EPIDEMIOLOGICAL STUDY OF RIFT VALLEY FEVER VIRUS IN KIGOMA, TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE EQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ONE HEALTH MOLECULAR BIOLOGY OF SOKOINE UNIVERSITY OF AGRICULTURE.

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ABSTRACT

Rift Valley fever (RVF) is an acute, zoonotic viral disease, caused by a *Phlebovirus* belonging to the *Bunyaviridae* family. RVF virus (RVFV) historically has been responsible for large explosive outbreaks of severe human and animal disease throughout Africa and recently in the Arabian Peninsula. In animals, it mainly affects domestic ruminants such as sheep, goats and cattle. RVFV outbreaks among livestock are economically devastating and often characterized by large sweeping abortion storms and significant mortality in adult livestock. This study was conducted to investigate RVF infection in Kigoma region. Regional wide serosurvey and conventional gel based single tube RT-PCR were conducted in Kigoma region on non- vaccinated small ruminants (sheep and goats). The study included 411 animals (32 sheep and 379 goats) sampled in 3 districts namely; Kigoma rural, Kasulu and Kibondo. Sera of animals were tested for the detection of immunoglobulins G (IgG) against RVFV using commercial enzyme-linked immunosorbent assays (ELISA) kit. Past infections were detected in 22 of 411 animals (5.4% at 95% CI 3.5 % to 8.1%) from all three districts. Kigoma rural recorded higher seroprevalence of 12.0% (CI 7.3% to 18.3%; P<0.0001) followed by Kibondo (2.3% [0.5% to 6.5%]; P>0.05) and Kasulu districts (0.8% [0.0% to 4.2%]; P>0.05). The prevalence was 12.5% and 4.7% for sheep and goats respectively. RT-PCR results indicated that only 8 samples were found positive (n=63) including 22 positive samples for IgG ELISA, where none was RT-PCR positive. This study has confirmed, for the first time, the presence of RVFV in Kigoma region, 4 years after the 2007 epizootic in Tanzania, and suggests further that the virus activity exists during the interepizootic period

(IEP) even in regions with no history of RVF. In-depth studies should be conducted to clarify the complex epidemiology of RVF in the country.

DECLARATION

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

……..……..……..….. **……**

Emmanuel George Kifaro **Date**

MSc. (OHM) Candidate

The above declaration is confirmed

…… **……..……..**

Dr. Christopher J. Kasanga **Date**

(Supervisor)

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I would like to extend my gratitude to my supervisors Dr. Christopher J. Kasanga of the Department of Veterinary Microbiology and Parasitology, and the Southern African Centre for Infectious Disease Surveillance (SACIDS) and Dr. George Dautu of the University of Zambia for their unconditional support, advice and proficient insight during the course of my research. Their guidance through numerous challenges associated with certain aspects of my project have aided me in expanding my academic knowledge and propensity for troubleshooting, subsequently enabling me to achieve a thoroughly successful and enjoyable research project.

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I also wish to express my sincere gratitude to Welcome Trust through SACIDS for their financial support of my postgraduate studies.

Finally, I thank my wife (Naojwa) and daughter (Ellen) for supporting and nurturing me while I spent numerous hours at home and away performing studies, analyzing data, and writing my dissertation.

DEDICATION

This work is dedicated to Prof. Mark Rweyemamu and his fellow senior scientists for their role in modelling on improving animal health, human health and livelihoods, under the concept of ‘’One Health” in East, Central and Southern Africa.

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RVF is a viral disease of animals and humans that occurs throughout sub-Saharan Africa, Egypt, and the Arabian Peninsula. Outbreaks of the disease are episodic and closely linked to climate variability, especially widespread elevated rainfall that facilitates RVFV transmission by vector mosquitoes (Linthicum et al., 1987). These mosquitoes are thought to initiate outbreaks among livestock, particularly susceptible breeds of sheep and cattle. Human infections follow as the result of either direct mosquito transmission or from percutaneous/ aerosol routes during the handling of aborted foetal materials or the slaughtering of infected livestock. The acute onset of large numbers of affected individuals and livestock during outbreaks can greatly strain public health and veterinary infrastructures (Bird et al., 2008). 1

The severity of RVFV, its ability to cause major epidemics among livestock and humans, and the lack of efficient prophylactic and therapeutic measures make infection with this pathogen a serious public health concern not only in endemic, developing countries, but also in many non-endemic industrial countries (Bouloy and Weber, 2010).2

The present study, reports the presence of RVF infections during IEP, and in a region known to be free from RVF. It is expected that, the output from this study will help in providing the scientific based evidence on the presence of RVF infections in such regions and that it will improve the national policy on control programs of the disease country wide. 2

have been reported in sub-Saharan and North Africa (Fig 1). In 1997-98, a major outbreak occurred in Kenya, Somalia and Tanzania and in September 2000, RVF cases were confirmed in Saudi Arabia and Yemen, marking the first reported occurrence of the disease outside the African continent and raising concerns that it could extend to other parts of Asia and Europe (WHO, 2007). 6

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2.4 RVF in Tanzania 7

In Tanzania, sporadic cases of RVF during the previous epidemics were mainly confined to livestock and mostly affecting northern parts of Tanzania (Woods et al., 2002). The latest disease epidemic expanded to cover wider areas (mostly northern and central zones) of the country and affecting both human and domestic ruminants (Corso et al., 2008). Periodic severe RVF outbreaks involving livestock and humans have occurred in Africa following heavy rainfall and flooding (Woods et al., 2002). In 2007, WHO reported that, From December 2006 to May 2007, RVF human cases reported in Somalia were 114 with 51 deaths. In Kenya 684 human cases were reported, with 155 deaths and Tanzania 290 cases were reported with 117 deaths. Initially the disease was concentrated in the northern parts of the country that borders Kenya i.e. Ngorogoro and Monduli in Arusha region. Between February and June 2007, other regions of Tanzania including Manyara, Tanga, Dodoma, Morogoro, Dar es Salaam, Coast, Iringa, Mwanza, and Singida reported cases of RVF (Fig.2). RVF Cases were reported until mid-June 2007 when rains ended (Nderitu et al., 2011). 7

2.5 Livestock keeping in Kigoma 7

Livestock keeping systems practiced in Kigoma region are both traditional and commercial in nature. A large proportion of cattle, goats and sheep are indigenous

The involvement of wildlife species during epidemics and the existence of sylvatic cycles involving wildlife and mosquitoes in maintenance and perpetuation of the virus during IEPs have never been investigated (Evans et al., 2008). The wildlife-mosquito cycling of RVFV could maintain the virus at low levels and might be difficult to detect if the wildlife reservoirs undergo mild or asymptomatic infections. This wildlife-mosquito cycling may involve low-level livestock infections since limited data in countries where RVFV outbreaks occur suggest that between 2.5% and 23% of livestock may have been infected by RVFV during an IEP (Swanepoel, 1981; Dohm et al., 2000). Evansi et al. (2008) reported the highest prevalence of RVFV antibodies in ruminant wildlife including Thomson's gazelle (87.5%), impala (62.5%), lesser kudu (50%), black rhino (32.6%), waterbuck (20%), and buffalo (15.6%). Buffalo, which provided the largest number of specimens (n=342), had both a high prevalence of anti-RVFV antibodies (16.95%) and also significantly higher titres of viral neutralizing antibodies compared to other animal species. 16

Since RVF antibodies may not be detectable during the first few days of disease and since the viremia often reaches high titers for several days, detection of viral genome or antigen may be the method of choice. 17

The RT-PCR provides a sensitive and specific detection of RVFV in cell culture and directly in serum samples from infected humans or animals. This RT-PCR based assay serves as a supportive diagnostic assay to the time consuming and cumbersome conventional virus isolation laboratory procedures which require high containment

laboratory facilities. The rapidity, sensitivity and specificity of the RT-PCR would greatly facilitate detection of RVFV during an outbreak of the disease among humans and susceptible animals (Elata and Aradaib, 2011). 17

Sample preparation and DNA extraction using QIAamp extraction kit is a simple procedure which takes half an hour. The thermal cycling profiles for amplification of the PCR products were consistently 3hours. Running of agarose gel and electrophoresis usually takes 1hour. The described assay is a rapid procedure as the time required from submission of samples to final results takes about 5hours. In contrast, the sensitivity studies of the conventional gel-based RT-PCR indicated that the described RT-PCR protocol was capable of detecting the amount of 1.0pg of total RVFV genomic ssRNA. This level of sensitivity is comparable to that of virus isolation (Elata and Aradaib, 2011). 17

A simple and reliable method for detection of RVFV is urgently needed for rapid diagnosis in developing countries. It is well known that the M segment of the virus is less conserved among cognate genes of RVFV strains and is frequently associated with reassortment. Therefore, it is suggested that detection of the virus targeting this M segment should enhance rapid diagnosis of RVFV and would facilitate differential diagnosis of hemorrhagic fever viruses (Salim et al., 2010). 17

A real-time rt-PCR method was developed to detect and specifically quantify the RVF virus either from cells or from sera targeting the NSs protein-coding region (Garcia et al., 2001) or by targeting the Gn (or Gc) glycoprotein coding region (Drosten et al., 2002). Obviously, the limitation of the RT–PCR method is the presence of virus. However, situations may be encountered when field samples had been exposed to

adverse storage temperatures, thus damaging the infectivity of the particles but not the genome. 18

The alternative method for nucleic acid amplification is loop-mediated isothermal amplification (LAMP) that was developed in 2000 (Notomi et al., 2000). LAMP amplifies specific sequences on nucleic acids using a set of six primers and relies on the strand displacement activity of the DNA polymerase. It is performed at a constant temperature (60 to 65°C), without cyclic denaturation of the template. RNA can be amplified simply by the addition of avian myeloblastosis virus reverse transcriptase to the reaction mix, keeping the same reaction conditions as for DNA amplification (Tomita et al., 2008). High amplification rates are observed, leading to the production of large amounts of double-stranded DNA (dsDNA) and leading as well to production of a white precipitate of magnesium pyrophosphate that can be observed with the naked eye. When primers are properly designed, the reaction is as specific and sensitive as traditional PCR or RT-PCR and is, moreover, faster. The RVFV-specific RT-LAMP primers demonstrated a high degree of specificity for RVFV by yielding negative results for all other tested phleboviruses. LAMP or RT-LAMP is thus efficient, fast, and inexpensive, and since isothermal reaction conditions are easy to provide, LAMP is of particular interest for field diagnosis of tropical diseases (Peyrefitte et al., 2008). 18

