MOLECULAR EPIDEMIOLOGY OF RIFT VALLEY FEVER AND ITS SOCIO-ECONOMIC IMPACT IN SELECTED AREAS OF TANZANIA

AUGUSTINO ALFRED CHENGULA

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MOLECULAR BIOLOGY AND BIOTECHNOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

2013

ABSTRACT

Rift Valley fever (RVF) is a viral notifiable zoonotic disease primarily of domestic ruminants that cause significant socio-economic impacts. Using the 2006-07 outbreak cases, this study aimed to establish the molecular epidemiology of Rift Valley fever virus (RVFV) and its socio-economic impact in selected areas of Tanzania. Data for awareness and socio-economic study were collected in Arusha, Manyara and Morogoro regions using questionnaires, focus group discussions and in-depth interviews with key informants. Molecular epidemiological study used samples that were collected during the outbreaks. Analysis of selected samples was done using RVF Inhibition Enzyme-Linked Immunosorbent Assay (ELISA) and Reverse Transcription Polymerase Chain Reaction (RT-PCR). Results indicate that there was little knowledge on disease (all clinical signs scored <50%) and the difference between the three regions was statistically significant (P=0.00459). Socio-economic impacts of RVF shown by this study included; animal and human deaths, disruption of livestock market chains, inability of pastoralists to achieve their daily demands, inability to obtain protein leading to malnutrition and monetary loss at individual and national level during control of the disease. The proportion of positive serum samples by RVF inhibition ELISA was 39.5% (n=200) and 17.6% (n=108) by RT-PCR. ELISA detected 41 (38.7%), 32 (39.0%) and 6 (50.0%), the RT-PCR detected 11 (0.2%), 7 (0.2%) and 1 (0.1%) positive results in cattle, goats and sheep respectively. These findings have demonstrated low knowledge of community on RVF and the presence of RVF virus in the country. Thus, more education and engagement is needed to the community together with further characterization of the virus from different geographical locations in order to

establish the profile of strains circulating in the country and develop more effective and efficient control strategies.

DECLARATION

I, **Augustino Alfred Chengula,** do declare to the Senate of Sokoine University of Agriculture that, this dissertation is my own original work done within the period of registration that it has neither been submitted nor concurrently being submitted in any other institution.

..... 06/11/2013

Augustino Alfred Chengula **Date**

(MSc. Molecular Biology and Biotechnology)

The above declaration is confirmed by:

..... 06/11/2013

Prof. Robinson H. Mdegela **Date**

(Supervisor)

..... 06-11-2013

Dr. Christopher J. Kasanga **Date**

(Supervisor)

COPYRIGHT

No part of this dissertation may be reproduced, stored in any retrieval system, or transmitted in any form or by any means without prior written permission of the author or Sokoine University of Agriculture in that behalf.

ACKNOWLEDGEMENTS

I am grateful to the Almighty God, Most Mercifully, the Creator of Heavens and the Universe for bestowing upon me the gift of life, strength and mostly, the chance to pursue my masters degree. My wife, Mary Alex Mpangala and our daughter Gabriela who kept on encouraging me throughout the course of this study; they were very understanding at times when I had to leave them behind and sometimes travel for field activities.

The Regional Universities Forum (RUFORUM), who sponsored my studies, is highly acknowledged. My great and sincere gratitude is accorded to my supervisors: Prof. Robinson. H. Mdegela and Dr. Christopher. J. Kasanga for their tireless supervision and constructive advice throughout my study.

My sincere thanks are also extended to all district veterinary officers and field officers in the study area, Arusha Veterinary Investigation Centre Officers (Dr. Emmanuel Swai and Dr. Winsford Moshy), and Tanzania Veterinary Laboratory Agency (TVLA) in Dar es Salaam (Director of TVLA (Dr. S. Das), Head of Department of Virology (Dr. M. Yongolo) and Raphael Sallu) for support in materials, reagents, technical support and conducive working environment.

My last sincere thanks go to all my colleague students who helped me in one way or another to accomplish my work.

DEDICATION

The dissertation is dedicated to my beloved parents; mother, Leonora Mgaya and father, Alfred Chengula for upbringing and building a strong background that enabled me achieve this far. It is also dedicated to my wife, Mary for her special support, care and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

µl	Microliter
ABTS	2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
bp	base pair
CBPP	Contagious Bovine Pleuropneumonia
CCPP	Contagious Caprine Pleuropneumonia
CDC	Centre for Disease Control and Prevention
cDNA	Complementary Deoxyribonucleic Acid
ECF	East Coast Fever
EDTA	Ethylene Diamine Tetra-acetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
EMPRES	Emergency Prevention System
FAO	Food and Agriculture Organization
FGD	Focus Group Discussion
HAI	Hemagluttination Inhibition test
HPRO	Horseradish Peroxidase
IgG	Immunoglobullin G
ILRI	International Livestock research Institute
LFOs	Livestock Field Officers
mRNA	messenger Ribonucleic acid
NSm	Non-Structural protein of medium segment
OD	Optical Density
OIE	Office International des Epizooties
ORF	Open Reading Frame

PBS	Phosphate Buffered Saline
PI	Percentage Inhibition
P-value	Probability (testing statistical significance)
rpm	Revolution per minute
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
SDS	Sodium Dodecyl Sulfate
TAE	Tris-Acetate EDTA
TBE	Tris-Borate EDTA
TVLA	Tanzania Veterinary Laboratory Agency
VIC	Veterinary Investigation Centre

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Rift Valley fever (RVF) is an acute vector-borne viral zoonotic disease affecting domestic animals and humans (FAO, 2003; Davies and Martin, 2006; Nachiket *et al.*, 2010). The disease is caused by the Rift valley fever virus (RVFV), a member of the genus *Phlebovirus* of the family *Bunyaviridae* (Elliott, 1990; Elliott, 1997; Robert, 2004; Labeaud, 2009; Nachiket *et al.*, 2010).

The RVFV virions are enveloped, low pleomorphic, composed of a lipid bilayer containing the carboxy-terminal glycoprotein (Gc) and the amino-terminal glycoprotein (Gn), measuring 8–120 nm in diameter (Huiskonen *et al.*, 2009; Bouloy and Weber, 2010; Pepin *et al.*, 2010). The viral genome (approximately 11.9 kb) contains single-stranded negative sense RNA composed of three segments designated as S (small), M (medium), and L (large) with 1690, 3885 and 6404 nucleotides respectively (Bird *et al.*, 2007b; Boulloy and Weber, 2010).

Rift Valley fever causes storm abortions in pregnant animals and a high mortality rate approaching 100% in young animals (Garcia *et al.*, 2001; Bird *et al.*, 2007a; Ikegami and Makino, 2009; Vuren and Paweska, 2009). Sheep are more susceptible with more effect than in other ruminants (Elfadil *et al.*, 2006). In humans, RVF causes a severe influenza-like illness characterized by fever (37.8–40 °C), headache, muscular pain, vomiting and extreme weight loss (Garcia *et al.*, 2001; Australian Government, 2007; Bird *et al.*, 2007a; Liu *et al.*, 2008). It is spread by a

bite of infected mosquito, typically the *Aedes* or *Culex* genera and thought to be maintained in nature at least in part by trans-ovarial transmission in flood water by Aedes mosquitoes during excess rainfall leading to floods referred to as 'dambos' (FAO, 2003, 2007; Labeaud *et al.*, 2007; Jost et al 2010; Nachiket *et al.*, 2010). In turn, it results into an abundance of vector mosquito species (Bird *et al.*, 2008; Breiman *et al.*, 2008). Elfadil *et al.* (2006) showed a positive association between RVF outbreaks and a dense mosquito population, high rainfall and the presence of lakes and/or ponds. Between epidemic waves, RVF virus circulates at very low incidence without noticeable clinical manifestation, neither in human nor in animals (FAO, 2007). Rift Valley fever epidemics have been observed at irregular intervals of about 5–20 years (FAO, 2007; Andriamandimby *et al.*, 2010; Jost *et al.*, 2010).

RVF disease is endemic in many African countries (Thiongane *et al.*, 1991; Garcia *et al.*, 2001; Bird *et al.*, 2008) and got out of Africa in 2000 onto Saudi Arabia and Yemen (Aziz, 2008; Vuren and Paweska, 2009). In East Africa the disease outbreak occurred in 1997/98 and 2006-07 an interval of about ten years and both outbreaks were associated with excessive rainfall (De La Rocque and Formenty, 2009; Jost *et al.*, 2010; Mohamed *et al.*, 2010; Munyua *et al.*, 2010) of an average of 1720 mm (2- to 3-fold higher than usual) that led to flooding in most parts of the plateau of Tanzania (Munyua *et al.*, 2010). During these outbreaks, high rates of illness and death resulting from haemorrhage among domestic animals in the area together with few human cases and massive economic hardship were observed (CDC, 1998, Breiman, 2008). In Tanzania, by the end of the outbreak in July 2007 there were 144 deaths of people out of 511 suspected cases (28.1% case fatality rate), whereby 186

(36.4%) were confirmed through laboratory tests and 124 (24%) classified as probable cases (Mohamed *et al.*, 2010). In the same year, similar extensive outbreak affecting domestic animals was reported in 29 out of 69 districts across six of the eight provinces of Kenya (Munyua *et al.*, 2009) and by the end of the outbreak in March 2007, 155 people died out of 684 reported cases (Dijkman *et al.*, 2009). Labeaud *et al.* (2008) and Munyua *et al.* (2010) reported the seroprevalence of RVF at 13% and 27.8% respectively in Kenya using the data of the 2006-07 outbreaks. Although Uganda is located in endemic zone of RVF disease in the list of East African countries, the outbreak of 2006-07 did not affect her (Magona *et al.*, 2009). Other countries that suffered RVF disease outbreaks during 2006-08 are Somalia, Sudan, Swaziland, South Africa and Islands in the Indian Ocean (Comoros, Mayotte and Madagascar) (De La Rocque and Formenty, 2009). In South Africa the disease persisted from 2008-11 and especially in the Free State and Northern Cape Provinces that were the most affected areas by floods (NICD, 2010; 2011).

1.2 Study Justification

Rift Valley fever remains to be a threat to livestock keepers and nations where the disease is occurring due to its major economic implications through the costs of the measures taken at individual, collective and international levels in order to prevent or control infection and disease outbreaks (Otte *et al.*, 2004; Dijkman *et al.*, 2009). The effects of infections on human health are usually greatest on herdsmen and farm workers who live in close proximity to their animals, veterinarians, abattoir workers and butchers as an occupational hazard by direct handling of infected animals and their products (Ericsson and Steffen, 2001; Aradaib *et al.*, 2008; FAO, 2009).

The socio-economic impacts, caused by morbidity and mortality of livestock and disruption of livelihoods, markets, and the meat industry that resulted into a ban of livestock slaughter and export of animals and animal products in Tanzania were not studied thoroughly during the 2006-07 outbreaks. In East Africa, during the outbreak, a detailed molecular epidemiology in domestic animals using RT-PCR antigen detection was done in Kenya only (Pepin *et al.*, 2010) while in Tanzania molecular epidemiology studies were done only in human (Mohamed *et al.*, 2010). There is lack of information on the factors associated with occurrence of RVF and the circulating RVFV field strains in Tanzania which could help vaccine matching. Thus, the main aim of this study was to asses awareness, socio-economic impact of RVF and factors associated with the occurrence of RVF as well as the circulating RVF virus strains (and their antigenic characteristics) in endemic and epidemic settings of Tanzania using the 2006-07 outbreaks as a case study. Since natural outbreaks of RVF disease are sporadic, explosive with a very small window to allow effective planning and proper management of the disease during the outbreaks, the information obtained in this study will help the government to design preparedness programmes for effective control strategies for RVF disease. This in turn will impact positively on the livelihoods of livestock keepers who either depend on sales of live animals in the pastoral areas or those who keep dairy cattle and lose milk revenue whenever there is an outbreak of RVF disease.

1.3 Study Objectives

1.3.1 Overall objective

To carry out the molecular epidemiological investigation of RVFV and establish the socio-economic impact of RVF during the 2006-07 outbreaks in Tanzania as a case study.

1.3.2 Specific objectives

i. To establish the socio-economic impact of RVF disease in selected areas which were affected by the 2006-07 outbreaks in Tanzania

ii. To assess the knowledge, attitudes and practices to RVF disease control practices in selected areas of Tanzania

iii. To establish the seroprevalnce of RVFV infection in cattle from selected areas of Tanzania

iv. To determine the presence of RVFV in serum bank samples collected during 2006-07 RVF outbreaks.

1.3.3 Study hypothesis

i. H0: There were no socio-economic impacts of RVF disease in areas that were affected during 2006-07 outbreaks in Tanzania

HA: There were socio-economic impacts of RVF disease in areas that were affected during 2006-07 outbreaks in Tanzania

ii. H0: There is poor knowledge in the community for proper handling and management of RVF disease in Tanzania

HA: There is good knowledge in the community for proper handling and management of RVF disease in Tanzania

iii. H0: There were no seropositive cases from serum samples that were collected during 2006-07 in different parts of Tanzania

HA: There were seropositive cases from serum samples that were collected during 2006-07 in different parts of Tanzania

iv. H0: There was no RVFV in the serum samples collected in various parts of Tanzania during 2006-07 RVF outbreaks.

HA: There was RVFV in the serum samples collected in various parts of Tanzania during 2006-07 RVF outbreaks.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rift Valley Fever (RVF)

Rift Valley fever (RVF) is an acute mosquito-borne viral zoonotic disease affecting domestic animals and humans leading to great losses socially and economically. Storm abortions in ruminants, high mortality approaching 100% in young animals and severe influenza-like illness in human being are the alarming signs of disease (FAO, 2003; Davies and Martin, 2006).

