vaccination to prevent human RVF outbreaks.

2. As Rift Valley fever expands its geographic range and becomes recognized as a disease of global importance for human and animal health, more research is needed to define the most accessible modes of transmission termination. In this study no serum samples were tested from domestic animals found in these two villages but studies on wild animals conducted by Evans et al., (2007) found out that African buffaloes from Ijara district had the highest prevalence (71%) of RVFV neutralizing antibodies out of the seven districts. This suggests that wild animals could also be playing a role in maintenance of the virus during inter epidemic periods and the use of GIS technology could also be applied in tracking of animal movements.

3. GIS can be used as an epidemiological tool to monitor the progression of RVFV by mapping areas of high antibody prevalence and high MIRs from year to year. Our data demonstrates the local abundance of mosquito vectors infected with arboviruses in Kenya.
5.3 Recommendations

1. Further studies are needed to examine the biologic and genetic basis for the increased risk among males, and to quantify the potential public health impact of modifying the rural environment which had a higher disease burden.

2. More research information is needed to understand the variability in RVFV exposure and seropositivity across villages and impact on age, gender, location and animal husbandry. Additionally more research information is required to understand why some RVFV infected cases get a life threatening disease while others only get a mild disease.

3. Improved vector surveillance and diagnosis of arboviral infection in animals and humans will lead to improved understanding and better estimates of the true burden of arboviral disease in animal and human populations. A greater understanding of how these important arboviruses are maintained in nature will improve targeted prevention in endemic regions and curtail introduction to new areas.

4. GIS surveillance system should be employed to monitor the progression of RVFV from location to location and from year to year depending on data from human seropositivity and mosquito MIRs.
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Annex 1

Information on adult consent to participate in the study

Purpose: The Head, Division of Vector-Borne Diseases (DVBD) in collaboration with Case Western Reserve University (CWRU) are inviting you to enroll in a research study. This research study is being done to understand how, why and when Rift Valley Fever (RVF) is spread. RVF is a disease that is spread by mosquitoes from animals to humans and causes an infected person to have fever, vomiting, headache, muscle pain and rarely blindness and death. Ijara District is a place where there have been large outbreaks of RVF in the past. The goal of this study is to examine Rift Valley Fever virus (RVFV) transmission and its related disease rates and infection attack rates during and after large outbreaks. We hope that the results of this study will help us predict and detect future RVF outbreaks.

Study procedures: Your participation in this study will last for three hours and will involve one visit. You will receive a structured interview asking questions about housing, occupation, animal exposure, vision, strength and abilities, and recent or past RVF related symptoms. You will also have a physical examination, a vision test, and an eye examination. During the eye exam, your pupils will be dilated with medicine drops. Then an eye doctor will look into your eyes with a machine that takes pictures of the back of your eyes. This eye exam will check for signs of current or previous eye inflammation, which can be side effects of RVF infection. After completing the exam, you will have approximately 1 teaspoon (5ml) of blood drawn by a needle from a vein in your arm. This blood will be sent to the Division of Vector-Borne Diseases labs in Nairobi and Case Western Reserve University to be tested for Rift Valley Fever virus infection.

Risks: Your participation in this study may involve a few possible risks. The insertion of the needle to draw blood is painful; however, this discomfort is brief. For most people, needle punctures to get blood samples do not cause any serious problems; however, they may cause bleeding, bruising, discomfort, infections, dizziness, or fainting. These risks are very uncommon and occur in less than 5% of the time. The dilating drops that are put
in your eyes will cause blurry vision for a few hours, and may also make you feel dizzy or nauseated. These effects wear off over a short time and there will be no permanent damage to your vision, but you should not operate heavy machinery when your vision is blurry. Free treatment will be given to any person experiencing the above symptoms secondary to blood drawing or pupil dilation.

**Benefits:** There will be no direct benefit to you by your participation in this research study. Your participation in this study may aid in our understanding of Rift Valley Fever and may help your community in predict and detect future outbreaks.

**Alternatives to participation:** Because of the nature of this research the only alternative is to not allow yourself to participate in this study.

**Financial Information:** There is no cost to you for participating in this study. You will not be paid for participating in this study.

**Confidentiality:** The results of your examinations will be collected in a data registry at the Division of Vector-Borne Diseases, Ministry of Health, Kenya. After the interview answers, physical examination results, eye exam pictures and blood are collected for study they will be identified by a study number and not your name or identifying examination. If information generated from this study is published or presented, your identity will not be revealed. Efforts will be made to keep the personal information in your research record private and confidential.