Early detection of RVF is a prerequisite to effective control of the disease (FAO, 2003). Sentinel herd monitoring has been used in different parts in Africa to monitor viral circulation in susceptible populations. Sentinel herd are important means of obtaining baseline epidemiological information on RVF. These are small ruminants herds located in geographically representative areas. Locations where mosquito breeding activities is likely to be greatest, e.g. near rivers, swamps and dams, should be selected. Activities should be directed towards active disease surveillance in order to build up baseline information on inter-epidemic virus transmission patterns, areas at risk and early warning of any increased virus activity or build up in vector mosquito populations (FAO, 2003). 20

Kigoma region is located on the shores of Lake Tanganyika at the North – West corner of Tanzania. The region is situated between Longitudes 29. 5 and 31.5 East and Latitudes 3.5 and 6.5 South of the Equator. It shares boundaries with Burundi and Kagera region to the North, Shinyanga and Tabora regions to the East, Congo to the West and Rukwa region to the South. It has a wonderful natural beauty, nestled under the hills of the western arm of the Great Rift Valley on the edge of the Lake. 24

Characteristically tropical with a distinct long wet rainy season beginning from late October to May, annual rainfall is variable ranging from 600 mm- 1500 mm. Kigoma region is a gently inclined plateau with steep hills rising very sharply from 800 metres at the level of lake Tanganyika to altitudes of 1, 750 metres to the East descending from the North and East into gently rolling hills with three major perennial rivers of Malagalasi, Luiche and Ruchugi. The first two rivers comprise the major drainage area. The descent leads to rivers valleys at 1000 metres, swampy and flat delta area at 800 meters where the rivers join the lake. The vegetation in Kigoma region comprise closed and open woodland which cover about 70 per cent of the land area, that includes bushy grassland and swamps of various coverage (Regional Commissioner's Office, Kigoma, 2008). 24

In Kibondo district, there is Moyowosi Game Reserve that was gazetted in 1981. It covers an area of 6000 sq km lying on the edge of the great central plateau between the East and western Rift Valley arms, with an altitude ranging from 800m to 1600m. Annual rainfall in the reserve varies between 1000mm and 1500mm. The dry season starts in mid May and ends in mid October with maximum temperature of 29◦C. Malagalasi-Moyowosi wetland is the large and excellent example of an East African floodplain wetland ecosystem in good condition. The reserve is recognized by supporting large populations of buffalo, Topi, Lion, Giraffe, Zebra, Hartebeest, Roan antelope, Greater kudu Waterbuck Sitatunga, Hippo and the Crocodile (MNRT, 2012).24

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LIST OF ABBREVIATIONS AND SYMBOLS

µL	Microlitre
ABTS	2,2'-azino di-ethyl-benzothiazoline-sulfonic acid
BDSL	Biological Diagnostic Supplies Limited
bp	Base Pairs
CDC	Centre for Disease Control and prevention
CI	Confidence Interval
CVL	Central Veterinary Laboratory
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immuno Sorbent Assay
HIV	Human Immunodeficiency Virus
HRPO	Horseradish Peroxidase
IEP	Inter Epizootic/epidemic Period
IgG	Immunoglobulin Gamma
IgM	Immunoglobulin Mega
KDa	Kilodaltons
MNRT	Ministry of Natural Recourses and Tourism

MoLFD	Ministry of Livestock and Fisheries Development
MW	Molecular Weight
nm	Nanometer
NSCA	National Sample Census for Agriculture
nt	Nucleotides
OD	Optic Density
OIE	World organization for Animal Health
ORF	Open Reading Frame
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PI	Percent Inhibition
RdRp	RNA dependent RNA polymerase
RNA	Ribonucleic Acid
RT PCR	Reverse Transcription Polymerase Chain Reaction
RVF	Rift Valley Fever
RVFV	Rift Valley Fever Virus
SDS	Sodium Dodecyl Sulfate
TAD	Transboundary Animal Disease

UV	Ultraviolet
VIC	Veterinary Investigation Centres
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 **Background information**

Tanzania has experienced several RVF epizootics/epidemics in different regions. The available records at the Ministry of Livestock and fisheries Development (MoLFD) indicate that RVF occurred for the first time in Tanzania in 1930. This was followed by periodic epidemics of 10-20 years i.e. 1947, 1957, 1977, 1997 and 2007 (Sindato *et al*., 2011). In 2007, the disease was initially concentrated in the northern parts of the country that borders Kenya i.e. Ngorogoro and Monduli in Arusha region. Between February and June 2007, other regions of Tanzania including Manyara, Tanga, Dodoma, Morogoro, Dar es Salaam, Coast, Iringa, Mwanza, and Singida had reported cases of RVF in humans and animal (Ndetiru *et al*., 2011).

RVF is a viral disease of animals and humans that occurs throughout sub-Saharan Africa, Egypt, and the Arabian Peninsula. Outbreaks of the disease are episodic and closely linked to climate variability, especially widespread elevated rainfall that facilitates RVFV transmission by vector mosquitoes (Linthicum *et al*., 1987). These mosquitoes are thought to initiate outbreaks among livestock, particularly susceptible breeds of sheep and cattle. Human infections follow as the result of either direct mosquito transmission or from percutaneous/ aerosol routes during the handling of aborted foetal materials or the slaughtering of infected livestock. The acute onset of large numbers of affected individuals and livestock during outbreaks can greatly strain public health and veterinary infrastructures (Bird *et al*., 2008).

The severity of RVFV, its ability to cause major epidemics among livestock and humans, and the lack of efficient prophylactic and therapeutic measures make infection with this pathogen a serious public health concern not only in endemic, developing countries, but also in many non-endemic industrial countries (Bouloy and Weber, 2010).

The present study, reports the presence of RVF infections during IEP, and in a region known to be free from RVF. It is expected that, the output from this study will help in providing the scientific based evidence on the presence of RVF infections in such regions and that it will improve the national policy on control programs of the disease country wide.

1.2 Problem statement and justification

RVFV is associated with large-scale epizootics/epidemics throughout Africa and the Arabian Peninsula. Virus infection can result in economically disastrous "abortion storms" and high newborn mortality in livestock. Human infections in most cases result in a flu-like illness, with 1 to 2% of patients developing severe complications, including encephalitis or hemorrhagic fever with high fatality rates (Bird *et al*., 2007). In 2007, WHO reported that from December 2006 to May 2007, RVF human cases in Somalia were 114 with 51 deaths. In Kenya 684 cases were reported, with 155 deaths and in Tanzania 290 cases were reported, with 117 deaths. RVF is one of the most important Transboundary Animal Disease (TAD), which has the ability to cross international borders and can cause devastating effect on both animal and human health and also food security. Furthermore it can lead to jeopardization of international trade, a reduction in confidence,

fewer tourists and loss of livelihoods. Huge economic losses are the consequences of such infections ascribed to mortality, reduced production and costs for controlling the disease (Domenech *et al*., 2006). However, there is no information on the disease status during interepizootic period, and on the existing knowledge on public health awareness about the disease outbreak both in human and animal populations. These two are of particular importance as "one health" concept to be achieved, in establishing rational control program of this serious emerging zoonotic disease. Also, there is scant information on the molecular characteristics of the circulating RVFV strains in the country. This study is thought to be a helpful tool for understanding RVF disease status and molecular epidemiology of RVFV in regions with no history of the disease in Tanzania.

1.3 **Main objective**

To investigate the RVF infection status in Kigoma region with the main focus on identifying the viral genome.

1.4 **Specific objectives**

- To determine interepizootic seroprevalence of RVFV in Kigoma, Tanzania.
- To identify RVF viral genome in Kigoma region.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Rift valley fever

RVF is considered a major zoonotic threat and is classified by the World organization for Animal Health (OIE) as one of the diseases that threatens of livestock trade, because it has the potential to spread at international level (OIE, 2008) as cited by Sindato et al. (2011)

In animals, RVFV is mainly transmitted by a range of mosquito species (*Aedes, Anopheles, Culex, Eretmapoites, Mansonia*), but has also been shown to be transmitted by other vectors e.g. sandflies (Moutailler *et al*., 2008). Biting flies such as midges, phlebotomids, stomoxids and simulids could possibly serve as mechanical transmitters of infection (Swanepoel and Coetzer, 2004). Livestock is highly susceptible to the virus and young animals die from acute hepatitis. Teratogenic and abortogenic effects are observed in pregnant adult animals (Moutailler *et al*., 2010). Infected animals develop necrotic hepatitis, haemorrhage and abortion, with death rates up to 100% among newborn animals (Bird *et al*., 2009).

Humans acquire RVF virus through bites from infected mosquitoes or through exposure to the blood, body fluids, or tissue of infected animals or other humans (Woods *et al*., 2002). The disease is associated with symptoms ranging from uncomplicated acute febrile illness to retinitis, hepatitis, renal failure, meningoencephalitis, severe hemorrhagic disease, and death (Bird *et al*., 2009)

2.2 Epidemiology of RVF

The epidemiology of RVF consists of both epizootic and interepizootic cycles (Meegan & Bailey, 1989). RVF is a climate-related infection and all outbreaks in East Africa have been reported to occur following periods of abnormal drought, followed by abnormal heavy rains and the consequent emergence of large numbers of Aedes and Culex mosquitoes (Linthicum *et al*., 1985). During an epizootic, virus circulates among infected arthropod vectors and mammalian hosts, particularly cattle and sheep, which represent the most significant livestock amplifiers of RVFV (Sall *et al*., 1998). The inter-epizootic survival of RVFV is believed to depend on transovarial transmission of virus in floodwater *Aedes* mosquitoes (Linthicum *et al*., 1985). Virus can persist in mosquito's eggs until the next period of heavy rainfall when they hatch and yield RVFV infected mosquitoes. Depending on factors such as availability of sufficient numbers of competent mosquito vectors, presence of susceptible vertebrates, appropriate environmental conditions, infected mosquitoes have the potential to infect a relatively small number of vertebrate hosts or to initiate a widespread RVF epizootic (Sall *et al*., 1998).