2.2 Rift Valley Fever Virus (RVFV)

The causative agent of RVF is the RVFV, a member of *Bunyaviridae* family, *Phlebovirus* genus and species is *RVFV* (Lopez *et al.*, 1995, Elfadil *et al.*, 2006; Andriamandimby *et al.*, 2010). The RVF virions are enveloped, slightly pleomorphic, composed of a lipid bilayer containing the Gc and Gn glycoproteins forming surface sub-units, 5–8 nm in length, regularly arranged on its surface, measuring 8–120 nm in diameter (Habijan *et al.*, 2008; Bouloy and Weber, 2010; Labeaudi, 2009).

The viral genome contains single-stranded negative or ambisense RNA polarity composed of three segments designated as S (small), M (medium), and L (large), packaged together in the virion in the form of RNP (Bird *et al.*, 2007b; Habijan *et al.*, 2008; Nachiket *et al.*, 2010). Each segment is enclosed in a separate nucleocapsid within the virion (Garcia *et al.*, 2001; Labeaud, 2009; Pepin *et al.*, 2010). The S segment codes for two proteins, namely; the nucleoprotein N and the non-structural NSs protein using an ambisense strategy and the L segment codes for

the 237-kDa viral RNA-dependent RNA polymerase in a single 6.4-kb open reading frame (ORF) (Muller *et al.*, 1991; Sall *et al.*, 1998; 1999; Bird *et al.*, 2007b; Bouloy and Weber, 2010). Whereas, M segment encodes at least four viral proteins in a single (ORF): the 14-kDa NSm of unknown function, two major envelope surface glycoproteins, the Gn and Gc (named the 55-kDa Gn and 58-kDa Gc synthesized as part of polyprotein precursors), and a 78-kDa fusion of the NSm and 14-kDa protein NSm of unknown function (Acardi *et al.*, 2001; Habjan *et al.*, 2008; Holman *et al.*, 2009; Lagerqvist *et al.*, 2009). Upon infection, RVFV attaches to specific receptors and enters susceptible host cells by low pH-primed endocytosis (Pepin *et al.*, 2010). The virus replicates in the cytoplasm of infected cells and virions mature by budding in the Golgi compartment (Habjan *et al.*, 2008; Liu *et al* 2008; Labeaud, 2009; Bouloy and Weber, 2010). During the replication cycle, each segment is transcribed into mRNA and is replicated by synthesis of the exact copy of the genome, called complementary RNA (cRNA) or antigenome (Liu *et al.*, 2008; Bouloy and Weber, 2010; Pepin *et al.*, 2010).

2.3 Transmission of RVF Disease

RVF is transmitted by at least 30 species of mosquitoes, where *Aedes* sp believed to be the major reservoir and vector; however *Culex* sp and *Mansonia* sp also are important vectors (Australian Government, 2007; Aziz, 2008; Labeaud, 2009; Kahlon *et al.*, 2010) which come in later during the outbreak. Figure 1 shows the theoretical life cycle of RVF where there are still unknown vectors transmitting the disease. In the periods between epidemics, the virus is believed to be dormant in eggs of the mosquito *Aedes mcintoshi (linneatopennis)* in the dry soil of grassland

depressions ('*dambos*') (Bird *et al.*, 2008; Breiman *et al.*, 2008) and hatches during heavy rainfall where flooding occurs (Bird *et al.*, 2008; Nachiket, *et al.*, 2010). Humans are also readily infected through aerosols from infected animals when humidity is high, or by exposure to infected animal tissues, aborted foetuses, mosquito bites, consumption of raw milk, and have the potential to introduce the disease (via mosquitoes) to animals in uninfected area (Ericsson and Steffen, 2001; Holman *et al.*, 2009; Nachiket *et al.*, 2010).

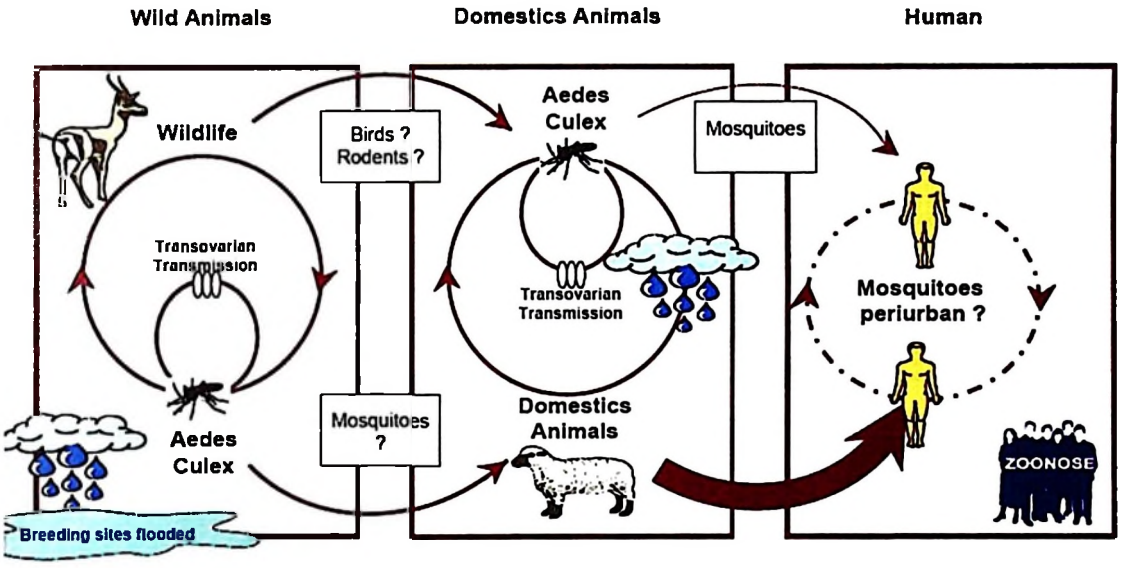

Figure 1: Theoretical life cycle of RVFV transmission

Source: Davies and Martin (2006)

2.4 Geographical Distribution of RVF Disease

2.4.1 RVF disease distribution in the world

RVFV was first isolated in 1931 near Lake Naivasha in Kenya by Daubney and his colleagues (Sall *et al.*, 1998; FAO, 2003; Ikegami and Makino, 2009; Labeaud, 2009), where they observed a number of abortions in ruminants and the presence of

a hyper acute lethal infection, characterized by necrotic hepatitis in lambs, and proposed the name Rift Valley fever (RVF) for the disease (Aziz, 2008). Countries which have shown to have endemicity and substantial outbreaks of RVF disease include Gambia, Senegal, Mauritania, Namibia, South Africa, Mozambique, Zimbabwe, Zambia, Kenya, Sudan, Egypt, Madagascar, Saudi Arabia, Yemen (Thiongane *et al.*, 1991; CDC, 2003; Bird *et al.*, 2008; Kahlon *et al.*, 2010; Nachiket *et al.*, 2010) whereas those known to have some cases, periodic isolation of virus, or serologic evidence of RVF disease are Botswana, Angola, Democratic Republic of the Congo, Congo, Gabon, Cameroon, Nigeria, Central African Republic, Chad, Niger, Burkina Faso, Mali, Guinea, Tanzania, Malawi, Uganda, Ethiopia, Somalia (CDC, 2003; Nachiket *et al.*, 2010).

During the rainy season of 2008/09, there were repeated outbreaks of the disease in Madagascar and analysis of partial sequences from RVFV strains showed that all were similar to the strains that circulated in Kenya during 2006–07 outbreaks (Andriamandimby *et al.*, 2010). The last reported outbreak of RVF disease took place early in 2011 in South Africa (NICD, 2010; 2011).

2.4.2 Spatial distribution of RVF outbreak of 2006-07 in Tanzania

In Tanzania the RVF outbreak of 2006-07 started on northern parts of Tanzania in Ngorongoro and Moduli districts, and by the end of the outbreak in June 15, 2007 it had affected 10 of the 21 regions of the Tanzania mainland and 25 of 126 districts (Swai and Schoonmanb, 2009; Mohamed *et al.*, 2010).

Table 1: The regions and districts that were affected by the 2006-07 RVF outbreaks in Tanzania

Regions	Districts
Mwaza	Magu and Kwimba
Arusha	Ngorongoro, Monduli, Longido and Arumeru
Manyara	Babati, Simanjiro, Hanang and Kiteto
Tanga	Mheza
Dodoma	Dodoma, Kondoa, Kongwa and Mpwapwa
Singida	Singida, Iramba and Manyoni
Morogoro	Kilosa, Morogoro, Kilombero and Ulanga
Iringa	Iringa Rural
Pwani	Bagamoyo, Kibaha and Rufiji and Mkulanga

Source: Mohamed et al., 2010

Since RVF virus replicates in a wide range of mosquito vectors, Swai and Schoonmanb (2009) reported the great chance of the virus spread to non-endemic areas of Tanzania especially due to inter-epidemic virus activity.

2.5 Clinical Signs of RVF

2.5.1 Clinical signs in animals

A sudden onset of storm abortions among sheep, goats, cattle or camels over a wide area is the most important sign; sudden deaths and disease with many fatalities in all species (approaching 100% in young lambs of susceptible breeds), high fever, lymphadenitis, nasal and lachrymal discharges in mature animals, profuse fetid diarrhoea (often haemorrhagic), vomiting, abdominal colic, severe prostration, dystocia, some teratology, hydrops amnii; anorexia, dysgalactia, jaundice, haemorrhagic enteritis are other signs that may be observed (FAO, 2000; Davis and Martin, 2006; Aziz, 2008; Holman *et al.*, 2009; Nachiket *et al.*, 2010).

2.5.2 Clinical signs in human

In humans incubation period is 2-6 days. Rift Valley fever in people may not show any clinical sign (in-apparent), or may have mild influenza-like illness, symptoms. Others may have fever (37.8–40 °C), haemorrhage, headache, muscular pain, vomiting, epigastric discomfort, extreme weight loss, encephalopathy and photophobia (Australian Government, 2007; Bird *et al.*, 2007a; Liu *et al.*, 2008; Mohamed *et al.*, 2010) may be seen. Mortality is about 1% and course of disease is 4 to 7 days leading to full recovery in 2 weeks (AUSVETPLAN, 1996; FAO, 2000; Aziz, 2008; Nachiket *et al.*, 2010).

2.6 Diagnosis of RVF Disease

Diagnosis of RVF disease is based on clinical signs, the plaque reduction neutralization test and complement fixation test in tissue culture; hemagluttination inhibition test (HAI), indirect immunofluorescence assay and Enzyme-linked immunosorbent assay (ELISA) (Aradaib *et al.*, 2008; OIE, 2008). Recently molecular techniques such as, Enzyme-linked immunosorbent assay (FAO, 2003; Paweska *et al.*, 2005; Wilson *et al.*, 2009) and nucleic acid sequencing have been developed for rapid detection and identification of viral RNA of RVFV (Paweska *et al.*, 2005) and are more sensitive, reproducible, applicable and provides a promising option for diagnosis and detection of viral RNA of RVFV (Le Roux *et al.*, 2008; OIE, 2008; Vuren and Paweska, 2009). Since antigen and IgM detection by RT-PCR and ELISA respectively are more rapid than virus isolation, Sall *et al.* (2002) recommended using them in parallel as a first-line diagnostic method for RVFV when an outbreak occurs.

2.6.1 Enzyme-linked Immunosorbent Assay (ELISA)

Serodiagnosis of RVF relies on the use of live or inactivated whole virus as antigens (Fafetine *et al.*, 2007). ELISA is the most widely used serological test and employs an inactivated antigen. It has been proved to be very specific and sensitive and by comparing with virus neutralisation and HAI, ELISA is more sensitive in the detection of early IgG responses in infected and vaccinated animals. It is less expensive, rapid and well suited to the needs of large scale testing, and as primary assays they can be well standardised, quality controlled and automated (Paweska *et al.*, 2003; 2005; Zaki *et al.*, 2006). Also the test can be used in RVF-free countries due to the use of inactivated antigen.

2.6.2 Reverse transcription polymerase chain reaction (RT-PCR)

This method is used to determine whether a specific DNA sequence is present in the sample containing RNA genome. The procedure of RT-PCR starts with transcription of RNA using transcriptase enzyme to form complementary DNA (cDNA) from which the regular PCR procedure proceeds. It is a highly sensitive technique for detection of RNA in the sample (Ma *et al.*, 2006). Reverse Transcription-PCR can be performed in one-step like in normal PCR where a single reaction mix for both cDNA synthesis and PCR occurring in the same tube or two-step where they are carried separately (Bustin, 2000; Ma *et al.*, 2006). Since RVF antibodies may not be detectable during the first few days of disease and the viremia often reaches high titers after several days, detection of viral genome or antigen by RT-PCR may be the method of choice. The test is highly accurate, rapid, and very simple nucleic acid detection format that can be used during the outbreak of RVF disease for early

diagnosis before production of antibodies (Shoemaker *et al.*, 2002; Vuren *et al.*, 2007; Le Roux *et al.*, 2008).

However, employing both tests (IgM-capture ELISA and one-step RT-PCR) to screen serum samples for IgM antibodies and subsequently identify the RVF virus genome in the IgM-positive samples has been recommended (Elfadil *et al.*, 2006).

2.7 Molecular Epidemiology of RVF

The overall genetic diversity of RVFV strains is low, with maximum difference of 5% and 2% at nucleotides and amino acid level respectively of the S, M and L segment (Table 2). This makes the molecular diversity of RVFV strains rather small despite the fact that segmented RNA viruses are expected to be unstable with high diversity. However, this has not been the case for RVFV as it has shown to be genetically stable probably due to low mutation tolerance and there is no statistical significant evidence of recombination (Bird *et al.*, 2007). Studies carried out by Bird and his colleagues (2008) demonstrated a direct link between 2006-07 RVF outbreaks with that of 1997-98. Another study carried out by Bird and his colleagues in 2007, obtained 33 ecologically and biologically different strains of RVFV showing to have a common ancestor isolated in 1800s during colonial rule in Africa. It has also being shown that there is an ongoing RVFV evolution and activity during the inter-epdermic period indicating potential public health and veterinary impact of the re-emergence of a single RVFV lineage leading to large epidemic events (Bird *et al.*, 2008).