**Summary of your rights as a participant in a research study:** Your participation in this research is voluntary. Refusing to participate will not will not alter your usual health care or involve any penalty or loss of benefits to which you are otherwise entitled. If you decide to join the study, you may decide to withdraw anytime for any reason. If information from this study is published or presented, your identity will not be revealed.
**Contact Information:** One of the team members has described to you what is going to be done, the risks, hazards, and benefits involved, and can be contacted at 20-725601. Further information with respect to illness or injury resulting from a research procedure as well as research subjects rights is available from the Office of the Chief Medical Officer at 1-216-844-3695.

**Signature:** The study described above has been explained to me. The consent form has been read to me and my questions have been answered to my satisfaction. I agree to participate in this research study. I understand that enrolling is voluntary. I understand that my decision to participate or not to participate in this study will not alter my usual health care. I understand that by signing this consent form, I do not waive any of my legal rights. I understand that I may withdraw from this study at any time. I understand that any information from this study will be kept confidential. I have received a copy this form.

Signature of Participant ..........................................................

Date..............................

Printed Name of Participant..................................................

Signature of Person Obtaining Consent.................................

Date..............................

Printed Name of Person Obtaining Consent............................

Signature of Principal Investigator........................................

Date..............................
Annex 2

Information on child informed consent

Purpose of Study
The Division of Vector-Borne Diseases (DVBD) in collaboration with Case Western Reserve University (CWRU) are inviting your child to enroll in a research study. This research study is being done to understand how, why and when Rift Valley Fever (RVF) is spread. RVF is a disease that is spread by mosquitoes from animals to humans and causes an infected person to have fever, vomiting, headache, muscle pain and rarely blindness and death. Ijara District is a place where there have been large outbreaks of RVF in the past. The goal of this study is to examine Rift Valley Fever virus (RVFV) transmission and its related disease rates and infection attack rates during and after large outbreaks. We hope that the results of this study will help us predict and detect future RVF outbreaks.

Study procedures
Your child’s participation in this study will last for three hours and will involve one visit. Your child will receive a structured interview asking questions about housing, occupation, animal exposure, vision, strength and abilities, and recent or past RVF related symptoms. Your child will also have a physical examination, a vision test, and an eye examination. During the eye exam, your child’s pupils will be dilated with medicine drops. Then an eye doctor will look into your child’s eyes with a machine that takes pictures of the back of your eyes. This eye exam will check for signs of current or previous eye inflammation, which can be side effects of RVF infection. After completing the exam, your child will have approximately $\frac{1}{4}$ - 1 teaspoon (1-5ml) of blood drawn by a needle from a vein in your arm. This blood will be sent to the Division of Vector-Borne Diseases labs in Nairobi and Case Western Reserve University to be tested for Rift Valley Fever virus infection.
Study Risks
Your child’s participation in this study may involve a few possible risks. The insertion of the needle to draw blood is painful; however, this discomfort is brief. For most children, needle punctures to get blood samples do not cause any serious problems; however, they may cause bleeding, bruising, discomfort, infections, dizziness, or fainting. These risks are very uncommon and occur in less than 5% of the time. The dilating drops that are put in your eyes will cause blurry vision for a few hours, and may also make your child feel dizzy or nauseated. These effects wear off over a short time and there will be no permanent damage to your child’s vision, but you should not operate heavy machinery when your vision is blurry. Free treatment will be given to any child experiencing the above symptoms secondary to blood drawing or pupil dilation.

Study Benefits
There will be no direct benefit to your child by participation in this research study. Your child’s participation in this study may aid in our understanding of Rift Valley Fever and may help your community in predict and detect future outbreaks.

Alternatives to participation
Because of the nature of this research the only alternative is to not allow your child to participate in this study.

Financial Information
There is no cost to you or your child for participating in this study. Your child will not be paid for participating in this study.

Confidentiality
The results of your child’s examinations will be collected in a data registry at the Division of Vector-Borne Diseases, Ministry of Health, Kenya. After your child’s interview answers, physical examination results, eye exam pictures and blood are collected for study they will be identified by a study number and not your child’s name or identifying examination. If information generated from this study is published or
presented, your child’s identity will not be revealed. Efforts will be made to keep the personal information in your child’s research record private and confidential.