Economically disastrous livestock epizootics often precede the detection of human illness and have been recorded since early 1900s (Findlay *et al*., 1931). Livestock epizootics usually manifest as sweeping "abortion storms" and high newborn mortality approaching 100% among sheep, goats, and cattle (Easterday., 1962; Coetzer, 1977) as cited by Bird et al. (2007).

It was first identified in the 1930's in Kenya after isolation from a sheep in the Rift Valley (Daubney *et al*., 1931). It is present throughout Africa, and has also caused outbreaks in

Madagascar off the Eastern coast of Africa as well as in Yemen and Saudi Arabia (Clements *et al*., 2007). RVF outbreaks reported in many African countries including Kenya, Somalia, Tanzania, Egypt, Senegal, Mauritania and South Africa (Sall *et al*., 1998, 1999, 2001). In 2000, RVF cases were confirmed in Arabia and Yemen marking the first reported occurrence of the disease outside the Africa (Ahmad, 2000; Shoemaker *et al*., 2002).

2.3 RVF in the World

RVFV is endemic to countries of East Africa, South Africa, and the Senegal River valley (CDC, 2002). The virus was first identified in 1931 during an investigation into an epidemic among sheep on a farm in the Rift Valley of Kenya. Since then, outbreaks have been reported in sub-Saharan and North Africa (Fig 1). In 1997-98, a major outbreak occurred in Kenya, Somalia and Tanzania and in September 2000, RVF cases were confirmed in Saudi Arabia and Yemen, marking the first reported occurrence of the disease outside the African continent and raising concerns that it could extend to other parts of Asia and Europe (WHO, 2007).

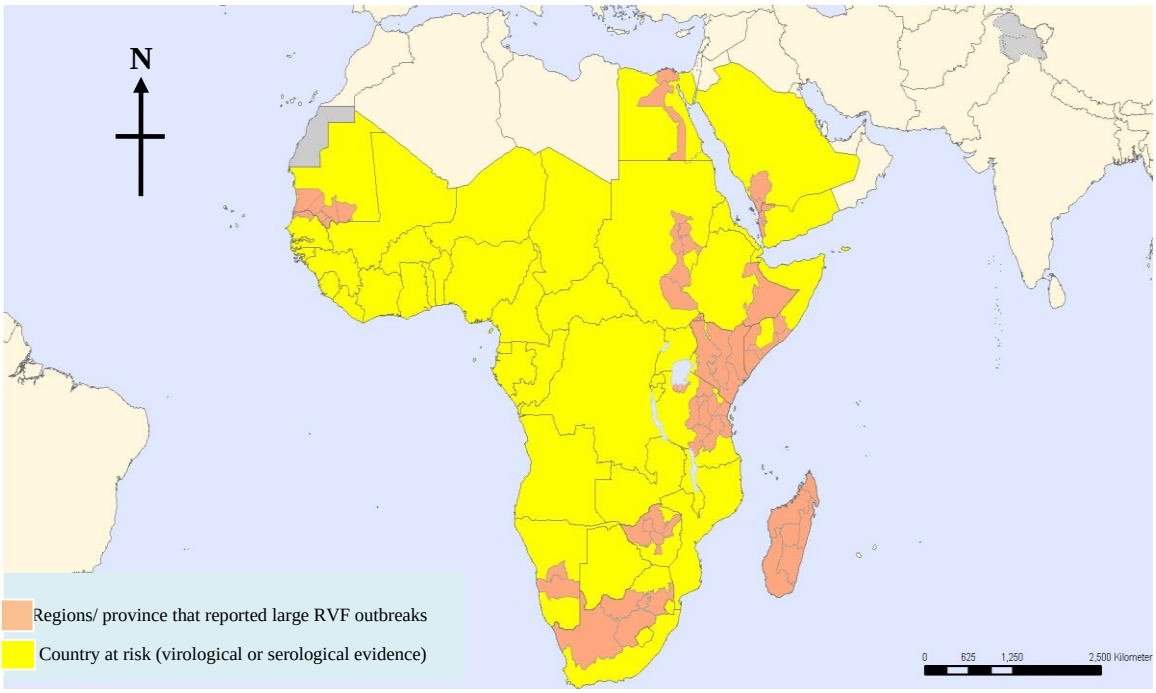

Figure 1: A map showing countries that has large RVF outbreaks and countries with virological or serological RVFV evidence. Source: (WHO, 2009).

2.4 RVF in Tanzania

In Tanzania, sporadic cases of RVF during the previous epidemics were mainly confined to livestock and mostly affecting northern parts of Tanzania (Woods *et al*., 2002). The latest disease epidemic expanded to cover wider areas (mostly northern and central zones) of the country and affecting both human and domestic ruminants (Corso *et al*., 2008). Periodic severe RVF outbreaks involving livestock and humans have occurred in Africa following heavy rainfall and flooding (Woods *et al*., 2002). In 2007, WHO reported that, From December 2006 to May 2007, RVF human cases reported in Somalia were 114 with 51 deaths. In Kenya 684 human cases were reported, with 155 deaths and Tanzania 290 cases were reported with 117 deaths. Initially the disease was concentrated in the northern

parts of the country that borders Kenya i.e. Ngorogoro and Monduli in Arusha region. Between February and June 2007, other regions of Tanzania including Manyara, Tanga, Dodoma, Morogoro, Dar es Salaam, Coast, Iringa, Mwanza, and Singida reported cases of RVF (Fig.2). RVF Cases were reported until mid-June 2007 when rains ended (Nderitu *et al*., 2011).

2.5 Livestock keeping in Kigoma

Livestock keeping systems practiced in Kigoma region are both traditional and commercial in nature. A large proportion of cattle, goats and sheep are indigenous dominated by pastoralists and agro pastoralists. Kigoma rural district has the highest percentage of goats at 56% of all goats reared in Kigoma region, while Kibondo district tops the list in sheep rearing with 111 212 sheep out of a total of 138 291 sheep in the region [Regional Commissioner's Office, Kigoma, 2006 (Table 1)].

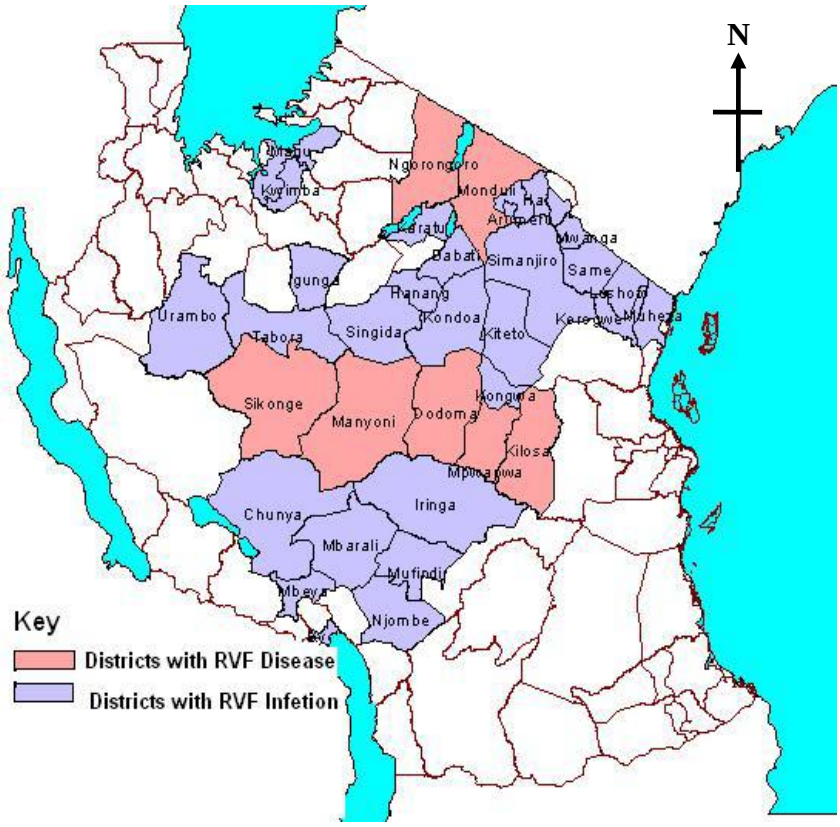

Figure 2: Map of Tanzania showing the distribution of domestic ruminant RVF cases by District during 2007 outbreak (Adopted from: MoLFD, 2007). Source: Sindato et al. (2011).

The predominance of the indigenous breed has resulted in low productivity of meat and milk. Apart from genetic potential, diseases, poor nutrition level and the rundown livestock infrastructure have contributed to this low productivity. Cattle are not kept primarily for slaughter but to supply milk, manure and to meet various social obligations. Kigoma region has three primary livestock markets namely Kakonko in Kibondo district, Buhigwe in Kasulu district and Nguruka in Kigoma district where livestock are brought for sale. In view of inadequate number of livestock for slaughter in the region, livestock

for slaughtering is augmented by livestock brought and transported by railways from the neighbouring regions (Regional Commissioner's Office, Kigoma, 2006).

Table 1: Estimated livestock population by district, Kigoma Region, 2004/05

District	Goats	Sheep
Kibondo	93 386	111 212
Kasulu	65 785	8 275
Kigoma (R)	210 945	17 468
Kigoma (U)	3 404	1 336
Total	**373 520**	**138 291**

Source: Regional Commissioner's Office, Kigoma, 2006

2.6 Etiology of RVF

Rift Valley fever (RVF) is an arthropod-borne disease caused by an RNA virus of the *Phlebovirus* genus in the family *Bunyaviridae* (Swanepoel and Coetzer, 2004). The family currently contains four genera of animal-infecting viruses (*Bunyavirus, Hantavirus, Nairovirus,* and *Phlebovirus* genera) and *Tospovirus* genus of plant-infecting viruses (Fields and Knipe, 1990). Members of the *Bunyaviridae* family are enveloped and have a tripartite segmented, single-stranded RNA genome of negative or ambisense polarity (Brigden *et al.,* 2001). The genus *Phlebovirus* is composed of two serogroups, the Sandfly fever viruses and the tick-transmitted uukuviruses (Murphy *et al.,* 1995). The viruses of the genus *Phlebovirus* are present throughout the world, with the exception of Australia, and are more diverse in terms of arthropod vector than those of the other arthropod-borne genera. Most virus members are associated with phlebotomine sandflies, hence the genus name *Phlebovirus.* However, there are prominent exceptions, such as Rift Valley Fever virus (RVFV), a medically and agriculturally important virus in Africa, which is primarily associated with *Aedes* species mosquitoes (Fields and Knipe, 1990).