Table 2: Maximum pairwise sequence identity differences in the 33 strains for S, M and L segment of RVFV

Maximum pairwise sequence identity differences	Difference at nucleotide level (%)	Difference at amino acid level (%)
S segment	4	1
M segment	5	2
L segment	4	1

Source: Bird *et al.* (2007)

2.8 Socio-economic Impact of RVF

RVF results in severe economic losses due to high mortality particularly in new-born lambs and kids and abortion in adult sheep, goats and cattle (Fafetine *et al.*, 2007). Catastrophic socio-economic and significant human disease consequences as a result of: delayed detection and response, lack of emergency plans, poor risk communication and inadequate information flow, inadequate collaboration between the sectors and lack of emergency fund were the lessons learnt from the 2006-07 RVF outbreak in East Africa (Breiman *et al.*, 2008; ILRI, 2009; Jost *et al.*, 2010; Munyua *et al.*, 2010).

2.8.1 Control and prevention of RVF disease

The most reliable method for controlling RVF disease is based on mass livestock vaccination programmes during inter-epidemic periods using modified live (attenuated) Smithburn neurotropic strain (SNS) vaccine (Davies and Martin, 2006; Ikegami and Makino, 2009). The vaccine confers immunity lasting for three years as compared to inactivated vaccine which require monthly and or/annual booster dose (FAO, 2003; Holman *et al.*, 2009; Nachiket *et al.*, 2010). Control of animal movements in and out from one local area or through the national borders should be prohibited immediately on suspicion of the disease (FAO, 2000; Aziz, 2008). After

introduction of RVF to a new area, effective quarantine and movement controls are essential to reduce spread, even if the virus has become established in an insect vector population (FAO, 2000; Azhar *et al.*, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

For awareness and socio-economic impact of RVF, the study was carried out in Arusha and Manyara where pastoral farming is practiced and Morogoro where dairy and agro pastoral farming systems are practiced in Tanzania (Fig. 2). Arusha, Manyaara and Morogoro have an altitude ranging from 482 to1368 m above sea level and are among the areas that experienced RVF outbreaks in 2006-07. These areas normally experiences two rainy seasons: a short rainy season between October and December, and a long rainy season between March and May. Typically, the annual precipitation averages between 500 and 1000 mm. The vegetation consists mainly of various shrubs and acacia bushes, and livestock species kept are primarily cattle, goats and sheep.

The Molecular epidemiological study, used archived sera samples collected during the outbreak of 2007 from cattle, goats and sheep in Mwanza, Arusha, Manyara, Dodoma, Tabora, Lindi, Mtwara, Iringa, Mbeya, Pwani and Tanga. Sera were collected under the supervision of Veterinary Investigation Centres (VICs) of Mwanza, Temeke, Tabora, Mpwapwa, Mtwara and Iringa serving for Northern, Eastern, Western, Central, Southern and Southern Highlands, respectively. Data analysis and report writing were done at Sokoine University of Agriculture, Morogoro and at Tanzania Veterinary Laboratory Agency (TVLA) in Dar es Salaam, Tanzania (Fig. 2).

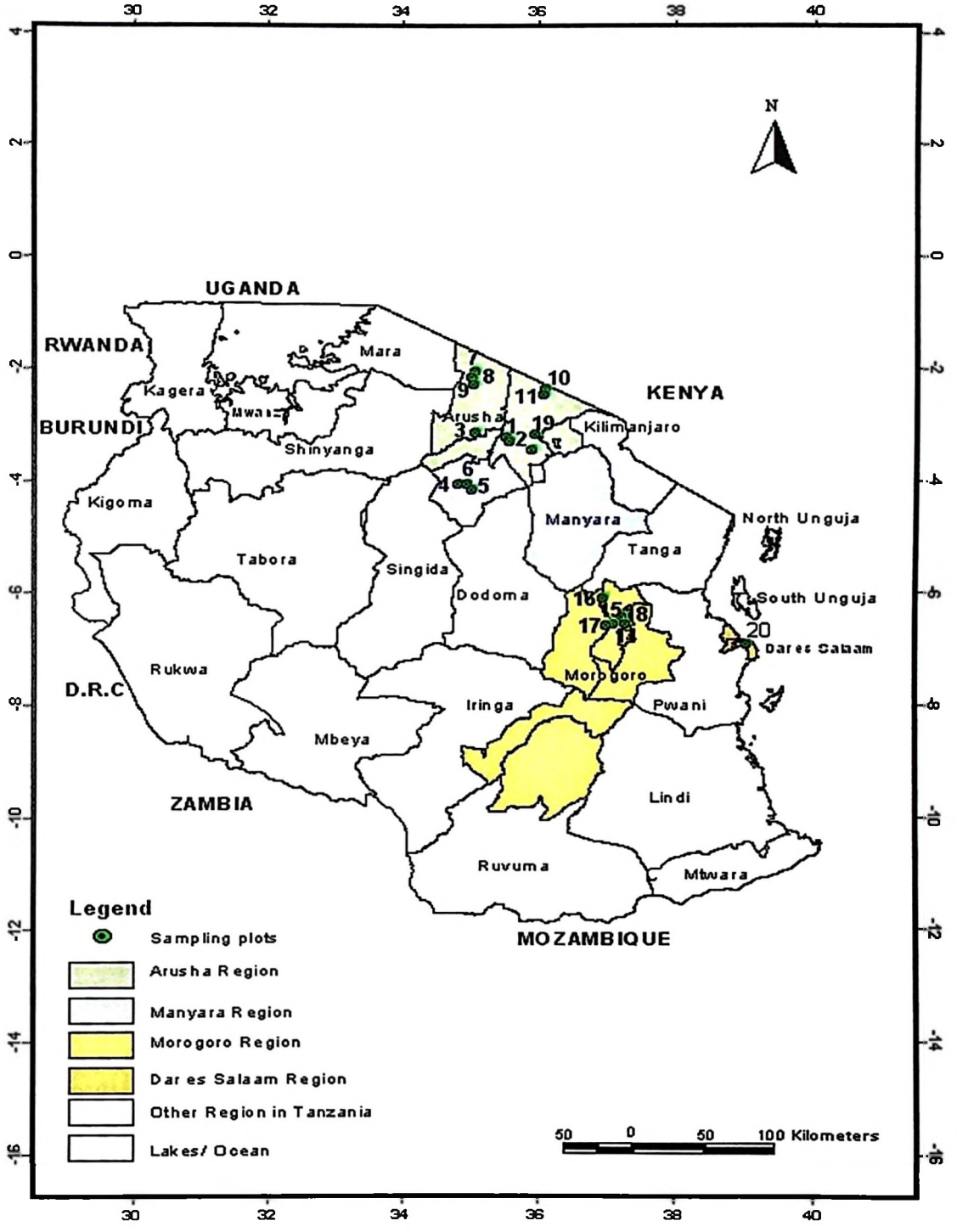

Figure 2: Map of Tanzania showing study areas: Number 1 to 19 shows areas where socio-economic study was conducted and number 20 shows location where stored samples were obtained.

3.2 Research Design

A cross-sectional study design that allows data to be collected at a single point in time was used in this study. The study was divided into two main areas: the socio-economic significance of RVF and the molecular epidemiological study of the virus. The following formula for sample size estimation as proposed by Naing and his colleagues (2006) was used for socio-economic study;

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where n = sample size, Z = Z statistic for a level of confidence of 95% (1.96), P = expected prevalence (0.13), and d = precision (0.05). This gave a total number of 174 livestock keepers.

3.3 Socio-economic Study

3.3.1 Data collection

During the study, quantitative data were collected using questionnaire while qualitative data were collected using in-depth interviews with key informants and focus group discussions.

Quantitative data: The questionnaire survey (Appendix 3) used 74 randomly selected respondents with age ranging from 21-79 years old. The participants were interviewed on socio-economic and cultural activities, type of livestock kept and livestock involvements regarding RVF disease impact on household livelihood activities. The seasonality of both human and livestock RVF issues, trade and marketing in livestock, their product and their perception on the occurrence of RVF on their livelihoods were assessed. The study determined the social organization of

production; livelihood constraints; household's income sources and average monthly expenditure; number of livestock-holding households and stock of livestock in the households including data on number, species and breeds as well as the quantity of livestock products (milk, meat, manure, traction power) produced and marketed by livestock keeping households through the year. Furthermore the community based knowledge for management of RVF was established to explore on what the community know and what was implemented during the 2006-07 outbreak. The interviews also collected descriptions of the clinical presentation of RVF in people and livestock, as well as its incidence relative to other diseases.

Qualitative data: These were general information about livestock diseases (disease outbreaks, specific view on RVF disease, control, and its significance to livestock, and government involvement to control of RVF). Also issues on livestock regulations and reasons for success and failure to implement recommended management procedures were explored during in-depth interviews. In-depth interviews were conducted with district veterinarians, veterinary investigation centre officers and LFOs who had been involved in the management of the 2006–07 outbreaks. The study also used focus group discussions (FGD) with agro-pastoralists and pastoralists in some villages where questionnaire was administered. The focus groups involved between 5 to 12 people, most of whom were men and most were ethnically Maasai with few Mbulu, Barbaig and other tribes. The focal group participants were interviewed on their economic and cultural activities, knowledge on the impact of livestock diseases and their management, responsibility for disease control, awareness on outbreak diseases especially RVF and how the community obtain general information about outbreak of diseases.

Data from government offices: Information on the areas affected by RVF, total number of animals died and aborted, emergency plans and the stake holders involved during the outbreak were obtained from district and regional veterinary offices, Arusha Veterinary Investigation Centre, Tanzania Veterinary Laboratory Agency and the Ministry of Livestock Development and Fisheries.

3.4 Molecular Epidemiological Study

3.4.1 Sample collection

Serum samples were collected by livestock field officers during the 2006-07 outbreaks and transported in cool boxes packed with ice packs to TVLA where they were stored between -50°C and -35°C.

3.4.2 RVF inhibition ELISA

Screening of serum samples for detection of IgG antibody levels against RVFV was done using RVF inhibition ELISA as described previously (Paweska *et al.*, 2005). Briefly, plates were coated with 100 µl polyclonal sheep anti-RVFV capture antibody and washed after incubation at 4°C overnight. Plates were blocked by adding 100 µl of 10% skim milk in PBS followed by 100 µl of serum-virus antigen mixture to rows of the top half of the plates (rows A-D: 1-12) and 100 µl of serum-control antigen mixture to the bottom half of the plates (rows E-H: 1-12), respectively. Then 100 µl of rabbit anti-virus rN, 100 µl of anti-rabbit IgG Horse raddish peroxidase (HRPO)-conjugate and 100 µl of 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) peroxidase substrate were added in series. Finally the reaction was stopped using concentrated sodium dodecyl sulfate (SDS) and optical density (OD) measured at 405 nm. Specific activity of each serum

(net OD) was calculated by subtracting the non-specific background OD in the wells with control antigen from the specific OD in wells with virus antigen. The mean OD readings for replicate tests were converted to a percent inhibition (PI) value using the equation: [100-(mean net OD of test sample/mean net OD of negative control) x 100]. Cut-off values (expressed as a PI of an internal negative control) used was 41.9, 41.4 and 38.4 for cattle, goat and sheep respectively. In this test, 200 sera were analysed for detection of IgG for RVF.

3.4.3 Extraction of RVFV RNA

Genomic viral RNA was extracted directly from 140 µl of 108 sera, 79 samples being positives from RVF Inhibition ELISA test above while 29 being negatives by using QIAamp® Viral RNA Mini Kit (QIAGEN, Germany) in accordance with the manufacturer's instructions. Briefly, 140 µl of sera were added to 560 µl of lysis buffer containing carrier RNA followed by incubation for 15 minutes. The lysis-buffer sample mixture was centrifuged then 560 µl of absolute ethanol were to precipitate protein added followed by another low speed centrifugation.

Afterwards, 630 µl of the mixture above was transferred in the QIAamp Min spin column mounted in a 2 ml collecting tube and centrifuged at 6 000 x g for 1 min (repeated two times to finish the mixture). The column was washed twice by adding 500 µl of wash buffers (AW1 and AW2) each time in a new collecting tube and centrifuged at 6 000 x g for 1 min and full speed at 20 000 x g for 3 min respectively. Finally, the column was transferred in a clean 1.5 microcentrifuge tube where RNAs were eluted by 60 µl of elution buffer containing sodium azide

equilibrated to room temperature, incubated for 1 min at room temperature and centrifuged at 6 000 x g for 1 min. The extracted RNA was then stored at -20°C until its use.

3.4.4 Reverse transcription polymerase chain reaction (RT-PCR)

A one-step RT-PCR protocol by Salim *et al.* (2010) was used with modification for synthesis and amplification of DNA. A reaction mix was prepared in a single tube by adding 12.5 µl of 2x reaction mix containing dNTPs and $MgSO_4$, 1.0 µl of 25x reverse transcriptase-Taq polymerase mix, 2.0 µl of 30 nanomoles of each M segment gene-specific oligonucleotide primer RVF1 (5'-GACTACCAGTCAGCTCATTACC-3') and RVF2 (5'-TGTGAACAATGGCATTGG-3'), and 1.7 µl of enhancer. The total volume of the reaction mix was brought to 20.0 µl by adding RNase free water. Target RNA (5.0 µl) was added last to make a final volume of 25 µl, ready for RT – PCR amplification. The cycling conditions were set at 37°C for 30 min (a reverse transcription step), an initial denaturation of 10 min at 95°C followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s, extension at 72°C for 30 s, a final extension of 10 min at 72°C and finally holding at 4°C. A live attenuated freeze dried Rift valley fever viral vaccine (RIFTVAX™, Nairobi, Kenya) and RNase free water were used as positive and negative controls respectively.