Contact Information
One of the team members has described to you what is going to be done, the risks, hazards, and benefits involved, and can be contacted at 20-725601. Further information with respect to illness or injury resulting from a research procedure as well as research subjects rights is available from the Office of the Chief Medical Officer at 1-216-844-3695.

Childs name………………………………….Date of Birth…………………………………
Childs Study ID Number………………………………………………

Parents / Guardian Statement
The study described above has been explained to me. The consent form has been read to me and my questions have been answered to my satisfaction. I agree to allow the participation of my child in this research study. I understand that enrolling the child is voluntary. I understand that my decision to participate or not to participate in this study will not alter the usual health care of my child. I understand that by signing this consent form, I do not waive any of my legal rights. I understand that I may withdraw my child from this study at any time. I understand that any information from the study will be kept confidential. I have received a copy of this form.

Parents/Guardians printed name……………………………………………………

Parents/ Guardians signature………………………………..Date…………………………

Relationship of Guardian to child…………………………

Witnessed by…………………………………………………..Date…………………………

Principal Investigator signature…………………………..Date…………………………
Child’s Assent form

What is a research study?
A research study is a way to find out new information about something. Children do not need to be in a research study if they don’t want to.

Why are you being asked to be part of this research study?
You are being asked to take part in this research study because we are trying to learn more about Rift Valley Fever. Rift valley Fever is an infection in which people get fever and feel very sick and weak. Sometimes it can even cause people to die. We are inviting you to be in the study because you live in Ijara District where there was a large outbreak of Rift Valley fever. About 50 children and 200 adults will be in the study.

Why is the study being done?
The results of the study will help us to understand more about Rift Valley Fever and how and why it is spreads. Our study may help prevent people from getting Rift Valley Fever in the future.

If you join in the study what will happen to you?
You will be asked to come to see one of doctors doing this study one time and you will need to stay for about 3 hours. One of the doctors will examine you. We will use a needle to take some blood from your arm one time. We will put medicine drops in your eyes, and then we will do a special eye test that takes about 20 minutes to complete.

Will any part of the study hurt?
Blood will be drawn from a vein in your arm once. You will feel a pinch when we use a needle to get the blood and this could leave a black and blue spot on your skin where the needle touched your skin. After the medicine drops are put in your eyes, your vision will be blurry, but this will go away within 6 hours.
Will the study help you?
When the doctor examines you, if he/she finds a problem, he / she will fix it.

Will the study help others?
This study might find out things that will help children and adults with Rift Valley Fever some day.

Do my parents know about this study?
This study was explained to your parents and they said that they could ask you if you want to be in it. You can talk this over with them before you decide. They may also be in the study.

Who will see the information collected about you?
The information collected about you during the study will be kept safely locked up. Nobody will know except the people doing the study.

Do you have to be in the study?
You do not have to be in the study. No one will be upset if you don’t want to be in the study. If you don’t want to be in the study, you just have to tell us. Its up to you. You can also take more time to think about being in the study.

What if I have any questions?
You can ask any questions that you have about the study. If you have a question later that you didn’t of now, you can call 020-725601. You can also take more time to think about being in the study and also talk some more with your parents about being in the study.

Other information about the study
If you decide to be in the study, please write your name below. You can change your mind and stop being part of it at any time. All you have to do is to tell the person in charge. If you would like, you can be given a copy of this paper to keep.
Write your Name.................................................................

I have discussed this clinical research study with ......................... using language, which is understandable and appropriate. I believe I have fully informed him/her of the nature of the study and its possible risks and benefits. I believe the participant understood the explanation and assented to participate in this study.

Witness .................................................................Date............................................
Annex 3