2.7 Genetic organization of RVFV

2.7.1 RVF virion

Like all bunyaviruses, RVFV is an enveloped RNA virus characterized by a genome composed of three segments designated L, M and S of negative or ambisense polarity. All the replication steps occur in the cytoplasm of infected cells and virions mature by budding in the Golgi compartment (Nichol *et al*., 2005) as cited by Pepin et al. (2010).

RVFV is an enveloped virus with a diameter of 90 to 110 nm (Ellis *et al*., 1988) and a core element of 80 to 85 nm. Its genome consists of three negative single stranded RNA segments denoted as L (large), M (medium) and S (small) respectively (Fig 3). The envelope is composed of a lipid bilayer containing the Gn and Gc glycoproteins forming surface sub-units, 5–8 nm in length, regularly arranged on its surface, similar to those reported for the related Uukuniemi phlebovirus (Von Bonsdorff *et al*., 1975).

Huiskonen et al. (2009) studied the structure of the RVFV virion using electron cryomicroscopy combined with three-dimensional image reconstruction and single particle averaging. The structure was solved to 2.2nm resolution. The structure revealed 110 cylinder-shaped glycoprotein hexamers and 12 pentamers organized on an icosahedral T=12 lattice.

2.7.2 RVFV genome

The L RNA segment (Fig 3) is 6404 nucleotides (nt) in length and consists of a single open reading frame which codes for a 243.6 kDa viral polymerase called the L protein

(RNA-dependent RNA polymerase). The L protein gene exhibits some sequence homologies to other viral RNA polymerases (Muller *et al*., 1994).

The M segment (Fig 3) is 3885 nt in length and codes for the two envelope glycoproteins Gn and Gc, as well as for two proteins of unknown function, a 14-kDa non structural protein (NSm) and a 78-kDa protein (Gentsch and Bishop, 1979). The Gn and Gc glycoproteins are responsible for eliciting and interacting with neutralising antibodies (Rozhon *et al*., 1981), and are the major determinants of virus virulence (Shope *et al*., 1981) as cited by Aitken (2008).

The S segment (Fig 3) codes for the nucleoprotein N and the non structural NSs protein using an ambisense strategy (Bouloy, 1991; Elliott *et al*., 1991; Schmaljohn, 1996).

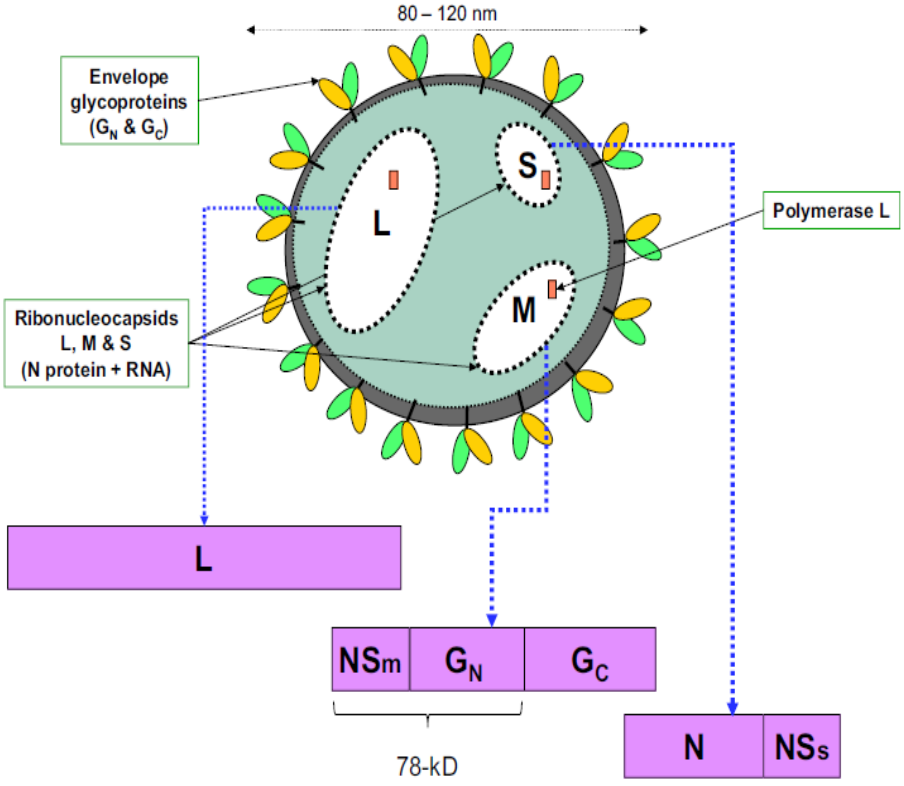

Figure 3: RVFV particle is enveloped and generally spherical, with spikes comprising Gn–Gc heterodimers arranged in genus specific arrays on the membrane exterior. Source: Pepin et al. (2010)

2.7.3 Virus replication and transcription

The general features of RVFV transcription and replication are similar to those of other negative stranded RNA viruses (Elliott, 1990). During the replication cycle, each segment is transcribed into mRNA and is replicated through a process which involves the synthesis of the exact copy of the genome, called complementary RNA (cRNA) or antigenome.

Transcription and replication of the viral genome is initiated through the recognition of promoter sequences in the 5' and 3' terminal untranslated regions (UTRs) of each genomic segment (Flick *et al*., 2004; Kohl *et al*., 2006). These processes occur in the cytoplasm and require both RNA dependent RNA polymerase (RdRp) and Nucleoprotein (N) (Lopez *et al*., 1995; Schmaljohn *et al*., 2007). The RdRp and N are translated in the cytoplasm, while the envelope glycoproteins enter the secretory system. Gn and Gc form a complex and localizes in the Golgi apparatus (Gerrard and Nichol, 2002, 2007; Wasmoen *et al*., 1988). The assembled virus buds into the lumen of the Golgi apparatus, and virions are released from the cell when elements of the Golgi fuse with the plasma membrane. As were cited by Piper. (2010).

The large (L) segment encodes the 237- kDa virus polymerase in a single 6.4-kb ORF (Muller *et al*., 1994). The medium (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 55-kDa Gn and 58-kDa Gc), and a 78-kDa fusion of the NSm and Gn proteins (Suzich *et al*., 1990). Like other members of the genus *Phlebovirus*, the small (S) ambisense segment (Fig 4) possesses one open reading frame (ORF) coding for the nucleoprotein (NP; 27 kDa) in the antigenomic strand and one coding for the nonstructural (NSs; 31-kDa) protein in the genomic strand (Nichol, 2001). These two regions are separated by a poly(C)-rich intergenic region (in the genomic sense) of approximately 81 nucleotides (nt). The NSs protein has been demonstrated to function in the down regulation of RNA polymerase II activity, resulting in host cell transcription shutoff and, via this mechanism, to cause antagonism of host cell interferon responses (Billecocq *et al*., 2004; Le May *et al*., 2004) as cited by Bird et al. (2007).

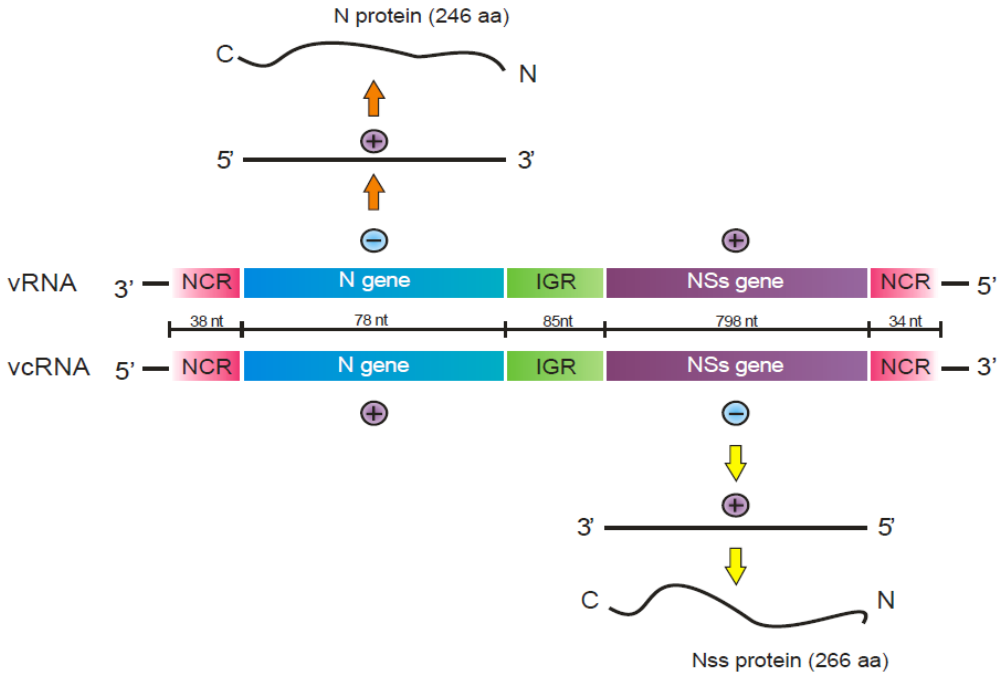

Figure 4: A schematic representation of the RVFV S segment ambisense coding strategy. Source: Simons et al. (1990) as adopted by Aitken (2008).

2.7.4 Strains of Rift Valley fever virus

The members of the *Bunyaviridae* family have been found to be genetically stable (Bishop and Shope 1979). In the case of RVFV, point mutations, deletions, and the reassortment between two or more strains have been shown to occur which resulted in the subsequent generation of new strains (Sall *et al.,* 1999).