Visualization of PCR products: PCR products were visualized and photographed after running agarose gel electrophoresis for 45 min at constant voltage of 100 volts and placed on an ultraviolet trans-illuminator. To achieve this, a gel tray was

prepared by sealing the ends with soletape and the comb inserted into the proper position. Agarose gel was prepared by adding 2 g of agarose to 200 ml of 1x Tris-acetate-EDTA (1x TAE) buffer (1% gel). The solution was heated in a microwave oven until the agarose appeared completely melted. After cooling to approximately 60°C, 5 µl of Ethidium bromide solution was added and mixed by shaking gently. The melted agarose was poured into the gel tray and left to solidify. Tapes from the tray were removed carefully and the tray was placed in the tank after which 1x Tris-borate-EDTA (1x TBE) buffer was added until the gel was covered. Combs were removed carefully to leave clear wells.

One microlitre of 10x loading buffer was added to each tube containing 9 µl of PCR amplified products and this mixture (10 µl) from each tube was loaded in a respective well, except the first well which was loaded by 5 µl of Molecular Weight DNA marker (2 kilo base pair). Finally, the power supply was connected and DNA samples moved towards a positive electrode.

3.5 Data Analysis

In this study, Statistical Package for Social Science (SPSS) version 17.0 was used for descriptive analysis (means, frequencies) and comparing the proportions for data collected using questionnaire. The MAXQDA 10 was used for analysis of focus group discussion transcripts. Analysis of variance (ANOVA) was used to compare means between populations.

Gel electrophoresis of PCR products was performed for analyzing reaction quality and yield. The products was electrophoresed in 1.0% agarose gel and a band of

approximately 551 bp corresponding to the gene encoding M protein was visualized on ultraviolet trans-illuminator with ethidium bromide stained agarose gels. Before analysis, quantitative data were entered, organized, coded and collated in Microsoft excel and qualitative data were entered in Microsoft office (Microsoft office 2010) and then imported to the respective analytical tool.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-Economic Study

4.1.1 Socio-economic activities and benefits

In this study, 15 households reported to be purely pastoralists and 59 to be agro-pastoralists and their main sources of income being livestock keeping 73 (98.6%), agriculture 58 (78.4%), business 18 (24.3%) and employment 26 (35.1%). The categories of livestock kept by majority of people in the area are cattle, goats, sheep and chickens and those kept by minority are donkeys, pigs, dogs and cats (Table 3). In this study 67.6% of the respondents reported to have inherited animals from their livestock keeping families.

Local breeds (Table 3) were kept by the majority livestock keepers. Cattle were the domestic animals that made by far the greatest contribution to livestock-based livelihoods in the study area. In the case of agriculture, crops that were cultivated included maize, beans, banana, potatoes, rice, finger-millet, sorghum, green gram, sunflower, pigeon peas, cow peas, chick peas, cassava, onions, and vegetables. Among the mentioned sources of income, livestock keeping gave them more income 53 (71.6%) followed by agriculture 12 (16.2%), while 9 (12.2%) thought that both livestock keeping and agriculture had equal contribution to income generated.

Table 3: Type of animals kept in the study area

Type of animal	Animals kept (n=74) Frequency (%)	Kept by Majority(n=74) Frequency (%)
Cattle	94.6	93.2
Goats	90.5	93.2
Sheep	78.4	78.4
Chickens	37.8	25.7
Donkeys	41.9	38.5
Dogs	27.0	6.8
Cats	16.2	2.7
Pigs	5.4	1.4

The minimum, average and maximum expenditure per month of livestock households in the study area were found to be 25 000, 120 000 and 3 000 000 Tanzanian shillings (TZS) respectively at the rate of US$ 1 to 1500 TZS. The highest expenditure was observed in Morogoro (from a pastoralist) and the lowest being in Manyara region from an agro-pastoralist (Fig. 3). Expenditure levels were not significantly different between the regions (P=0.414) and within the regions (Arusha P= 0.0564, Manyara P= 0.0668 and Morogoro P= 0.3522).

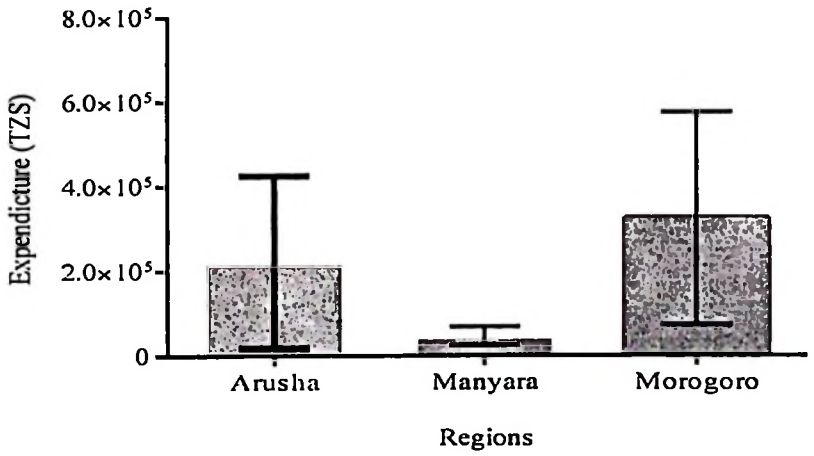

Figure 3: Average monthly expenditure of livestock households in the study area (TZS).

The main benefits derived from livestock keeping were reported to be food (meat, milk, ghee, and fat), socio-cultural roles such as paying dowry, school fees and buying school needs, draft power, buying household requirements and health (Table 4). Other benefits included transport for donkeys, skins, manure for crop production and building houses.

Thirty (40.5%) livestock keepers used manure for crop production and the majority left it in the yard (47, 63.5%); few disposed off (11, 14.9%), sold to others (5, 6.8%) or use for decorating their houses (10, 13.5%). The amount of manure used for crop production ranged from 100 kg to 20 tonnes annually. Drought cattle were used for cultivation and or tracking luggage for an average of 3 to 5 hours per day. The average amount of milk obtained per household per day was 19.4 litres; the amount differed from one household to another depending on the number of animals kept. Only 36 (48.6%) sold their milk for a price ranging from 300 to 2000 TZS (US$ 1 to 1500 TZS). Livestock keepers earned most of their income from selling animals. The price before, during and after RVF outbreak of 2006-07 varied significantly depending on the animal species (Table 8).

Table 4:The purpose of keeping livestock as reported by livestock keepers

	Arusha (n=36)	Manyara (n=16)	Morogoro (n=22)
Advantage	%	%	%
Paying dowry	81	44	64
School needs	97	94	95
Food	100	94	100
Agriculture	67	56	5
HHR	100	88	100
Health care	97	81	100
Transport	25	13	5
Get manure	8	25	49

HHR = Household requirements

4.1.2 Livelihood constraints

The main constraints in the area were animal diseases, drought, inadequate pasture, water availability and lack of dipping tanks. Important diseases in the area include East Coast fever (ECF), Malignant catarrhal fever (MCF), Trypanosomosis, Contagious Bovine Pleuropneumonia (CBPP), Contagious Caprine Pleuropneumonia (CCPP), Peste des Petits Ruminants (PPR), RVF, Fasciolosis, Helminthosis, Anaplasmosis, Babesiosis, Anthrax, three day sickness, Myiasis, Foot and Mouth Disease (FMD), Lumpy Skin disease, Heartwater, Black quarter (BQ) and brucellosis. Among the mentioned diseases, the following were declared by the majority to cause great impact to their animals; ECF, Trypanasomosis, CCPP, Myiasis and CBPP (Appendix 1). Two (22.2%) villages during focus group discussions reported to use dipping tanks and six (66.7%) to use spray pumps to control vector borne diseases. As reported by the FGD, inadequate water and pasture in 2009 caused high mortalities in animals. Consequently, pastoralists were forced to become agro-pastoralists in northern part of Tanzania in order to cope with the losses.

4.1.3 History of RVF in the study area

The District Veterinary Officer (DVO) in Ngorongoro District first reported an abnormal disease (suspected RVF) in Ngorongoro district to the Arusha VIC on 21st January 2007 suspecting to be RVF. Copies were sent to the Director of Veterinary Services (DVS) in Dar es Salaam, Regional administrative secretary (Arusha) and to the District Council Executive Director (Loliondo). This official report was followed by the local investigations done by the District Veterinary Officer. Reported cases from the livestock keepers in the district were after observing cases of abortions that

started in December 2006 during rainy season. After epidemiological and clinical investigations by the veterinary district office, areas which had massive abortions and deaths in animals included; Pinyinyi, Monic, Engaresero, Matale A and B, and Malambo in Ngorongoro district. The first three villages lie along the floor of the rift valley along shores of Lake Natron (594-637 m above sea level) while Malambo and Matale A and B villages are on the escapement of the rift valley and all affected villages had heavy rainfall that started in December 2006. Engaresero village was also the first area to report RVF in 1998 outbreak. A team of experts from the VIC (Arusha) were sent in the suspected areas in the district to carry out investigations and to collect specimens from suspected clinical cases of RVF. Specimens were dispatched to Onderstepoort Veterinary Institute, South Africa and at TVLA (Tanzania) where both laboratories confirmed RVF based on samples submitted. Apart from Arusha, there were other areas in the country that at the same time were reporting unusual abortion cases in sheep and goats. Cases were reported in Manyara, Kilimanjaro, Tanga, Dodoma, Iringa and Morogoro regions and at different time intervals. The first two human RVF suspected cases were admitted on 28th January 2007 at Mount Meru hospital being from Terrat (Simanjiro district) and Makuyuni (Monduli district) in Manyara region. Sadly both of them died on 31st January 2007. Samples from these two patients were carefully collected and sent for detailed diagnosis to the Centers for Disease Control and Prevention (CDC) laboratory in Nairobi, Kenya and both were confirmed to be positive for RVF. Rift Valley fever was officially declared to the community in the country and OIE on February 7 and 12, 2007 respectively.

4.1.4 Governmental emergency plan to control RVF outbreak of 2006-07

After the involvement of human confirmed cases, an inter-ministerial meeting was held in Arusha in early February 2007, after which the District Commissioners were given tasks to prepare strategies to control the disease in their districts. One of the strategies was to provide education to the community on clinical appearance of the disease, spread of disease (transmission) and the effect of disease to human and their animals. Also education was given in slaughter premises to all people who were involved in handling and slaughtering animals. Livestock keepers were emphasized to make sure they did not move animals from one village to another and that they were to participate fully in vaccination campaigns.

During this time the government was ordering vaccines abroad and organizing funds and human resources. The first vaccine doses were received by the government in the end of February 2007, 116 600 were distributed to districts with reported cases namely Monduli, Ngorongoro, Simanjiro, Longido, Hai, Babati, Mkinga and Kilosa. Also equipments and funds to run vaccination campaigns were provided by the Government. Additional, 370 400 vaccine doses were distributed to all districts as well as in other two districts namely Iringa rural and Mvomero in which RVF cases were reported. Vaccination campaigns to animals started early on March 2007 to all ages of cattle, sheep and goats except those under six months and pregnant animals were vaccinated. Emphasis was put on sheep and goats when the amount of vaccine was not enough. Vaccinations started on the high risk areas for RVF and ended on the low risk areas based on the known history of RVF outbreaks.

4.1.5 Community based knowledge on handling and control practices of RVF

The government used the community meetings to educate people on the presence of RVF disease in the country, how people get the disease and preventing them from eating uninspected meat. All people involved in slaughtering animals or handling slaughtered meat and livestock products were told to take all the necessary precautions. Great emphasis was given to livestock keepers to send their animals for vaccination. The community in the study area in addition received information on managing common and new diseases in the area from radio, few from LFOs and local government authorities. During the study, 69 (93.2%) reported to have heard about RVF in their life time and only 27 (36.5%) knew that it was an outbreak disease. Also 26 (35.1%) of the respondents reported that RVF disease outbreak happened in the study area and only 22 (29.7%) indicating the exact year of the last outbreak of 2006-07 with few (3, 4.1%) reporting the 1997-98 and majority failed to remember the year of the last outbreak. When asked on how RVF manifest in animals, some were able to mention the following signs; storm abortions, high fever, high mortality in lambs and kids, ocular and nasal discharge, haemorrhagic diarrhoea, vomiting, abdominal pain, jaundice and body swelling (Table 4).

Respondents who reported to have the disease in their household were the one who could remember significantly the clinical signs of the disease (P=0.002) and especially storm abortions, high fever, high mortality in young animals, and oculonasal discharges. On the side of the animals affected by RVF that were significantly identified in the household were goats (P=0.001) and sheep (P=0.002) probably because are the ones that were severely affected. Those who heard the

disease from neighbours, mass media, and local government authority or livestock experts, most of them did not remember the clinical signs of the disease. This study has indicated that there was little knowledge on clinical signs of RVF and the difference in the three regions was statistically significant (P=0.00459).

The presence of mosquitoes in villages lying on the shores of Rift Valley especially in the evening together with floods was associated with the outbreak of the disease. Cattle, sheep, goats and human being were reported to be affected by RVF and man could get the disease from eating meat and drinking milk of RVF sick animal. Many respondents could not remember the exact year of the 2007 RVF outbreak. Livestock keepers who experienced the disease treated animals themselves using oxytetracycline, but there was no response. Respondents reported that animals were not vaccinated against RVF before the outbreak of 2006-07 as the government did not have such a control programme in their area. Livestock keepers understood that vaccination was important for controlling livestock diseases and most of them were ready to vaccinate and fully participate in the programme. During the outbreak, vaccinations were done in areas where there was no disease and targeted goats and sheep which were severely affected.