Information on General and Demographic Questions

1. Village Name □ Nomadic □ Moved here? □ When?

2. Name of person entering Data

3. Name of Data Collector

4. First Name

5. Second name

6. Third name

7. Other name

8. Head of household

9. Relationship to HH

10. House number

11. Do you sleep at this house? □ Do you stay in this house?

12. Village number

13. Date of registration

14. Year of birth

15. Sex (0=Female, 1=Male)

16. Age in years

17. Level of education
   (Primary/secondary/university/ Never attended)
Annex 4

Information on RVFV Exposure Factors

A. Non-Animal Exposures

1. What type of settlement do you live in?  
   ________________________________
2. Was your home ever flooded? □  
   ________________________________
3. When was it flooded?  
   ________________________________
4. Have you ever been displaced by a flood? □  
   ________________________________
5. When were you displaced?  
   ________________________________
6. Do you use mosquito net? □  
   ________________________________
7. How often do you use the net?  
   ________________________________
8. Do you use fire? □  
   ________________________________
9. How often do you use fire?  
   ________________________________
10. Do you use other forms of mosquito control? □  
    ________________________________
11. Have you had a recent mosquito bite? □  
    ________________________________
12. Have you had any personal illness? □  
    ________________________________
13. When were you ill?  
    ________________________________
14. Have you had an ill family member? □  
    ________________________________
15. When was your family member ill?  
    ________________________________
16. Have you had contact with a dead human body? □  
    ________________________________
17. When was your contact with a dead body?  
    ________________________________

B. Animal Exposures Factors

1. Please check any animal contact: Sheep contact □          Cow contact □
   Goat contact □          Camel contact □
2. Have you ever sheltered livestock in your home? □
   Camel ○ Sheep ○          Goat ○          Cow ○ Other
3. Have you ever killed an animal? □
4. Have you ever butchered an animal? □
   Camel ○ Sheep ○ Goat ○ Cow ○ Other

5. Have you ever skinned an animal? □
   Camel ○ Sheep ○ Goat ○ Cow ○ Other

6. Have you cooked with meat? □
   Camel ○ Sheep ○ Goat ○ Cow ○ Other

7. Have you ever milked an animal? □
   Camel ○ Sheep ○ Goat ○ Cow ○ Other

8. Have you ever drunk raw animal milk? □
   Camel ○ Sheep ○ Goat ○ Cow ○ Other

9. Have you ever cared for birthing animal? □
   Camel ○ Sheep ○ Goat ○ Cow ○ Other

10. Have you ever disposed of an aborted animal fetus? □
    Camel ○ Sheep ○ Goat ○ Cow ○ Other

28. Have you ever had any of the following symptoms? If yes, please indicate when.
    Fever □ Red eyes □ Hard to arouse □
    Sick Feeling □ No appetite □ Coma □
    Muscle aches □ Flushing □ Neck stiffness □
    Chills □ Nausea □ Poor Vision □
    Backache □ Vomiting □ Nose bleedings □
    Eye pain □ Painful eyes to light □ Vomiting Blood □
    Headache □ Confusion □ Bloody stool □
    Rash □ Spinning □ Bruising □
Annex 5

Information on Physical Examination

1. RVFID

Weight in kg (  ) Height(cm) (  )

2. General

Wasted □

3. Head

Healed burn scars to the face

4. Eyes

Scleral hemorrhages □ Scleral icterus □

5. Ears

6. Nose

7. Throat

8. Neck

Normal movement □

9. Chest

10. Heart

Murmur □

11. Abdomen

Hepatomegaly □ Splenomegaly □

12. GU

13. Neuro

14. Skin

Jaundice □ Petechiae □ Purpura □ Ecchymosis □

15. Lymphadenopathy

Cervical □ Auxiliary □ Inguinal □

16. Other
Annex 6

Information on Ophthalmologic Examination

1. RVFID

2. Visual Acuity – OS | Visual Acuity -OD

3. Anterior Chamber-OS
   Anterior Chamber –OD
   Anterior Uveitis – OD

4. Posterior Chambers – OS
   Posterior Chambers - OD
   Vitreous reaction -OS

5. Retina - OS
   Retina – OD

   Paramacul ar - OS ○ Paramacul ar - OD ○

7. Retinitis – Hemorrhage - OS □ Retinitis – Hemorrhage - OS □
   Zone – OS
   Zone - OD
   Area –OS
   Area –OD

8. Optic disc edema – Os □ Optic disc edema – OD □
9. Retinal vasculitis - Os □ Retinal vasculitis – OD □

10. RVF Related Disease OS □ RVF Related Disease OS □

11. Comments

12. Ophthalmologists Name | Data Enterer Name
A submerged traditional grass thatched hut in the study area during a flooding event.
Annex 8

Sheep and goat slaughtering in an abattoir
Annex 9

Movement of a cattle herd in Ijara district
A field worker filling in demographic data in one of the villages
Eye examination procedure being carried out during the study.
Annex 12

Setting of mosquito traps near one of the animal shelters
Annex 13

Sorting and identification of mosquitoes in the laboratory