2.8 Seroprevalence of RVFV

In Madagascar, Jeanmaire et al. (2011) reported the prevalence of past infections they detected in 244 small ruminants was (24.7%) n= 989 after 2008 epidemic. When considered at the district scale, prevalence of the past infections in small ruminants ranged from 0.4% (0–1.2) to 60.1% (54.0–66.2), highlighting high circulation areas in the country. They also reported that, recent infections (IgM against RVFV) were detected in 33 small ruminants (3.3%). In Madagascar, a total of 894 bovines were sampled between early May and mid-June 2009, belonging to 258 breeders coming from 43 villages of 51 villages existing in the area. Cattle ages ranged from 1 to 18 years, with the majority of sampled animals less than 8 years old. Overall anti-RVFV IgG seroprevalence rate was 28% (IC 95% 25–31). This rate varied among villages, from 0 to 71.4%. Out of 533 bovines belonged to breeders that did not purchase any animal. The anti-RVFV IgG seroprevalence within this population was 27.8%. The anti-RVFV IgG seroprevalence of bovines belonging to breeders that purchase to renew their herd was 28.1%. In the multivariate model, belonging to owners who buy animals to renew (at least partially) their herd was a risk factor, p = 0.04 (Chevalier *et al*., 2011).

In Senegal, RVF seroprevalence reached a peak of around 70% after the 1987 epizootic, dropped to 30.8% in 1988 and then decreased continuously until 1993. This fall in RVF prevalence in animal population corresponded to a period of low rainfall. During a period of heavy rainfall (1993/95) RVF activity re-emerged as epizootics among herds of the lower Senegal River basin (Thonnon *et al*., 1999). The serosurvey conducted in sheep and goats showed a significant increase of RVF IgG and IgM antibodies between June 1994 and December 1995 in all herds. In these RVF epizootics (40 sera were tested in 1994), the RVF IgG prevalence reached a peak of 40% with 12.5% of RVF IgM (Thonnon *et al*., 1999).

In Baringo district in Kenya, 30 herds drawn from 14 sub-locations were assessed, most consisting of cattle, sheep, and goats. The seroprevalence of RVF virus IgM among livestock was 25.2% (36 of 143), whereas IgG was 27.8% (22 of 79). It is important to note that of the 22 specimens that tested positive for IgG antibodies, 21 (95.4%) were also positive for IgM antibodies, indicating that this was a naive ecology that had no prior virus activity. By species, the seroprevalence of RVF virus IgM was highest in sheep sampled 38.8% followed by goats 14% and cattle 10.5% (Munyua *et al*., 2010). Among animals that were born after the 1997–98 epizootic in Kenya, 18% (34/188) of sheep were seropositive for IgG against RVFV, compared with 3% (2/75) of goats (Rostal *et al*., 2010)

The involvement of wildlife species during epidemics and the existence of sylvatic cycles involving wildlife and mosquitoes in maintenance and perpetuation of the virus during IEPs have never been investigated (Evans *et al*., 2008). The wildlife-mosquito cycling of

RVFV could maintain the virus at low levels and might be difficult to detect if the wildlife reservoirs undergo mild or asymptomatic infections. This wildlife-mosquito cycling may involve low-level livestock infections since limited data in countries where RVFV outbreaks occur suggest that between 2.5% and 23% of livestock may have been infected by RVFV during an IEP (Swanepoel, 1981; Dohm *et al*., 2000). Evansi et al. (2008) reported the highest prevalence of RVFV antibodies in ruminant wildlife including Thomson's gazelle (87.5%), impala (62.5%), lesser kudu (50%), black rhino (32.6%), waterbuck (20%), and buffalo (15.6%). Buffalo, which provided the largest number of specimens (n=342), had both a high prevalence of anti-RVFV antibodies (16.95%) and also significantly higher titres of viral neutralizing antibodies compared to other animal species.

2.9 RVFV genome detection

Since RVF antibodies may not be detectable during the first few days of disease and since the viremia often reaches high titers for several days, detection of viral genome or antigen may be the method of choice.

The RT-PCR provides a sensitive and specific detection of RVFV in cell culture and directly in serum samples from infected humans or animals. This RT-PCR based assay serves as a supportive diagnostic assay to the time consuming and cumbersome conventional virus isolation laboratory procedures which require high containment laboratory facilities. The rapidity, sensitivity and specificity of the RT-PCR would

greatly facilitate detection of RVFV during an outbreak of the disease among humans and susceptible animals (Elata and Aradaib, 2011).

Sample preparation and DNA extraction using QIAamp extraction kit is a simple procedure which takes half an hour. The thermal cycling profiles for amplification of the PCR products were consistently 3hours. Running of agarose gel and electrophoresis usually takes 1hour. The described assay is a rapid procedure as the time required from submission of samples to final results takes about 5hours. In contrast, the sensitivity studies of the conventional gel-based RT-PCR indicated that the described RT-PCR protocol was capable of detecting the amount of 1.0pg of total RVFV genomic ssRNA. This level of sensitivity is comparable to that of virus isolation (Elata and Aradaib, 2011).

A simple and reliable method for detection of RVFV is urgently needed for rapid diagnosis in developing countries. It is well known that the M segment of the virus is less conserved among cognate genes of RVFV strains and is frequently associated with reassortment. Therefore, it is suggested that detection of the virus targeting this M segment should enhance rapid diagnosis of RVFV and would facilitate differential diagnosis of hemorrhagic fever viruses (Salim *et al*., 2010).

A real-time rt-PCR method was developed to detect and specifically quantify the RVF virus either from cells or from sera targeting the NSs protein-coding region (Garcia *et al.*, 2001) or by targeting the Gn (or Gc) glycoprotein coding region (Drosten *et al.*, 2002). Obviously, the limitation of the RT–PCR method is the presence of virus. However,

situations may be encountered when field samples had been exposed to adverse storage temperatures, thus damaging the infectivity of the particles but not the genome.

The alternative method for nucleic acid amplification is loop-mediated isothermal amplification (LAMP) that was developed in 2000 (Notomi *et al*., 2000). LAMP amplifies specific sequences on nucleic acids using a set of six primers and relies on the strand displacement activity of the DNA polymerase. It is performed at a constant temperature (60 to 65°C), without cyclic denaturation of the template. RNA can be amplified simply by the addition of avian myeloblastosis virus reverse transcriptase to the reaction mix, keeping the same reaction conditions as for DNA amplification (Tomita *et al*., 2008). High amplification rates are observed, leading to the production of large amounts of double-stranded DNA (dsDNA) and leading as well to production of a white precipitate of magnesium pyrophosphate that can be observed with the naked eye. When primers are properly designed, the reaction is as specific and sensitive as traditional PCR or RT-PCR and is, moreover, faster. The RVFV-specific RT-LAMP primers demonstrated a high degree of specificity for RVFV by yielding negative results for all other tested phleboviruses. LAMP or RT-LAMP is thus efficient, fast, and inexpensive, and since isothermal reaction conditions are easy to provide, LAMP is of particular interest for field diagnosis of tropical diseases (Peyrefitte *et al*., 2008).

2.10 Other methods for diagnosis of RVFV

2.10.1 Field diagnosis

Rift Valley fever (RVF) should be considered when the following group of conditions occurs: Usually the environmental signals of heavy rain, high abortion rates especially in sheep, cattle and other ruminants, high mortalities in young ruminants, severe hepatic

necrosis at necropsy of young animals and foetuses, flu–like symptoms in humans, high numbers of mosquitoes; and rapid spread of disease signals a RVF outbreak (OIE, 2008).

2.10.2 Cell culture (Viral isolation)

Approximately 5 ml of blood collected during the febrile stage of the disease or approximately 5 g of liver, spleen and brain collected after death should be presented for virus isolation A variety of cell monolayers including African green monkey kidney (Vero), baby hamster kidney (BHK), and primary kidney or testis cells of calves and lambs may be inoculated with 1 ml of clarified sample supernates and incubated at 37°C for 1 hour. The cultures are observed microscopically for 5–6 days. RVF virus induces a cytopathic effect (CPE) characterised by slight rounding of cells followed by destruction of the whole cell sheet within 12–24 hours (OIE, 2005a) Specific identification of RVF virus antigen may be made 18–24 hours after infection by immunofluorescent staining of the cover-slip preparations (OIE, 2005a). Culture remains the gold standard for diagnosis (Flick and Bouloy, 2005). Virus isolation is considered as the gold standard but it requires biosafety type three or four laboratory and although very sensitive and specific, they have limitations because of the short duration of viremia which is in general 2-4 days. In addition, manipulation of RVFV generates biohazards (Zeller *et al.*, 1997).

2.10.3 Histopathological examination

Histopathological examinations specimens from liver, brain, kidney, heart, and spleen should be collected in 10% buffered formalin. This can provide the first clues that the

disease is RVF as they reveal characteristic liver necrosis in all susceptible animals with variable degree of severity related to species and age of the affected animal (Wood *et al*., 1990).

2.10.4 Serological diagnosis (Detection of anti-RVFV antibodies)

Paired sera are needed; one collected during the acute phase of illness and one convalescent sample obtained 14-28 days after onset of symptoms.

Confirmatory diagnosis can be achieved by detecting specific anti-RVFV IgM antibody in a single convalescent serum sample or by detecting four-fold or greater rise in IgG antibody between the paired serum samples. Mouse and plaque reduction neutralization tests (PRNT), Enzyme linked immunosorbent assay (ELISA), Indirect Immunofluorescent assay (IFT), Complement Fixation Test (CFT), Hemagglutination Inhibition Test (HI), and AGID are commonly used to detect anti-RVFV antibody in serum samples (Wood *et al*., 1990). A study to determine the accuracy of serological methods (PRNT, ELISA, HI, IFA, and CFT) in detecting anti-RVFV antibodies was carried out and indicated that HI and ELISA are the most precise of the four serological methods compared with PRNT for measuring anti-RVFV antibodies. IFA was less accurate, and CFT was the least sensitive of the four methods (Scott *et al.*, 1986).

2.11 Prevention and control of RVFV

Early detection of RVF is a prerequisite to effective control of the disease (FAO, 2003). Sentinel herd monitoring has been used in different parts in Africa to monitor viral circulation in susceptible populations. Sentinel herd are important means of obtaining

baseline epidemiological information on RVF. These are small ruminants herds located in geographically representative areas. Locations where mosquito breeding activities is likely to be greatest, e.g. near rivers, swamps and dams, should be selected. Activities should be directed towards active disease surveillance in order to build up baseline information on inter-epidemic virus transmission patterns, areas at risk and early warning of any increased virus activity or build up in vector mosquito populations (FAO, 2003).