Table 5: Knowledge of livestock keepers on clinical signs of RVF in livestock in the three regions

	Arusha (n=36)	Manyara (n=16)	Morogoro (n=22)
Clinical sign	%	%	%
Storm abortions	44	19	27
High fever	28	6	0
High mortality	36	6	14
Ocular and nasal discharges	39	13	23
Haemorrhagic diarrhoea	19	0	18
Vomiting	3	0	0
Abdominal pain	6	0	0
Jaundice	25	13	14

4.1.6 Socio-economic impact of RVF

The disease posed a great threat not only to the livestock keepers but also to the Government due to its social and economic implications. There were costs incurred due to measures taken at different levels in order to prevent or control infection and disease outbreaks. Rift Valley fever affected people in the study area two-fold; directly and indirectly. Directly, livestock keepers lost their animals through deaths (Table 6 and Table 7) and massive abortions (Table 7), and lost all the benefits mentioned previously (Table 4).

Table 6: Status of livestock in the study households during RVF disease outbreak in the three regions (Arusha, Manyara and Morogoro)

Animal category	Total No	Diseased animals	Treated animals	Animals died	Vaccinated animals
Bulls	594	37	21	21	302
Oxen	145	0	0	0	85
Cows	2398	38	38	20	1383
Heifers	403	21	21	13	248
Calves	1127	249	236	121	9
Goats	2721	128	108	50	1759
Kids	1276	205	199	164	0
Sheep	2516	49	49	35	0
Lambs	1191	133	123	119	0

Non-livestock keepers were affected directly from lack of red meat as most of the markets were closed (45, 60.8%) in many areas of the country and also they were also affected by fear stress as the disease was politically exaggerated. Also indirectly non-livestock keepers were affected by competing in other sources of food which replaced the red meat such as chicken, sardines; pork, vegetables, fish and beans. The price for these replacements became high making low income people failing to buy them creating another big problem of malnutrition due to lack of protein-rich food.

However, none of the respondents reported a household which stopped keeping animals because of the impact caused by RVF in the study area. Turning to agro-pastoralist was their copying strategy after the outbreak.

Table 7: Overall deaths and abortions in domestic ruminants in the study districts

Region	District	Cattle			Goats			Sheep		
		N	Death (%)	Abortion (%)	N	Death (%)	Abortion (%)	N	Death (%)	Abortion (%)
Arusha	Ngorongoro	424 780	0.29	0.34	437 103	0.34	0.40	327 424	0.39	0.40
	Longido	302 272	0.27	0.34	391 953	0.26	0.32	305 797	0.32	0.33
	Monduli	283 428	0.21	0.27	368 223	0.20	0.26	294 395	0.28	0.29
	Arumeru	128 355	0.11	0.11	318 095	0.05	0.06	240 915	0.04	0.04
Manyara	Simanjiro	482 810	0.10	0.16	295 883	0.20	0.32	148 064	0.23	0.37
Morogoro	Mvomero	132 560	1.10	0.92	98 245	1.83	1.51	19 797	3.23	2.82
	Kilosa	156 246	1.48	0.75	122 609	2.33	1.16	38 542	3.25	1.35

Source: District veterinary offices and Veterinary Investigation Centre (Arusha). **N** = Total number of animals in a district and the same N for each category of animals was used to calculate the proportions of animals died and aborted.

During the RVF outbreak the average price for the different category of animals went down and became higher after the outbreak except in calves where the price increased progressively (Table 8).

Table 8: Average price of selling animals before, during and after RVF outbreak of 2006-07

Animal category	Before RVF	During RVF	After RVF	P-Value	Comment
Bulls and Oxen	507 373	398 571	611 864	1.28×10^{-5}	Significant***
Cows	328 276	267 059	404 068	6.77×10^{-4}	Significant**
Heifers	196 316	156 061	251 754	1.44×10^{-6}	Significant****
Calves	125 714	136 935	186 786	0.0337	Significant*
Goats and sheep	40 918	36 135	61 475	1.86×10^{-9}	Significant*****

* Indicates increasing strength of significant different in price of selling animals as the number of star increases, where P>0.05 considered significant. In all categories of animals the price dropped during the outbreak except for calves. For bulls and cows the significant different was due to drop in price during the outbreak, and for heifers, calves and goats and sheep the significant different was due increase in price after the outbreak.

4.1.7 Challenges of controlling RVF disease outbreaks

4.1.7.1 Housing

Most human and animal housing in the pastoral and agro-pastoral systems in Tanzania are not reliable and more so for animals. As it was observed during the study (Fig. 4), some communities had their houses at the centre of the animal's house while others were just close to the human houses and open. This was reported during in-depth interview with the key informants as one of the factors that

contributed to the occurrence of the first RVF human cases in the pastoral settings as in the intensive farming systems animal houses were in good condition and well closed.

Figure 4: Maasai community shared premises with domestic animals at Monic village, Ngorongoro district within the Rift Valley during the outbreak

4.1.7.2 Inadequate knowledge on control methods for RVF

This study has indicated that only 2.7% (2) of the respondents got knowledge about RVF and that 10 (13.5%) knew that there was a control measure currently in place that involved vaccination. The level of literacy in the study community was low due to nomadic lifestyle with 37.8% (28) being illiterate, 39.2% (29) standard seven, 5.4% (4) form four, and 1.4% (1) of the respondents being college graduates. This has an impact not only in the transmission and implementation of control strategies for RVF but also in the control of other livestock diseases.

4.1.7.3 Control of animal movements

Animal movements contribute very much on the spread of RVF from one village to another. During the 2006-07 outbreaks in Tanzania, animal movements were

restricted and local government authorities reinforced regulation about animal movements. However there were some people who moved animals from one village to another in search for pastures and water with few for search of livestock markets in near village, district, region or country (Kenya). Only five (6.8%) respondents reported to control animal movement in their households during the outbreak and they were agro-pastoralists.

4.1.7.4 Treatment of animals by livestock keepers

Veterinary services in agro-pastoral and pastoral communities in Tanzania are mainly provided by LFOs who are found at least in each ward. However, provision of service to animals has been very minimal due to uncontrolled animal movements and treatment by farmers. During the outbreak of RVF in 2006-07, 17 (23%) of the households treated their animals either themselves (14, 18.9%) or LFOs (2, 2.7%). Famers during focus group discussions said that they treated animals because it was expensive to call LFOs demands payments for fuel and drugs. The knowledge for treating animals by themselves was acquired from the family members and other livestock keepers. Free market economy for veterinary drugs led to easy access. Some veterinary drugs are sold in open markets ('*minada*') sometimes in direct sun rays. This has great impact in the control of diseases especially outbreaks as they will report after so many trials while the disease is spreading to other animals and households or villages. The main drug for treatment for many diseases in the households was Oxytetracycline (OTC). This drug was used to treat RVF cases but there was no response at all.

4.1.7.5 Consumption habits of meat and milk

In pastoral and agro-pastoral communities meat inspection is not commonly practiced hence respondents reported consumption of meat without inspection as a normal practice. Both confirmed human cases in Arusha and Manyara as reported by key informants and focus groups was due to consumption of meat from dead sheep. Despite the local government authorities prohibiting people from eating meat without inspection and drinking unpastoralized milk during the outbreak, some people continued to eat meat and drink raw milk.

4.1.7.6 Insufficiency of dipping Tanks

The use of acaricides to control ticks and other ectoparasites in the pastoral and agro-pastoral farming systems is not effective due to their nomadic life styles. Focus groups indicated that only two villages (N=9) had dipping tanks and the remaining villages controlled ticks by spraying animals (Appendix 2).

4.1.7.7 Delay on emergency plans for controlling RVF

There was a complaint from some key informants that they were not involved during initial stages of preparations of emergency plans despite the fact that they knew the areas of outbreaks very well. Being experts in the areas and they could advise the government appropriately regarding important areas for vaccinations. Because of that vaccinations were done in areas where it was not necessary or of priority. There was a delayed response following communication with government officials. This was attributed to either long chain of command or slow acting of the responsible people (administrators) along the chain or not having emergency plan for RVF. Early warning message were issued by EMPRES in November 2006 predicting RVF

outbreak in sub-Saharan Africa based on predictive climatic models like NDVI (Normal differential vegetation index), elevated temperatures in the Pacific and Indian oceans that indicated heavy rains, elevated humidity, and cloud cover favouring increased population of mosquitoes that support and spread RVF virus (EMPRES WATCH, 2006; Martin *et al.*, 2007; Dijkman *et al.*, 2009; Munyua *et al.*, 2010). This information was not acted upon on time in Tanzania. The disease outbreak started in late November 2006 while vaccinations started early march 2007. The human resource was available but the problem was a delay in accessing funds, equipments, and vaccines. During the outbreak, vaccines came very late and were insufficient to cover the number in infected areas. In some occasions vaccines came but there were no funds to allow vaccination campaigns to start.

4.1.7.8 Lack of coordination and inter-sectorial collaboration

During in-depth interviews the key informants reported that the control of RVF was highly influenced by politicians. Vaccinations were carried out in areas which did not qualify based on vaccination regimes. In this case areas with infections could be vaccinated boosting up the disease. There was no clear mode of coordination between the central government (ministry), and local governments (districts) on inputs distributions. Some inputs were distributed directly to districts, some to veterinary investigation centres with no or little harmonization and coordination. The link between livestock sector and public health sector was inadequate especially on disease diagnosis and control.

4.2 Molecular Epidemiological Study

In this study two tests (RVF Inhibition ELISA and RT-PCR) were performed using serum samples. ELISA method was used to test a total of 106, 82 and 12 samples while RT-PCR was used to test 54, 36 and 18 samples from cattle, goats and sheep respectively. Test results for samples from each test clustered by location where samples were collected are shown in Table 9.

Table 9: Proportion (%) of positive serum samples based on ELISA (N=200) and RT-PCR (N=108) tests clustered by regions

Region	Date of collection	ELISA	RT-PCR
		n (%)	n (%)
Dodoma	29/03-04/04/2007	33 (45.5)	20 (10.0)
Tabora	26-30/03/2007	53 (34.0)	34 (44.1)
Manyara	21/10/2007	20 (50.0)	10 (0.0)
Arusha	21/10/2007	10 (30.0)	7 (0.00)
Tanga	09/05/2007	5 (20.0)	2 (50.0)
Mwanza	12/04/2007	11 (9.1)	7 (0.0)
Mtwara	12-18/04/2007	17 (58.8)	10 (0.0)
Iringa	17/09/2007	11 (36.4)	4 (0.0)
Mbeya	18/04/2007	15 (33.3)	5 (0.0)
Lindi	12/04/2007	6 (50.0)	3 (33.3)
Pwani	22/11/2007	19 (47.4)	6 (0.0)

N= Total samples of all regions, and n = Total samples in each region

All positive samples by RVF ELISA Inhibition test were negative by RT-PCR and 65.5% (n=29) of negative samples by ELISA Inhibition test were positive by RT-PCR. The prevalence of RVF based on ELISA Inhibition test and RT-PCR as clustered by species is shown in Table 10.

Table 10: Proportion of positive serum samples from different animal species tested by ELISA and RT-PCR methods

	ELISA		RT-PCR	
Species	Total sample	Positive (%)	Total sample	Positive (%)
Cattle	106	38.7	53	20.8
Goat	82	39.0	38	18.4
Sheep	12	50.0	17	5.9
Overall	200	39.5	108	17.6

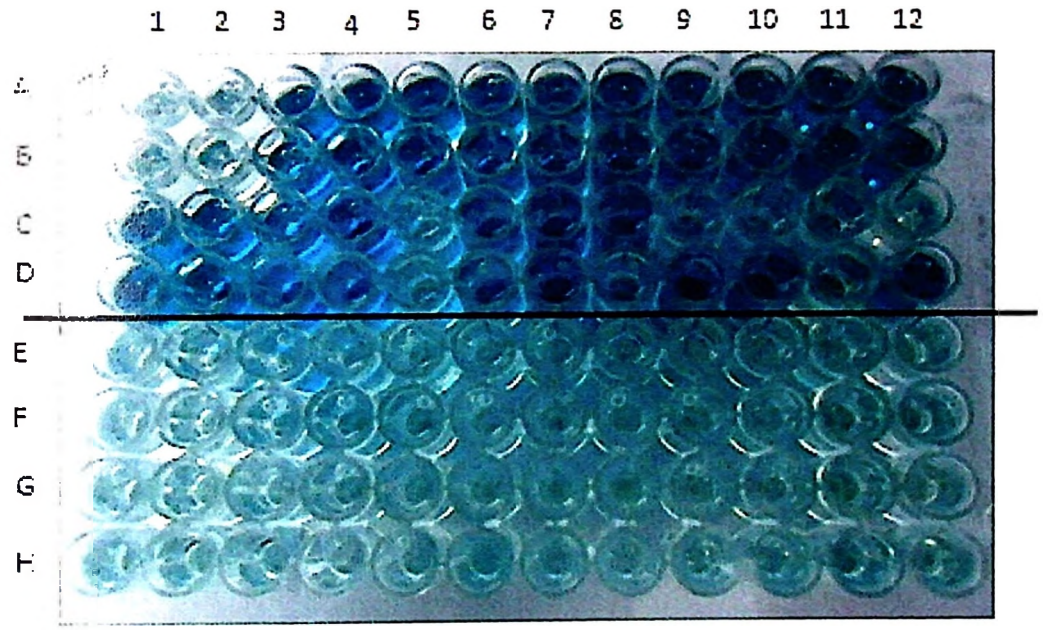

Figure 5: ELISA Plate for reading OD. A-D: 1-12- Test serum-virus antigen mixture (upper half), E-H: 1-12- serum-control sera/virus antigen mixture (lower half), A-B: 1-2- Conjugate control added diluent buffer only.