2.11.1 Controlling RVF in animals

Outbreaks of RVF in animals can be prevented by a sustained program of animal vaccination. Both modified live attenuated virus and inactivated virus vaccines have been developed for veterinary use (WHO, 2010). This is the most effective means to control RVF. The most effective vaccine is the modified live smithburn neurotropic strain (SNS). This vaccine is immunogenic but has the disadvantage that it can cause fetal pathology and abortion in pregnant sheep of susceptible genotypes (FAO, 2003). Only one dose of the live vaccine is required to provide long-term immunity, the inactivated virus vaccine does not have this side effect, but multiple doses are required in order to provide protection which may prove problematic in endemic areas (WHO, 2010).

- Animal immunization must be implemented prior to an outbreak if an epizootic is to be prevented. Once an outbreak has occurred animal vaccination should NOT be implemented because there is a high risk of intensifying the outbreak.

- Restricting or banning the movement of livestock may be effective in slowing the expansion of the virus from infected to uninfected areas.

- As outbreaks of RVF in animals precede human cases, the establishment of an active animal health surveillance system to detect new cases is essential in providing early warning for veterinary and human public health authorities (WHO, 2010).

2.11.2 Public health education and risk reduction

During an outbreak of RVF, close contact with animals, particularly with their body fluids, either directly or via aerosols, has been identified as the most significant risk factor for RVF virus infection. In the absence of specific treatment and an effective human vaccine, raising awareness of the risk factors of RVF infection as well as the protective measures individuals can take to prevent mosquito bites, is the only way to reduce human infection and deaths.

- Reducing the risk of animal-to-human transmission as a result of unsafe animal husbandry and slaughtering practices. Gloves and other appropriate protective clothing should be worn and care taken when handling sick animals or their tissues or when slaughtering animals.

- Reducing the risk of animal-to-human transmission arising from the unsafe consumption of fresh blood, raw milk or animal tissue. In the epizootic regions, all animal products (blood, meat and milk) should be thoroughly cooked before eating.

- The importance of personal and community protection against mosquito bites through the use of impregnated mosquito nets, personal insect repellent if

available, wearing light coloured clothing (long-sleeved shirts and trousers) and by avoiding outdoor activity at peak biting times of the vector species (WHO, 2010).

2.11.3 Vector control

Other ways in which to control the spread of RVF involve control of the vector and protection against their bites. Larviciding measures at mosquito breeding sites are the most effective form of vector control if breeding sites can be clearly identified and are limited in size and extent. During periods of flooding, however, the number and extent of breeding sites is usually too high for larviciding measures to be feasible (WHO, 2010).

2.11.4 RVF forecasting and climatic models

Forecasting can predict climatic conditions that are frequently associated with an increased risk of outbreaks, and may improve disease control. In Africa, Saudi Arabia and Yemen RVF outbreaks are closely associated with periods of above-average rainfall. The response of vegetation to increased levels of rainfall can be easily measured and monitored by Remote Sensing Satellite Imagery. In addition RVF outbreaks in East Africa are closely associated with the heavy rainfall that occurs during the warm phase of the El Niño/Southern Oscillation (ENSO) phenomenon (WHO, 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

Kigoma region is located on the shores of Lake Tanganyika at the North – West corner of Tanzania. The region is situated between Longitudes 29. 5 and 31.5 East and Latitudes 3.5 and 6.5 South of the Equator. It shares boundaries with Burundi and Kagera region to the North, Shinyanga and Tabora regions to the East, Congo to the West and Rukwa region to the South. It has a wonderful natural beauty, nestled under the hills of the western arm of the Great Rift Valley on the edge of the Lake.

Characteristically tropical with a distinct long wet rainy season beginning from late October to May, annual rainfall is variable ranging from 600 mm- 1500 mm. Kigoma region is a gently inclined plateau with steep hills rising very sharply from 800 metres at the level of lake Tanganyika to altitudes of 1, 750 metres to the East descending from the North and East into gently rolling hills with three major perennial rivers of Malagalasi, Luiche and Ruchugi. The first two rivers comprise the major drainage area. The descent leads to rivers valleys at 1000 metres, swampy and flat delta area at 800 meters where the rivers join the lake. The vegetation in Kigoma region comprise closed and open woodland

which cover about 70 per cent of the land area, that includes bushy grassland and swamps of various coverage (Regional Commissioner's Office, Kigoma, 2008).

In Kibondo district, there is Moyowosi Game Reserve that was gazetted in 1981. It covers an area of 6000 sq km lying on the edge of the great central plateau between the East and western Rift Valley arms, with an altitude ranging from 800m to 1600m. Annual rainfall in the reserve varies between 1000mm and 1500mm. The dry season starts in mid May and ends in mid October with maximum temperature of 29°C. Malagalasi-Moyowosi wetland is the large and excellent example of an East African floodplain wetland ecosystem in good condition. The reserve is recognized by supporting large populations of buffalo, Topi, Lion, Giraffe, Zebra, Hartebeest, Roan antelope, Greater kudu Waterbuck Sitatunga, Hippo and the Crocodile (MNRT, 2012).

3.2 Study design

A simple cross sectional study was conducted among livestock (sheep and goats) in three out of four districts that formally made Kigoma region.

3.3 Sample collection and preparation

3.3.1 Blood sample collection

All blood samples were collected from small ruminants during IEP after the fall of rain season and early winter from three districts in Kigoma region. Blood were collected from 45 villages in the region, a total of 32 sheep and 379 goats were bled by venipuncture.

3.3.2 Preparation of serum

Serum used in this study was collected in May and June, 2011 and were kindly provided by Dr. Japhet Nkangaga, the Veterinary Investigation Centre (VIC) currently (TVLA), Tabora, Tanzania. The blood were left to coagulate and later separated to obtain sera, which was transported to the laboratory under cold condition and stored at -35°C until were used for detection of anti RVF viral IgG and viral genome (M segment) by RT-PCR.

3.4 Laboratory analysis of samples

3.4.1 An inhibition ELISA for detection of anti RVFV IgG antibodies

The ELISA kit (Biological Diagnostic Supplies Limited (BDSL), Scotland, UK) was used according to the manufacturer protocols and published procedures by Paweska *et al*., 2005 (Fig 5).

3.4.2 Extraction of viral nucleic acid from serum

Viral RNAs were extracted from the serum samples using a QIAamp Viral RNA Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer's instructions. Briefly, 140 µL of serum were added to 560 µL AVL buffer containing carrier RNA into a 1.5 mL micro-centrifuge tube and mixed by pulse-vortexing for 15 sec followed by incubation at room temperature for 10 min. About 560 µL of absolute ethanol were added and mixed by pulse-vortexing for 15 sec.

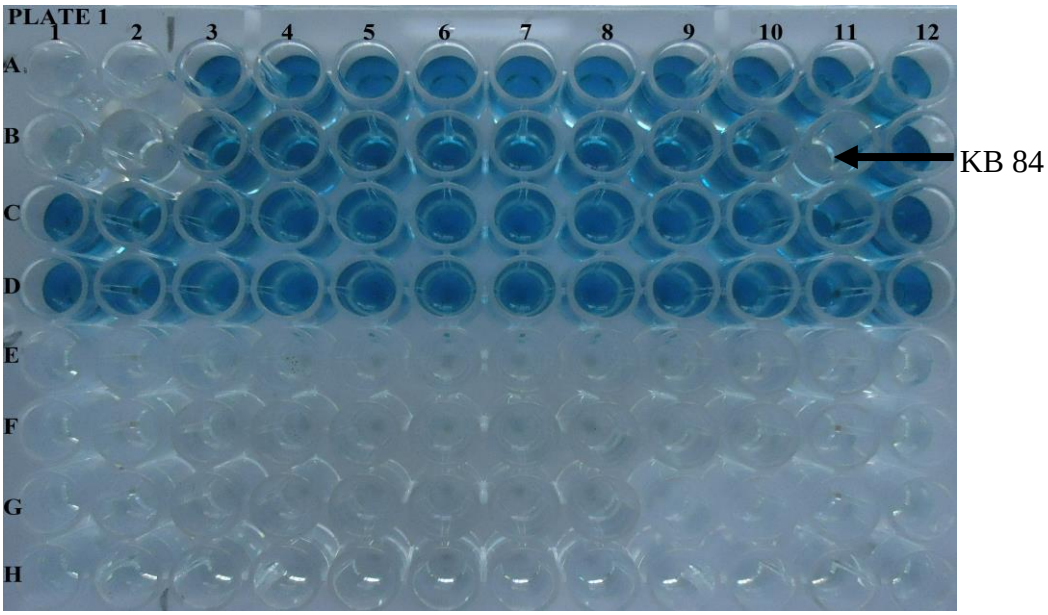

Figure 5: ELISA plate indicating sample layout whereby; Rows A-D 1-12 RVFV Ag, Rows E-H 1-12 Control Ag, Well 1A and 2A; Conjugate control, Well 1B and 2B; High positive control serum, Wells 1C, 1D and 2C, 2D; Negative control serum, Well 11B; Positive serum sample (KB 84, from an adult goat).

About 630 µL of the mixture were transferred to QIAamp spin column mounted on 2 mL collection tube and centrifuged at 6000x (8000 rpm) for 1 min. The column was then transferred to another collection tube and the other 630 µL of the mixture were passed through it. The column was then washed twice with 500 µL of washing buffers WB1 and WB2, respectively. Finally, RNAs were carefully eluted by 60 µL of buffer AVE equilibrated temperature.

3.4.3 Reverse transcriptase (RT) polymerase chain reaction (RT-PCR)

Single-tube RT-PCR amplification was carried out using One-Step Access RT-PCR system (AgPath-ID™ One-Step RT-PCR Kit, Applied Biosystems, USA). Briefly, a 25µL reaction mixture contained in final concentration with 12.5µL of 2x RT-PCR buffer,

2.0µL of 25picomole of both forward and reverse primers, 1.0µL of 25x Enzyme mix, 1.7µL of Detection enhancer and 5.0µL of target RNA were used. The total volume was brought to 25.0µL using RNase free water. Target amplified in low-profile 0.2mL tube (Axygen Inc, California, USA). The cycling program consisted of a reverse transcription step at 37°C for 30min, followed by 45 cycles of denaturation at 94°C for 30sec, annealing temperatures at 65°C for 30 sec each, extension at 72°C for 30sec. The reaction mixture in each PCR tube was then subjected to a final extension step at 72°C for 7min. All PCR amplifications were carried out at a 25µL volume per tube. Thermal profiles were performed on a GeneAmp PCR system 9700 (Applied Biosystems, USA). One set of primer RVF 1 FWD Forward: 5′-GAC TAC CAG TCA GCT CATT ACC-3′ and RVF 2 REV Reverse: 5′-TGT GAA CAA TAG GCA TTG G-3′ targeting a 551 base pair fragment of the M segment of RVFV was used during the PCR testing. Following amplification, 10 µL from each PCR tube containing amplified product were loaded onto gels of 2.0% (SeaKem agarose Bioproduct, Rockland, Maryland, USA) and electrophoresed. The gels were stained with ethidium bromide and the PCR products were visualized under UV light.

3.5 Data analysis

All the data were compiled in Microsoft Office Excel 2007. The serological data were analyzed using Epi info version 3.5.1 software. Chi-square test was used to analyse data by comparing different groups of collected data for statistical significance. The individual serological status was the binomial response, and the previously mentioned variables (age, sex and species) were the explicative factors.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Seroprevalence of RVF

The overall RVF seroprevalence was 5.4% (CI 95%, 3.5 – 8.1) in the three districts (Table 1). Kigoma rural district recorded higher seroprevalence 12.0% (CI 95%, 7.3 – 18.4) compared to the seroprevalence of 2.3% and 0.8% in Kibondo and Kasulu districts respectively. The difference was also statistically significant ($p < 0.0001$).

Table 2: Seroprevalence of Rift Valley Fever Virus infections in Kigoma, Tanzania May-June, 2011

District	Sample size	Positive samples	Prevalence (in %)	P Value
Kigoma rural (KG)	150	18	12.0	P< 0.0001
Kibondo (KB)	132	3	2.3	P= 0.322
Kasulu (KS)	129	1	0.8	P= 0.322
Total	**411**	**22**	**5.4**	

Univariate analysis showed that (Table 2) there was no statistical difference in the seroprevalence recorded in goats (4.7%) compared to that in sheep (12.5%). Similarly, there was no statistical difference in the RVF seroprevalence in female (4.5%) compared to that recorded in male (8.2%) animals sampled in this study. Age-wise, 5.5% of the adults and 4.8% of the kids or lambs were seropositive, however, there was no statistical difference.

Table 3: Univariate analysis between seroprevalence of Rift Valley Fever Virus infections and age, sex, and species in Kigoma, Tanzania May-June, 2011

Demographic data		Sample size	Positive samples	Negative samples	Prevalence (in %)	P Value
Age	Adults	348	19	329	5.5	1.0000
	Sub-adults	63	3	60	4.8	
	Total	**411**	**22**	**389**		
Sex	Males	97	8	89	8.2	0.1473
	Females	314	14	300	4.5	
	Total	**411**	**22**	**389**		
Species	Sheep	32	4	28	12.5	0.0815
	Goats	379	18	361	4.7	
	Total	**411**	**22**	**389**		

Legend: Sub-adult, animals below 1year of age

4.1.2 Single tube RT-PCR

RVF viral RNA was detected in serum samples by RT-PCR from 63 samples that showed high PI on ELISA assay (Fig 6).

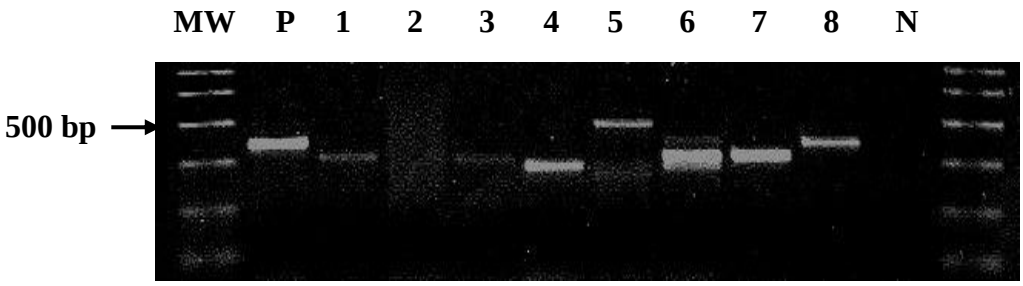

Figure 6: MW: 100 bp DNA ladder, P; RNA extracted from RVFV vaccine strain (Positive control), Lane 1-8 RNA samples extracted from Serum samples, N; RNase free water sample (Negative control).

Out of 63 serum samples with high PI, RVFV genome was detected in 8 samples (12.7%)

District	Sample ID	Species	Sex	Age	ELISA Results	PI-OD	PCR Results
KG	KG 126	Goats	F	Adult	_	4.08	+
KB	KB 28	Goats	F	Adult	_	9.97	+
	KB 61	Goats	F	Adult	_	2.47	+
	KB 80	Goats	F	Adult	_	2.73	+
	KB 87	Goats	F	Sub-Adult	_	20.91	+
KS	KS 20	Sheep	F	Adult	_	4.27	+
	KS 26	Goats	F	Adult	_	4.05	+
	KS 34	Goats	F	Adult	_	5.22	+

from all three districts (Table. 3). The entire 22 samples confirmed positive for RVF IgG antibodies by ELISA, were negative for viral RNA by RT-PCR.

Table 4: Serum samples that RVFV M segment was detected using RT-PCR
Legend: Sub –adult, animals below 1 year of age

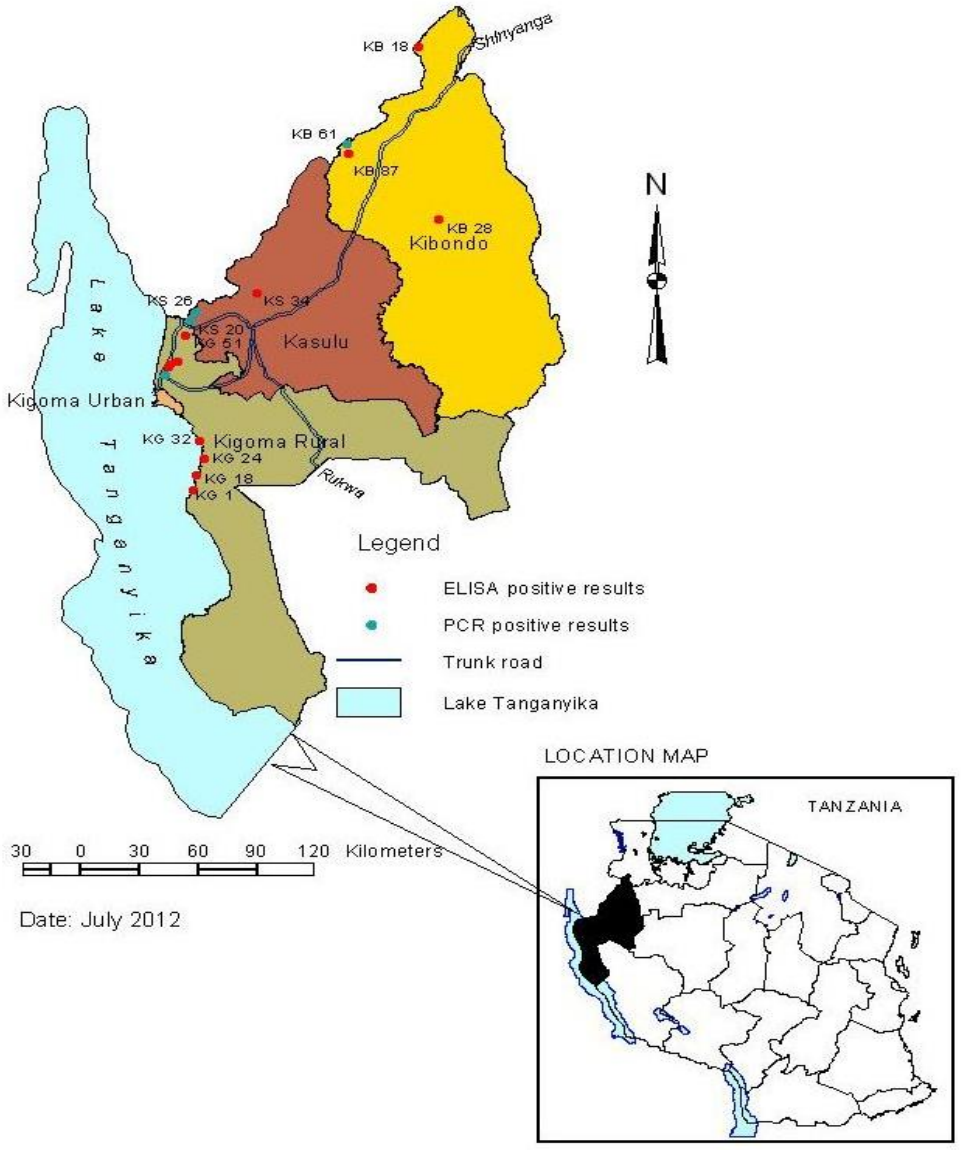

Figure 7: Map of Kigoma region showing the three districts where some of the RVFV-positive sheep and goats were identified

4.2 Discussion

The results of this serosurvey and genome detection targeting M segment in domesticated small ruminants suggest that RVFV has circulated in all the three sampled districts of Kigoma region. A serological surveillance system was chosen according to local

conditions, especially the convenience of getting samples, short-time frame for the study, cost and effectiveness. RVF surveillance can also be accomplished by a variety of other approaches including case finding, virus isolation from animal or entomological specimens, geographical and meteorological information systems (Thonnon *et al*., 1999).

This study was conducted 4 years after the 2006/07 outbreak, and was the largest RVF outbreak documented in Tanzania (Mohamed *et al*., 2010). It affected 10 of 21 regions of the country and 25 of 126 districts, resulting in over 300 suspected cases and a case fatality rate of 47%, this was higher than the previously reported outbreaks of 2000/01 in Saudi Arabia (14%) and 2006/07 in Kenya (26.4%) (Balkhy and Memish, 2003; Mohamed *et al*., 2010).