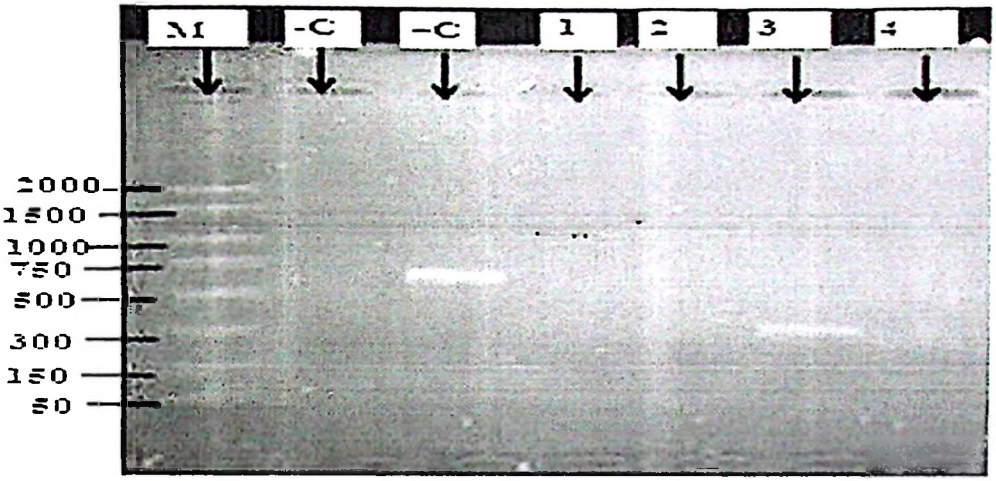

Figure 6: Agarose gel electrophoresis of amplified M segment gene of RVFV. M =2 kb Marker, -C =negative control, +C = Positive control (producing a 700 base pair), and 1 to 4 are test samples with lane 3 producing 400 base pair (bp) band.

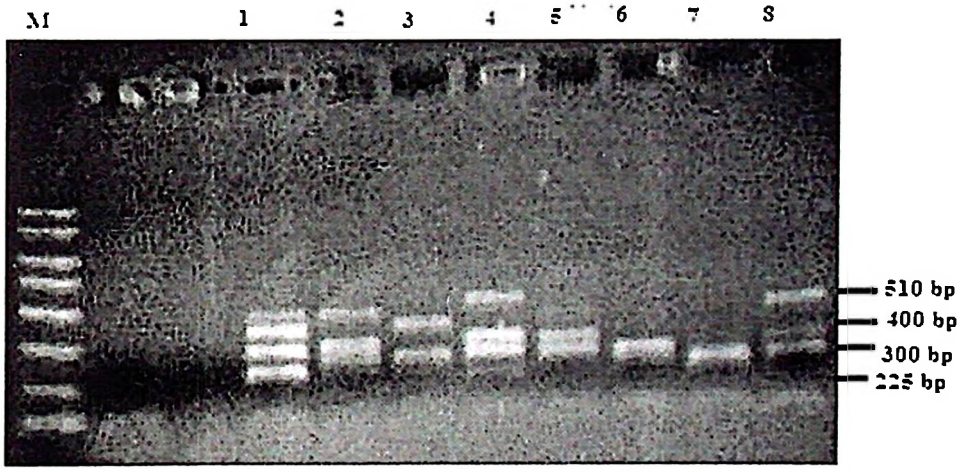

Figure 7: Agarose gel electrophoresis PCR amplification profile of the M segment gene of RVFV. M =2 kb Marker, Lane 1 to 8 are test samples. Lane 1 producing four bands (225, 300, 400 and 500 bp), lane 2 three bands (280, 350 and 500 bp), lane 3 two bands (280 and 400 bp), lane 4 three bands (300, 350 and 700 bp), lane 5 two bands (300 and 350 bp), lane 6 one band (300 bp), lane 7 one band (280 bp) and lane 8 two bands (350 and 700 bp).

CHAPTER FIVE

5.0 DISCUSSION

5.1 Socio-economic Survey

Findings from this study have revealed that the majority of farmers think livestock keeping give more income than cropping. Farmers in the study area depend on livestock as the main source of income. However, diseases and drought pose serious threats to livestock keepers. Losses are attributable to morbidity, mortality and costs of disease treatment and control measures to meet national and international requirements. Epidemic diseases such as RVF, with few natural factors to limit their spread and experience in managing them bring great threat to livestock keepers.

Tanzania has an estimated livestock population of 17 million cattle, 11 million goats, and 3.6 million sheep (Mohamed *et al.*, 2010) most of which are located in the north and central regions of the country where the 2006-07 RVF outbreak affected much leading to disrupted socio-economic setting of all Tanzanians and more so livestock keepers who are completely dependent on livestock and their products. The 2006-07 RVF disease outbreaks in Tanzania started on the northern part in Ngorongoro and Monduli districts, and by the end of the outbreak it affected 10 of 21 regions of the country and 25 of 126 districts (Azizi, 2008; Swaia and Schoonmanb, 2009; Mohamed *et al.*, 2010).

This study has revealed that, during the outbreak minimal education was given to the community in the study area and more so to the pastoralists who live nomadic life. Pastoralists depended much on radios to get information about RVF as they can carry with them even to the remote grazing areas. The government used community

meetings, posters, newspapers and seminars to educate communities. Since during this study, most of community members were found with low knowledge about RVF may imply that, the education provided was not effective. In livestock keeping community, the majority of them had not gone to school and therefore posters were not suitable for them. In this regard, providing education via their local leaders and radios could be the best option for livestock keeping community to get education easily during RVF outbreak. Vernacular languages should be used when providing education and information to the pastoralist as it has been seen that there was good proportion of people who could not understand and speak the national language (Kiswahili). This observation was also noted in Kenya (Munyua *et al.*, 2010) as among obstacles for efficient dissemination of information and extension of knowledge to livestock keepers. With advancement of communication technology, the use of automated messages via cell phones that would provide information on outbreak of diseases may be useful. In addition, if cell phones are used by the livestock keepers and veterinary professionals to exchange information on livestock diseases, this may also help to contain the disease as early as possible during outbreaks.

During the 2006-07 RVF outbreaks, some farmers went on eating meat without inspection and proper cooking that led to more human cases especially in some parts of Dodoma. This was due to their socio-cultural behaviour of eating meat not inspected or from dead animals. This calls further educational intervention at community levels. On the other hand in some communities it was observed that human and animals shared the same housing that also predisposed them to zoonotic diseases. Based on this, community members were at high risk of acquiring RVF.

Hence more education is needed and interventions that will enable the community live in separate houses from animals.

In pastoral communities, animals that get sick are often treated by themselves due to unavailability of livestock disease professional and para-professionals that can take charge in disease diagnosis, treatment and other disease management. Other factors include high treatment cost linked to calls of veterinary doctors and buying of drugs, keeping large number of animals just for prestige, nomadic lifestyle and insufficient knowledge on best ways to control diseases (Appendix 2). Apart from livestock experts being few, nomadic lifestyle contributes by far for the limited access to veterinary services that would provide service on time. Pastoralists have limited knowledge about dosage and routes for drug administration. Free market economy for veterinary pharmaceutical in Tanzania contributes greatly to self-treatment of animals by and mishandling of drugs. Easy access to drugs and self-treatment procedures have great impact on control of livestock diseases especially during outbreaks as pastoralists will report after so many trials, while the disease is progressing to spread.

During the outbreak, the quarantine was not executed properly as pastoralists could still move their animals from one village to another to search for pastures. Farmers were still selling animals to nearby country (Kenya) via unauthorized routes, a practice that facilitated further spread of the disease to unaffected areas (Azizi, 2008; Nicoletti and Ciufolini, 2008). It was observed that good pastures were found in the low land areas where mosquitoes were also found in large numbers and facilitated the disease transmission. However, the short difference in time of occurrence of

disease in different regions of Tanzania is an indication that, those foci of outbreaks were caused by other factors other than animal movement as it was also highlighted by FAO (2000). The results also indicated that few farmers used dipping tanks and the majority used spray pumps to control vector borne diseases. Since some of the farmers owned large number of animals, it was not possible to effectively spray all of them. According to FAO (2003) effective use of dipping tanks also reduces the magnitude of mosquito borne diseases like RFV.

Individual respondents in this study reported a total death of 175 cattle, 214 goats and 154 sheep, while reports in District Veterinary Offices and Arusha Veterinary Investigation Centre in the study area indicate a total death of 7013 (0.22%) cattle, 8641(0.30%) goats and 5409 (5.65%) sheep due to 2006-07 RVF outbreaks. Reports from TVLA indicated that there were deaths of 16973 (0.10%) cattle, 20913 (0.18%) goats and 12124 (0.31%) sheep and 15726 (0.09%) abortions in cattle, 19199 (0.16%) in goats and 11085 (0.28%) in sheep. Both reports from District veterinary offices and Arusha VIC and TVLA show that sheep were highly affected followed by goats then cattle. These findings are in agreement to the one reported in Kenya (Jost *et al.*, 2010) following similar outbreaks. Also disease led to disruption of whole market chains system in the country similar to what it was reported in other countries that experienced the disease (Holleman, 2002). This was contributed by lack of emergency plans that led to delayed control of RVF in the country. This was a similar observation in Kenya (FAO EMPRES WATCH, 2006; Martin *et al.*, 2007; Dijkman *et al.*, 2009; Munyua *et al.*, 2010).

5.2 Molecular Epidemiological Study

In this study RVF inhibition ELISA test detected 39.5% (n=200) positive serum samples that were collected during the 2006-07. All serum samples (79) which were IgG positive and 29 IgG negative were further tested using conventional RT-PCR for which, 17.6% (n=108) of samples tested were found to be positive from RVF disease. All RT-PCR positive samples that tested negative by RVF inhibition ELISA test indicated presence of an acute infection as IgG antibodies starts to appear after approximately one month or more (Bird *et al.*, 2008; OIE, 2008).

An interesting finding from this study is that, band size of the positive control did not match to that of the test serum samples and even within test samples there were variations in band size. The positive control used in this study was live attenuated freeze dried Rift valley fever viral vaccine (RIFTVAX^{TM}, Nairobi, Kenya) which was taken from the field when there was an on-going vaccination campaigns (April, 2012) in Mwanga, Tanzania. These variations could be due to insertion and/or deletion mutations of the M segment gene at various positions (Bird *et al.*, 2007b). This could lead to change of the primer annealing sites that would amplify the DNA fragment with different band size from the positive control. Variations in the M segment gene that code for surface glycoproteins has a significant implication as it is responsible for production of neutralizing antibodies and influences the viral cell attachment and tissue tropism. In the study carried out by Bird *et al.* (2007b; 2008), complete genome sequence showed little variations in the overall nucleotide lengths of the S, M, and L segments among the 2006-07 viruses. Although no variation was detected for the virus M RNA segments in the study done by Bird *et al.* (2007b) as

they expected, that does not undermine the differences in band size of M segment gene obtained in this study.

Another interesting finding observed in RT-PCR is the presence of multiple bands in seven (6.5%) of the samples tested. Experience observed by Ma *et al.* (2006), show that for short amplicons (< 150 bp), very weak (and fussy) bands migrate ahead of the major specific bands. These weak bands are super-structured or single-stranded version of the specific amplicons in equilibrium state and therefore should be considered specific. In this study, two possibilities can be suggested; one may be due to different short fragments which are still specific and from the same strain or due to mixed infection (more than one RVF strains) because both bands are seen to be equally strong. The existence of different strains of RVF in Tanzania that are different from the vaccine (Kenya) which is currently used in the country is possible as it has been observed in other countries (Bird *et al.*, 2007b; 2008). Therefore, further studies that include sequencing of M segment gene of the samples are required to clarify the differences of band sizes and multiple bands obtained in this study.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has revealed inadequate knowledge in communities on the control measures for RVF disease that led to great socio-economic loses during the 2006-07 outbreaks that otherwise could be minimized. Control measures that were put in place by the government could not be implemented properly because time frame for the disease to spread in a wider area was very short to enable the government to provide education as fast as possible. Inadequate numbers of livestock disease experts in the livestock keeping community led to livestock keepers treat their animals. This led to delayed reporting of the disease outbreaks. Also lack of collaboration between and within the livestock sectors led to difficulties in effective implementing control measures during the outbreak. Thus, there is high chance that the disease disappeared naturally.

This study has also found that there were RVFV in the serum samples collected during the 2006-07 outbreaks. The genome of these viruses has shown to have different band sizes and some with multiple bands. These variations could be due to insertion and/ or deletion and may be one among factors contributing to the vaccine failure reported by key informants in this study. There is high chance of using different strain (s) of the vaccine that may not protect animals in our country as the information on the circulating strains is still unknown.

6.2 Recommendations

This study recommends that education to the community need to be provided regularly regardless of the presence or absence of the RVF as during the disease there is minimal time for providing education. Education messages should be developed in different languages because of heterogeneity of communities living in RVF prone regions and should be disseminated by radio, a widely used medium of communication for most parts of the country. In addition, village elders, chiefs, and religious leaders should be educated through public meetings. Early warning systems should be used that will help effective planning for preventing disease outbreaks. Also active disease surveillance and monitoring should be carried out routinely in the field in order to create baseline information on inter-epidemic virus transmission patterns, areas at risk and early warning of virus activity or increased mosquito populations. It is high time for Tanzania to build capacity for all its VICs so that they can be used effectively for surveillance and monitoring of disease outbreaks.

Annual vaccinations with matching vaccines to the field strains of Tanzania to all animals in highly susceptible areas that will be identified by experienced livestock stakeholders will play a great role for preventing outbreak of RVF. Since RVFV strains circulating in Tanzania are not known, the study suggests carrying out detailed molecular studies to characterize the viruses circulating in the country. In countries like Tanzania where outbreaks of diseases are common, an emergency unit needs to be established that will deal with them to the interest of individuals and the national economy at large.