These results confirm that RVFV had circulated in livestock in Kasulu, Kibondo and Kigoma rural districts in Kigoma region. This information will complement what was known regarding RVF infection in the country after 2006/07 outbreaks in the country. Initially the disease was concentrated in the northern parts of the country that borders Kenya i.e. Ngorogoro and Monduli in Arusha region. Between February and June 2007, other regions of Tanzania including Manyara, Tanga, Dodoma, Morogoro, Dar es Salaam, Coast, Iringa, Mwanza, and Singida reported cases of RVF (Sindato *et al*., 2011).

The presence of significant levels of anti-RVFV IgG antibody against RVFV in the districts studied indicates that the virus could be circulating during this IEP. RVF vaccine had not been used in any of the districts during a period of at least four years prior to the

study. In most locations, RVFV antibodies were detected in animals aged both less than one year and over one year of age. This showed endemic presence of the virus in different parts of the region. Similar results were observed by Olaleye et al. (1996) in Nigeria.

These results also showed an overall seroprevalence of RVFV IgG antibody of the sampled districts. The remarkable higher seroprevalence rate was observed in Kigoma rural district compared to Kibondo and Kasulu districts. Similar results have been reported in Senegal with RVF IgG prevalence of 40% (n=40) and IgM 12.5% in 1994, ten years after epizootic (Thonnon *et al*., 1999). Species wise we found that sheep had higher seroprevalence of 12.5% compared to goats 4.7%. This has been observed in several other studies. Olaleye et al. (1996); Rostal et al. (2010) and Jeanmaire et al. (2011) from Nigeria, Kenya and Madagascar respectively, observed that sheep had higher seroprevalence than goats. This shows how sheep do acts as a good primary amplifying host of RVFV. Apart from sheep, RVF can affect many other species of animals including cattle, buffalo, camels, and monkeys, as well as gray squirrels and other rodents. The primary amplifying hosts are known to be sheep and cattle (OIE, 2007).

Kigoma rural district is joined together with Ujiji/Kigoma town, the regional headquarters as well as largest port of Lake Tanganyika, which is also linked to Dar es Salaam by the railway line and trunk road. This makes it a business city with a lot of activities including livestock trade (NSCA, 2008). This could be a major contributing factor for higher RVFV IgG antibody prevalence, as livestock importation to the region for trade could have contributed to introduction of RVF infection.

In view of inadequate number of livestock for slaughter in the region, livestock for slaughtering is augmented by livestock brought and transported by railways and road from the neighbouring regions to different destinations in the region (Regional Commissioner's Office, 2008). So, animal transportation for trade and looking for animal pastures have probably played a major role in the spread of the disease in Kigoma. RVFV could be transferred from endemic areas to other parts of the country in a very short period, via viremic animals (Jeanmaire *et al*., 2011). Chevalier et al. (2011) in Madagascar observed that the anti-RVFV IgG seroprevalence of bovines belonging to breeders who purchased livestock to renew their herd was 28.1%. Belonging to owners who buy animals to renew their herd was a risk factor ($p = 0.04$). Introduction of RVFV to new areas has been linked to livestock movement, which was previously implicated as a possible route for the introduction of RVFV into Egypt during 1977 outbreak (Gad *et al*., 1986).

In Kibondo and Kasulu districts, the seroprevalence between the districts was not significant. We speculate that animal movement coupled with the presence of Moyowosi game reserve that covers an area of 6000 sq km in Kibondo district, and support large populations of wildlife species like buffalo, Topi, Lion, Giraffe, Zebra, Hartebeest, Roan antelope, Greater kudu, Waterbuck, Sitatunga, Hippo and the Crocodile could be the supporting factors of RVFV transmission in Kibondo district (MNRT, 2012).

Moyowosi game reserve in Kibondo district might be playing important role in RVF infection in the region. The presence of wildlife species in the game reserve that have been proved to have RVFV antibodies during IEP might be a risk factor. Evans et al. (2008)

reported the highest prevalence of RVFV antibodies in ruminant wildlife in Kenya. Buffalo, which provided the largest number of specimens (n=342), had both a high prevalence of RVFV antibodies (16.95%) and also significantly higher titres of viral neutralizing antibodies compared to other animal species. Furthermore, wildlife-mosquito cycle may result into low-level livestock infections. Data in countries where RVFV outbreaks occur suggest that between 2.5% and 23% of livestock may have been infected by RVFV during an IEP (Swanepoel, 1981; Dohm *et al*., 2000).

In this study there was no significant difference observed in the seroprevalence among the three demographic characteristics namely species, sex and age. This is accounted to low number of sampled animals, which could not justify significance of the univariate analyses done. As compared to other studies, the prevalence of recent infections per district ranged from 5.9% (0–12.4) to 10.6% (5.6–15.5) in sheep and from 0.8% (0–1.9) to 3.3% (0–6.9) in goats, prevalence of past infections was significantly higher in males than in females in cattle ($P<0.001$) and in small ruminants ($P<0.001$) in a study done in Madagascar (Jeanmaire *et al*., 2011).

The RT-PCR provides a sensitive and specific detection of RVFV in cell culture and directly in serum samples from infected humans and animals. Currently, there are several RT-PCR assay protocols targeting different viral genes and a good example is the RT-PCR amplification assay targeting the S segment (Sall *et al*., 1999). It is well known that the L segment of the virus is highly conserved among the cognate genes of RVFV strains. Therefore, detection of viral genome targeting this L segment should enhance rapid diagnosis of RVFV and would facilitate differential diagnosis of hemorrhagic fever

viruses (Elata and Aradaib, 2011). The current study targeted the M segment in the detection of the viral genome using gene specific primers.

This study does not find any correlation between positive ELISA results and RT-PCR. All ELISA positive samples became negative on PCR. This is due to the fact that, after incubation period of 4 to 6 days, virus is usually demonstrated in the blood during the febrile period (3-4 days), where as neutralizing antibodies also start appearing around the 4^{th} day of onset of symptoms (Findlay and Daubrey, 1931). In case of RVF, PCR is more sensitive in the detection of early acute viraemic phase of infection that occurs between 4-8 days after infection. This explains the presence of past RVF infection in small ruminants in Kigoma for IgG ELISA positive results, and a recent RVF infection transmission due to single tube RT-PCR positive results. This could have been clearly explained by performing IgM ELISA together with IgG ELISA as it detects recent infections (Jeanmaire *et al*., 2011).

The mismatch of DNA bands we got in this study could be attributed due to several factors. The mechanisms of RNA virus variation include mutation, homologous and non homologous recombination and genome segment reassortment. It is worthwhile to note that different virus families exploit these mechanisms to different extents. The feature that most distinguishes RNA genetic elements from cellular DNA is the high mutation rate operating during genome replication. Miss-insertion errors during RNA replication and retro transcription have been estimated to be in the range of 10^{-3} to 10^{-5} substitutions per nucleotide and per round of copying (Batschelet *et al*., 1976; Drake, 1993). The main factor contributing to such high mutation rates is the absence or the low efficiency of

proofreading-repair activities that are associated with RNA replicases and transcriptases enzymes (Steinhauer *et al*., 1992). The crystal structures of reverse transcriptase and RNA polymerases do not reveal the presence of a domain that could be assigned to a 3' to 5' exonucleolytic proofreading activity, such as those found in cellular enzymes like the DNA-dependent DNA polymerase from *Escherichia coli* (Steinhauer *et al*., 1992). Also, mismatch repair mechanisms are unlikely to operate on replicating RNA (Modrich and Lahue, 1996) and cannot operate on single-stranded RNA progeny genomes.

Multiple infections from different RVFV strains, is another possibility for this and it could have due to the instability of the M segment. The M segment of the virus is less conserved among cognate genes of RVFV strains and is frequently associated with reassortment (Salim *et al*., 2010). In Kenya, Bird et al. (2008) reported co-circulation of Kenya 1 and Kenya 2 lineages over the same time period which showed contrasting pattern of virus evolution between the two closely related groups. Strong evidence was also found for a reassortment event occurring within the M segment of lineages D and E. Lineage D contains two subgroups, a West African group (D1) and a group comprised of strains collected in Southern Africa and Kenya (D2), while these 2 subgroups are monophyletic based on S and L segments tree topology, the M segment of the Southern Africa/Kenya group (D2) is monophyletic with lineage E viruses. Together these findings indicate that co-infection with different RVF viral genotypes do occur and have resulted in the generation of reassortant viruses multiple times over the evolutionary history of these virus strains (Bird *et al*., 2007). The detection of a reassortant virus containing both Kenya 1 and Kenya 2 genome segments indicates that interaction and the exchange of genetic material between each lineage via superinfection are possible (Bird *et al*., 2008).

Mismatches on primer binding sites, could be another contributing factor for variable in band sizes. A major problem in designing oligonucleotides for diagnostic PCR of RNA viruses is the considerable genetic variability of these viruses. In order to reduce the risk of PCR failure due to mismatches on primer binding sites, it is important to include as many sequences as possible during primer design (Drosten *et al*., 2002). To resolve this uncertainty, other sets of primers targeting other genes are also needed, but the most important thing is to sequence PCR products/bands as this will provide an insight of what this study could have been dealing with.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study has confirmed, for the first time, the presence of the RVFV in Kigoma region; 4 years after the 2007 epizootic in the country indicating that the virus activity exists during the IEP even in regions with no history of RVF.

Therefore, the study was of importance for gaining more knowledge on RVF infection in none RVF known regions. For the seck of improving national control strategies for this emerging disease which has devastating effect on animal and human health, food security, jeopardization of international trade, loss of livelihoods and huge economic losses being the consequences, and policy making regarding such kind of disease in the country.

5.2 Recommendations

Therefore, it is recommended that,

1. In-depth studies should be conducted to clarify the complex epidemiology of RVF and to ascertain extend of the problem country wide.

2. Viral isolation and sequencing researches for identification of the local circulating strains. This will have importance in vaccine development for rational control programs.
3. Public health awareness studies and campaigns should be conducted throughout the country, as we have confirmed the presence of active viral activities in

areas with no history of the disease. This will be very important when planning for proper RVF control programs.

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