REFERENCES

Accardi, L., Prehaud, C., Bonito, P. D., Mochi, S., Bouloy, M. and Giorgi, C. (2001). Activity of Toscana and RVFV transcription complexes on heterologous templates. *Journal of General Virology* 82: 781–785.

Andriamandimby, S. F., Randrianarivo-Solofoniaina, A. E., Jeanmaire, E. M., Ravololomanana, L., Razafimanantsoa, L. T., Rakotojoelinandrasana, T., Razainirina, J., Hoffmann, J., Ravalohery, J., Rafisandratantsoa, J., Rollin, P. E. and Reynes, J. (2010). Rift Valley Fever during Rainy Seasons, Madagascar, 2008 and 2009. *Emerging Infectious Diseases* 16 (6): 963-970.

Aradaib, I. E., Karrar, A. E., Al-Dubaid, M. A., Musa, H. A., Kafi, S. K., El-Kadarou, A., Ashmaig, A. and Abu-Aisha, H. H. (2008). Hemorrhagic Fever caused by rift valley fever virus: Rapid detection based on viral small (S) RNA genome segment. *International Journal of Tropical Medicine* 3(1): 5-9.

Australian Government (2007). Rift Valley Fever. In: Exotic Animal Diseases Bulletin No. 100. *Australian Veterinary Journal* 85(6): 18-19.

AUSVETPLAN (1996). Australian veterinary emergency plan: Disease Strategy. RVF *AUSVETPLAN Edition 2.0.*

Azhar, E. I., Madani, T. A., Kao, M. and Jamjoom, G. A. (2010). A seroepidemiological study of rift valley fever virus among slaughter houses' workers in Saudi Arabia. *International Journal of Medicine and Medical Sciences* 2(4): 111-115.

Aziz, M. I. A. (2008). Rift Valley Fever: The story unfolds. *Sudanese Journal of Public Health* 3(1): 5-10.

Bird, B. H., Bawiec, D. A., Ksiazek, T. G., Shoemaker, T. R. and Nichol, S. T. (2007a). Highly Sensitive and Broadly Reactive Quantitative Reverse Transcription-PCR Assay for High-Throughput Detection of RVFV. *Journal of clinical microbiology* 45 (11): 3506–3513.

Bird, B. H., Githinji, J. W. K., Macharia, J. M., Kasiiti, J. L., Muriithi, R. M., Gacheru, S. G., Musaa, J. O., Towner, J. S., Reeder, S. A., Oliver, J. B., Stevens, T. L., Erickson, B. R., Morgan, L. T., Khristova, M. L., Hartman, A. L., Comer, J. A., Rollin, P. E., Ksiazek, T. G. and Nichol, T. S. (2008). Multiple virus lineages sharing recent common ancestry were associated with a large RVF outbreak among livestock in Kenya during 2006-2007. *Journal of Virology* 82 (22): 11152-11166.

Bird, B., Khristova, M. L., Rollin, P.E., Ksiazek, T. G. and Nichol, S. T. (2007b). Complete Genome Analysis of 33 Ecologically and Biologically Diverse Rift Valley Fever Virus Strains Reveals Widespread Virus Movement and Low Genetic Diversity due to Recent Common Ancestry. *Journal of Virology* 81(6): 2805-2816.

Bouloy, M. and Weber, F. (2010). Molecular Biology of Rift Valley Fever Virus. *The Open Virology Journal 4:* 8-14.

Breiman, R., Njenga, M. K., Cleaveland, S., Sharif, S.K., Mbabu, M. and King, L. (2008). Lessons from the 2006-07 Rift valley fever outbreak in East Africa: Implications for prevention of emerging infectious diseases. *Future Virol*ogy 3(5): 411-417.

Bustin, S.A. (2000). Review. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *Journal of Molecular epidemiology*, 25: 169-193.

Centre for Disease Control and prevention, CDC. (1998). Rift Valley Fever in East Africa, 1997-1998. *Centres for Disease Control and Prevention* 47(13): 261-264.

Centre for Disease Control and prevention, CDC. (2003). Rift Valley Fever: Distribution. [http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/rvfmap.htm] site visited on 18/08/2011.

Davies, F.G. and Martin, V. (2006). Recognizing Rift valley fever. *Veterinaria Italiana* 42 (1): 31-53.

De La Rocque, S. and Formenty, P. (2009). Rift valley fever ecology and early warning. In: *FAO/IAEA International Symposium on Sustainable Improvement of Animal Production and Health* 8–11 June 2009 Vienna, Austria. IAEA-CN-174-268.

Dijkman, J., Hall, A., Steglich, M., Sones, K., Keskin, E., Adwera, A. and Wakungu, J. (2009). Innovation response capacity in relation to livestock-related emergencies in Africa. *IGAD LPI Working Paper* No. 03 – 10.

Elfadil, A. A., Hasab-Allah, K. A. and Dafa-Allah, O. M. (2006). Factors associated with Rift Valley fever in south-west Saudi Arabia. *Rev. Sci. tech. Off. Int. Epiz.* 25 (3): 1137-1145.

Elliott, R.M. (1990). Review article. Molecular biology of Bunyaviridae. *Journal of Virology* 71: 501-522.

Elliott, R.M. (1997). Emerging viruses: The Bunyaviridae. *Molecular Medicine* 3(9): 572-577.

Emergency Prevention System for the Transboundary Animal Diseases (EMPRES WATCH), FAO. (2006). Possible RVF activity in the Horn of Africa. [ftp://ftp.fao.org/docrep/fao/011/aj218e/aj218e00.pdf] site visited on 21/09/2011.

Ericsson, C. D. and Steffen, R. (2001).Viral Hemorrhagic Fever Hazards for Travelers in Africa. *Clinical Infectious Diseases* 33:1707–1712.

Fafetine, J. M., Tijhaar, E., Paweska, T. J., Neves, L. C.B.G., Hendriks, J., Swanepoel, R., Coetzer, J.A.W., Egberink, H. F. and Rutten, P.M.G. (2007). Cloning and expression of RVFV nucleocapsid (N) protein and evaluation of a N-protein based indirect ELISA for the detection of specific IgG and IgM antibodies in domestic ruminants. *OpenUP (July 2007).*

FAO EMPRES WATCH. (2007). Rift Valley Fever could spread with movement of animals from East Africa. [ftp://ftp.fao.org/docrep/fao/011/aj215e/aj215e00.pdf] site visited on 25/08/2010.

Food and Agriculture Organization, FAO. (2000). Emergency preparedness planning for RVF. *FAO Animal Health Manual* 15: 1-67.

Food and Agriculture Organization, FAO. (2003). Recognizing Rift valley fever. *FAO Animal Health Manual No. 17.*

Food and Agriculture Organization, FAO. (2009). Socio-economic consequences for poor livestock farmers of animal diseases and veterinary public health problems. In: Improved animal health for poverty reduction and sustainable livelihoods. [http://www.fao.org/DOCREP/005/Y3542E/y3542e04.htm] site visited on 20/07/2010.

Garcia, S., Crance, J. M., Billecocq, A., Peinnequin, A., Jouan, A., Bouloy, M. and Garin, D. (2001). Quantitative Real-Time PCR Detection of Rift Valley Fever Virus and Its Application to Evaluation of Antiviral Compounds. *Journal of Clinical Microbiology* 39(12): 4456-4461.

Habijan, M., Penski, N., Spiegel, M. and Weber, F. (2008). 7 RNA polymerase-dependent and - independent systems for cDNA-based rescue of Rift Valley fever virus. *Journal of General Virology* 89: 2157-2166.

Holman, D. H., Penn-Nicholson, A., Wang, D., Woraratanadharm, J., Harr, M-K., Luo, M., Maher, E. M., Holbrook, M. R. and Dong, J. Y. (2009). A Complex Adenovirus-Vectored Vaccine against Rift Valley Fever Virus Protects Mice against Lethal Infection in the Presence of Pre-existing Vector Immunity. *Clinical and Vaccine Immunology* 16(11): 1624-1632.

Ikegami, T. and makino, S. (2009). Rift Valley fever vaccines. *Vaccine* 27: 69–72.

International Livestock Research Institute (ILRI). (2009). Decision-support tool for prevention and control of Rift Valley fever epizootics in the Greater Horn of Africa. *Manuals and Guides* 7(1).

Jost, C. C., Nzietchueng, S., Kihu, S., Bett, B., Njogu, G., Swai, E. S. and Mariner, J. C. (2010). Epidemiological Assessment of the Rift Valley Fever Outbreak in Kenya and Tanzania in 2006 and 2007. *American Journal of Tropical Medicine and Hygiene* 83(2_Suppl): 65-72.

Kahlon, S. S., Peters, C. J., LeDuc, J., Muchiri, E. M., Muiruri, S., Njenga, M. K., Breiman, R. F., White, A. C. and King, C. H. (2010). Case Report: Severe Rift Valley Fever May Present with a Characteristic Clinical Syndrome. *American Journal of Tropical Medicine and Hygiene* 82(3): 371–375.

Knowles, N. J. and Samuel, A.R. (1998). RT-PCR and Sequencing Protocols for the Molecular Epidemiology of Exotic Virus Diseases of Animals. *Protocol. WPD Molecular Epidemiology group.*

Labeaud, A. D., Muchiri, E. M., Ndzovu, M., Mwanje, M.T., Muiruri, S. and Peters, C. J. (2008). Interepidemic Rift Valley fever virus seropositivity, northeastern Kenya. *Emerging Infectious Diseases*.

Labeaud, D. A. (2009). New understanding of the epidemiology of rift valley fever virus in Kenya. http://etd.ohiolink.edu/send-pdf.cgi/LaBeaud%20Angelle.pdf. Site visited on 07/08/2010.

Labeaud, D. A., Ochiai, Y., Peters, C. J., Muchiri, E. M. and King, C. H. (2007). Spectrum of rift valley fever virus transmission in Kenya: Insights from three distinct regions. *American Journal of Tropical Medicine and Hygiene* 76(5): 795–800.

Lagerqvist, N., Näslund, J., Lundkvist, A., Bouloy, M., Ahlm, C. and Bucht, G. (2009). Characterisation of immune responses and protective efficacy in mice after immunisation with Rift Valley Fever virus cDNA constructs. *Virology Journal* 6:6.

Le Roux, C. A., Kubo, T., Grobbelaar,A. A., Vuren, P. J. V., Weyer, J., Nel, L. H., Swanepoel, R., Morita, K., Paweska, J. T. (2008). Development and Evaluation of a Real-Time Reverse Transcription-Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Rift Valley Fever Virus in Clinical Specimens. *Journal of clinical Microbiology* 47(3): 645–651.

Liu, L., Celma, C. P. and Roy, P. (2008). Rift Valley fever virus structural proteins: expression, characterization and assembly of recombinant proteins. *Virology Journal* 5:82.

Lopez, N., Muller, R., Prehaud, C. and Bouloy, M. (1995). The L Protein of RVFV Can Rescue Viral Ribonucleoproteins and Transcribe Synthetic Genome-Like RNA Molecules. *Journal of virology* 69 (7): 3972–3979.

Ma, H., Shieh, K., Chen, G., Qiao, X.T., Chuang, M. (2006). Application of Real-time Polymerase Chain Reaction (RT-PCR). *The Journal of American Science* 2(3): 1-15.

Magona, J. W., Kabi, F., Walubengo, J. and Ssekitto, C.M.B. (2009). Surveillance of Rift valley fever in cattle, goats and sheep in Uganda. In: *FAO/IAEA International Symposium on Sustainable Improvement of Animal Production and Health* 8–11 June 2009 Vienna, Austria. IAEA-CN-174-25.

Martin, V., Simone, L. D., Lubroth, J., Ceccato, P. and Chevalier, V. (2007). Perspectives on using remotely-sensed imagery in predictive veterinary epidemiology and global early warning systems. *Geospatial Health 2(1): 3-14.*

Mohamed, M., Mosha, F., Mghamba, J., Zaki, S. R., Shieh, W., Paweska, J., Omulo, S., Gikundi, S., Mmbuji, P., Bloland, P., Zeidner, N., Kalinga, R., Breiman, R. F. and Njenga, M.K. (2010). Epidemiologic and Clinical Aspects of a Rift Valley Fever Outbreak in Humans in Tanzania, 2007. *American Journal of Tropical Medicine and Hygiene* 83(Suppl 2): 22–27.

Muller, R., Argentini, C., Bouloy, M, Prehaud, C. and Bishop, D. H. L. (1991). Completion of the genome sequence of Rift Valley fever phlebovirus indicates that the LRNA is negative sense or ambisense and codes for a putative transcriptase-replicase. *Nucleic Acid Research* 19 (19): 5433.

Munyua, P., Murithi, R. M., Wainwright, S., Githinji, J., Hightower, A., Mutonga, D., Macharia, J., Ithondeka, P. M., Musaa, J., Breiman, R. F., Bloland, P., and Njenga, M. K. (2010). Rift Valley Fever Outbreak in Livestock in Kenya, 2006–2007. *American Journal of Tropical Medicine and Hygiene* 83(2_Suppl): 58-64.

Nachiket, S. D., Pattan, S. R., Bhawar, S. B., Gaware,V. M., Hole,M. B., Waman, S. and Butle, S. R. (2010). RVF: A Review. *Journal of Chemistry and Pharmarcological Research* 2(1): 228-239.

Naing, L., Winn, T. and Rusli, B. N. (2006). Practical Issues in Calculating the Sample Size for Prevalence Studies. *Archives of Orofacial Sciences* 1: 9-14.

National Institute for Communicable Diseases, NCD. (2010). Preliminary report on an outbreak of Rift valley fever, South Africa. *Communicable diseases*. Volume 9, No. 5 Additional Issue (1).

National Institute for Communicable Diseases, NCD. (2011). Interim report on the 2011 Rift Valley fever outbreak in South Africa. *Communicable diseases*

Office International des Epizooties, OIE. (2008). Rift valley fever. *OIE Terrestrial Manual* Chapter 2.1.1 4 .pp 324-333.

Otte, M. J., Nugent, R. and McLeod, A. (2004). Transboundary Animal Diseases: Assessment of socio-economic impacts and institutional responses. *Livestock policy discussion paper* No. 9.

Paweska, J. T., Mortimer, E., Leman, P. A. and Swanepoel, R. (2005). An Inhibition Enzyme-linked Immunosorbent assay for the detection of antibody to RVFV in humans, domestic and wild ruminants. *Journal of Virological Methods* 127: 10–18.

Paweska, J.T., Burt, F.J., Anthony, F., Smith, S.J., Grobbelaar, A.A., Croft, J.E., Ksiazek, T.G. and Swanepoel, R. (2003). IgG-sandwich and IgM-capture enzyme-linked immunosorbent assay for the detection of antibody to Rift Valley fever virus in domestic ruminants. *Journal of Virological Methods* 113:103–112.

Pepin, M., Bouloy, M., Bird, B. H., Kemp, A. and Paweska, J. (2010). Review article for RVFV (Bunyaviridae: Phlebovirus): An update on pathogenesis, molecular epidemiology, vectors, diagnostics and prevention. *Veterinary Research* 41:61.

Peyrefitte, C., Boubis, L., Coudrier, D., Bouloy, M., Grandadam, M., Tolou, H. and Plumet, S. (2008). Real-Time Reverse-Transcription Loop-Mediated Isothermal Amplification for Rapid Detection of RVFV. *Journal of Clinical Microbiology*, 46 (11): 3653–3659.

Robert, S. (2004). Bunyaviridae. In: Principles and Practice of Clinical Virology, Fifth Edition. "Edited by" (A. J. Zuckerman, J. E., Banatvala, J. R., Pattison, P. D., Griffiths and Schoub, B.D.) John Wiley & Sons Ltd, West Sussex, England pp 556-588.

Salim, R. W., Khairalla, K. M. S., Eljamal, A. A.,Karrar, A.E., Aradaib, A. E. (2010). A Single Tube RT-PCR amplification for detection of Rift valley fever virus. *Research journal of Medical Sciences* 4 (3): 146-151.

Sall, A. A., Zanotto, P. M.A., Vialat, P., Sène, O. K. and Bouloy, M. (1998). Molecular Epidemiology and Emergence of RVF. *Mem Inst Oswaldo Cruz, Rio de Janeiro* 93(5):609-614.

Sall, A. A., Zanotto, P. M.A., Sène, O. K., Zeller, H. G., Digoutte, J. P., Thiongane, Y. and Bouloy, M. (1999). Genetic Reassortment of Rift valley fever virus in Nature. *Journal of Virology* 73 (10): 8196-8200.

Sall, A.A., Macondo, E. A., Sène, O. K., Diagne, M., Sylla, R., Mondo, M., Girault, L., Marrama, L., Spiegel, A., Diallo, M., Bouloy, M. and Mathiot, C. (2002). Use of Reverse Transcriptase PCR in Early Diagnosis of RVF. *Clinical and Diagnostic Laboratory Immunology* 9(3): 713–715.

Shoemaker, T., Boulianne, C., Vincent, M. J., Pezzanite, L., Al-Qahtani, M. M., Al-Mazrou, Y., Khan, A. S., Rollin, P. E., Swanepoel, R., Thomas G. Ksiazek, T. G., T. Nichol, S. T. (2002). Genetic Analysis of Viruses Associated with Emergence of Rift Valley Fever in Saudi Arabia and Yemen, 2000-01, *Emerging Infectious Diseases*, 8 (12): 1415-1420.

Swaia, E. S. and Schoonmanb, L. (2009). Prevalence of Rift valley fever IgG antibody in various occupational groups before the 2007 outbreak in Tanzania. In: *FAO/IAEA International Symposium on Sustainable Improvement of Animal Production and Health* 8–11 June 2009 Vienna, Austria. IAEA-CN-174-24.

Thiongane, Y., Gonzalez, J.P., Fati, A. and Akakpo, J. A. (1991). Changes in Rift Valley fever neutralizing antibody prevalence among small domestic ruminants following the 1987 outbreak in the Senegal River basin. *Reseaarch of Virology* 142: 67-70.

Vuren, P. J., Paweska, J. T. (2009). Laboratory safe detection of nucleocapsid protein of Rift Valley fever virus in human and animal specimens by a sandwich ELISA. *Journal of Virological Methods* 157: 15–24.

Vuren, P. J.; Potgieter, A. C.; Paweska, J. T.; Dijka, A. A. (2007). Preparation and evaluation of a recombinant Rift Valley fever virus N protein for the detection of IgG and IgM antibodies in humans and animals by indirect ELISA. *Journal of Virological Methods* 140: 106–114.

Wilson, W., Jiang, J., Weingartl, H., Rodriguez, L., Murieta, C. and Miller, M. (2009). Molecular detection technologies for Arboviruses including Bluetongue and Rift valley fever viruses. In: *FAO/IAEA International Symposium on Sustainable Improvement of Animal Production and Health* 8–11 June 2009 Vienna, Austria. IAEA-CN-174-40.

Zaki, A, Coudrier, D, Yousef, A. I, Fakeeh, M, Bouloy, M, Billecocq, A. (2006). Production of monoclonal antibodies against Rift Valley fever virus Application for rapid diagnosis tests (virus detection and ELISA) in human sera, *Journal of Virological Methods* 131: 34-40.

APPENDICES

Appendix 1: Frequency distribution (%) of common and outbreak diseases reported in the study area by individual household (N=74).

Disease	Diseases encountered	Diseases with great loss	Diseases in outbreak form
ECF	79.7	47.3	12.2
MCF	12.2	4.1	1.4
Trypanosomosis	50.0	6.8	1.4
CBPP	45.9	24.3	13.5
CCPP	60.8	40.5	9.5
PPR	13.5	12.2	0.0
RVF	2.7	0.0	36.5
Fasciolosis	10.8	4.3	0.0
Helminthosis	21.6	4.3	0.0
Anaplasmosis	17.6	6.8	1.4
Babesiosis	9.5	2.7	0.0
Anthrax	23.0	5.4	24.3
Myiasis	40.5	21.6	2.7
FMD	32.4	2.7	6.8
LSD	20.3	1.4	0.0
Heartwater	2.7	0.0	1.4
Black Quarter	5.4	1.4	6.8
Brucellosis	0.0	0.0	1.4
Swine flue	0.0	0.0	1.4

Appendix 2: Focus group discussion responses (N=9 groups)

Category	Code	% of codes
Activities	Livestock keeping	22.2
	Cropping	22.2
	Both	11.1
Main constraints	Drought	55.6
	Livestock diseases	55.6
	Lack of dipping tanks	55.6
	Insufficient drugs	11.1
Disease control methods	Treating themselves	66.7
	Vaccination deworming	33.3
	Dipping	44.4
Dipping methods	Spray pumps	66.7
	Dipping tanks	22.2
	Neither of the two	11.1
Challenges to vaccination programme	Poor turn-up	22.2
	No regulation	33.3
	Sending few animals	11.1
	Satisfied	22.2
Usefulness of experts	Unsatisfied	11.1
	Very little	44.4
Source of information	Veterinary experts	22.2
	Government	22.2
	Neighbors	11.1
Advice needed	Regular visits by veterinary experts	22.2
	More education on livestock keeping	33.3
	Building dipping tanks	11.1

Appendix 3: Questionnaire survey at household level on RVF disease

Questionnaire No..... Date.....
District..... Region.....

1.0. Respondent information

1.1. Name of Respondent .....Head of Household.....Yes/No
1.2. Age..... 1.3. Sex..... F (emale)/M (ale)
1.4. Village.....
1.5. Education Level.... .....A (None), B (standard 4), C (standard 7), D (form 4), E (form 6), F (College or University), G (others, specify).....
1.6. Grid References.....

2.0. Community based knowledge on handling and disease control practices

2.1. In your family is there anyone who is employed by the government or private organization? ..... Yes/No

2.2. (a) List all activities carried out in your family that give you income.....
(b) What about what is used for subsistence? .....
2.3. Among the mentioned activities, which one do you think gives you more income? .....

2.4. What is your average monthly expenditure? .....
2.5. How many children are in primary school.....and secondary school .....now?

2.6. (a) When did you start keeping livestocks? ..... (Year)
(b) What types of animals are kept by your household? Tick
A (cattle)
B (goats)
C (sheep)
D (chickens)
E (donkeys)
F (ducks)
G (others, specify).....

(c) What types of animals are kept by the majority of the livestock keepers in the area? Tick
A (cattle)
B (goats)
C (sheep)
D (chickens)

E (donkeys)
F (ducks)
G (others, specify).....
(d) Why? .....

2.7. What advantage(s) do you get from keeping livestock? Tick
A (paying dowry)
B (paying school fees and buying school needs)
C (gets milk and meat)
D (use for cultivation)
E (buying household requirements)
F (health care)
G (others, specify).....
2.8. (a) What is the total amount of milk (litres) do you get per day from your animals?.....

(b) How much money do you get when you sell a litre of milk? .....

2.9. (a)What are the average working hours per day of draught cattle? .....

(b) Which activities? .....
3.0. (a) How do you use livestock manure? Tick
A (crop production)
B (throw away)
C (leave in the yard)
D (sell to others)
E (others, specify).....

(b) If for crop production, how much do you use per year? .....

(c) What is the price of manure if sold per given unit? .....

3.1. (a) List all diseases you think affects your livestocks.....

(b)Among the diseases you have mentioned, which diseases do you think have great effect to your animals? .....
(c) Have you ever heard of outbreak diseases?.....Yes/No?

(e) Can you mention, outbreak diseases that you know.....

3.2. Have you ever heard of Rift valley fever (RVF) disease? .....Y (es)/N (o)
(if not listed in 2.8e above, otherwise go to question 3.0)

3.3. If Yes, How does the disease manifest itself in the affected area? Tick
A (storm abortions)
B (high fever)
C (high mortality in lambs)
D (nasal and ocular discharges)

E (haemorrhagic diarrhoea)
E (vomiting)
F (abdominal pain)
G (jaundice)

3.4. Tick animals that are affected by RVF disease
A (cattle)
B (sheep)
C (goats)
D (dogs and cats)
E (human)
F (others, specify).....

3.5. How did you come to know Rift valley fever? Tick
A (told by neighbour),
B (happened here),
C (livestock experts),
D (Mass media),
E (others, mention).....

3.6. When did the first outbreak of RVF occur in your area? ---------- yyyy

3.7. When did the last outbreak of RVF occur in your area? ---------- yyyy
3.8. How did you manage to handle the disease during outbreak to? Tick

(i) Your livestocks
A (treated),
B (vaccinated),
C (controlled animal movement),
D (others, specify).....

(ii) Your family
A (treated)
B (vaccinated)
C (stopped eating meat)
D (others, specify).....

3.9. If you treated, who treated your animals? Tick
A (district veterinary officer)
B (livestock field officer)
C (yourself)
D (others specify).....

4.0. (a) Did you have animals vaccinated against RVF before outbreak of 2006-07?..... Y (es)/N (o)

(b) If yes, when? ..... yyyy, and how many times per year?.....

(c) Do you think vaccination was an effective way of controlling the disease? ..... (Y) es/ (N) o

(d) Do you still vaccinate your animals against this disease? .....Y (es)/N (o)

(e)If no, give reason(s).....

4.1. (a) Do you know any control measure(s) which is (are) in place now for RVF disease in your area? .....Y (es)/N (o)

(b) If yes, (Tick)
A (vaccination)
B (monitoring and surveillance)
C (control animal movement)
D (others, specify).....

4.2. (a) Have you ever got an education about Rift valley fever? ..... Y (es)/N (o)

(b) If yes, from whom (Tick)
A (livestock experts)
B (government leaders)
C (fellow farmers)
F (mass media)
G (others, specify).....

5.0. Socio-economic impact of RVF disease

5.1. Treatment and vaccination status during the outbreak

Animal type	Total no.	No. treated	No. responded	Cost/animal	No. vaccinated	Cost/animal
Cattle						
Goats						
Sheep						

5.2. Status of the herd during the outbreak

Animal category	Cattle category	Total No	Got sick	Died	Survived	Sold
Cattle	Bulls					
	Cows					
	Calves (0-9 months)					
	Oxen					
	Heifers					
Sheep	Lambs					
	Adults					
Goats	Kids					
	Adults					

5.3. Selling price per animal before, during and after the outbreak.

Animal category	Cattle category	Selling price/animal before outbreak	Selling price/animal during outbreak	Selling price/animal after outbreak
Cattle	Bull			
	Cow			
	Calf			
	Oxen			
	Heifer			
Sheep	Lambs			
	Adults			
Goats	Kids			
	Adults			

5.4. Where did you sell your animals during outbreak? .....
A (middle men)
B (you slaughtered)
C (transported to market) --- (village, ward, division, district or regional market)
Tick
D (others, specify).....

5.5. What was the response of people in your village during the outbreak towards?
Tick
(a) **Beef**
A (high)
B (normal)
C (low)
D (you and your family)

(b) **Sheep and goat meat**
A (high)
B (normal)
C (low)
D (you and your family) .....

(c) **Drinking milk**
A (high)
B (normal)
C (low)
D (you and your family) .....

5.6. What do you think was the replacement for beef, (red meat)? Tick
A (fish)
B (pork)
C (chicken)
D (sardines)
E (others, specify).....

5.7. Household status during and after the outbreak

Age category	Total No.	Got sick	Recovered	Died
Adults				
Children				
Relatives				
Employees				

5.8. Can you estimate the number of households that stopped keeping livestock in your village because of the 2006-07 outbreaks?

.....