SEROPREVALENCE OF RIFT VALLEY IN HUMANS AND THE ASSOCIATED RISK FACTORS IN SOME SELECTED DISTRICTS OF CENTRAL AND WESTERN ZAMBIA

By

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A dissertation submitted to the University of Zambia in partial fulfilment of the requirements for the award of the degree of Master of Science in One Health Analytical Epidemiology

THE UNIVERSITY OF ZAMBIA

Lusaka

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DECLARATION

I, **Chilufya Chola Kasongamulilo** do hereby declare that the contents of this dissertation being submitted herein represent genuine research I carried out and that no part of this dissertation has been previously submitted to this or another university or institution for the award of a degree or any other qualification.

Students Name: ……..

*Date : ……**.....***

Signature: ……

CERTIFICATE OF APPROVAL

This dissertation submitted by **Chilufya Chola Kasongamulilo** is approved as fulfilling part of the requirements for the award of the degree of Master of Science in One Health Analytical Epidemiology at the University of Zambia.

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ABSTRACT

Rift Valley fever (RVF) is an important viral zoonotic disease that not only affects ruminants but can cause serious morbidity and mortality in humans. In humans, symptoms range from mild flu-like to severe form such as retinal damage, meningo-encephalitis to haemorrhagic fever. From previous studies a prevalence of 9.43% of RVF antibodies was recorded in Lusaka abattoir workers close contact with cattle, while in Mazabuka district 18.63% tested were sero-positive of RVFV antibodies. However, Zambia has not recorded an outbreak of RVF for over 30 years, and as such little is known about the current sero-prevalence and risk factors associated with RVFV in human populations in the country. The aim of this study was to determine the extent of human exposure to RVFV and its associated risks in the selected districts of Zambia.

In this study, 202 blood samples were collected via the cephalic vein from healthy individuals who were at high risk of exposure to RVFV in central and western parts of Zambia. The sera tested using competitive multi-species ELISA and IgM capture ELISA.

This study revealed an overall seropositivity of 9.90%. The seropositivity was shared between two occupations, i.e. 16.67% was among abattoir workers while 14.41% was among livestock farmers. All seropositive results were IgG positive and none were IgM positive. Risk that were associated with RVF seropositivity ($p=<0.250$) at bivariate analysis were further analysed using a forward stepwise logistic regression analysis. However, only the movements of livestock in search of pasture and water was significantly associated to RVF seropositivity. This suggests that there was silent circulation of the virus indicative of an inter-epidemic period in Zambia. Since movement of livestock in search of pasture is a major risk factor for exposure to the RVFV as was found in this study, this would put other disease free areas at risk of having the virus spreading there. Therefore, controlled animal movements can be of great help in controlling the spread of this virus. This study recommends that public education will lead to increased understanding of RVF in Zambia thereby, achieving effective control of the disease.

DEDICATION

This dissertation is dedicated to my husband Innocent Mwape, my three children Chebo Mary Mwape, Chibote Charles Mwape and Chellah Katebe Mwape, my siblings Mukuka, Shula, Kombe, Kasonde and Mutale, my dear mother Bibiana Bungoni Kasongamulilo, my late father Charles Kasonde Kasongamulilo and to my late sister Kangwa Chola Kasongamulilo with endless love and good memories.

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LIST OF ACRONYMS AND ABBREVIATIONS

µl	microliters
Abs	Anti-bodies
c-ELISA	capture Enyme-linked Immunosorbent Assay
ELISA	Enzyme-linked Immunosorbant Assay
Gc	Carboxyterminal Glycoprotein
Gn	Amino-terminal Glycoprotein
ID	Innovative Diagnostics
Ig	Immunoglobulin
LFT	Lateral Flow Test
NSP	Non-structural Protein
OD	Optical Density
OR	Odds Ration
RNA	Ribonucleic acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
USD	United States Dollar
VRPRVF	Rift Valley Fever virus replicon particles

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Rift Valley fever (RVF) is an important viral zoonotic disease that affects both humans and animals such as sheep, goats, cattle and camels. It is caused by the Rift Valley fever virus (RVFV) of the genus *Phlebovirus,* a member of *Bunyaviridae* family (Hartman, 2017) . It is transmitted via infected mosquito (*Culex* and *Aedes* species) bites, humans to human contact or from contact with infected animal tissues and fluids (Linthicum, Britch and Anyamba, 2016; Nyakarahuka et al., 2018). In humans, symptoms range from mild flu-like to severe form such as retinal damage, meningo-encephalitis to haemorrhagic fever (Madani *et al.*, 2003). Case fatality can be as high as 14% as was seen in an outbreak in Saudi Arabia in 2003 (Madani et al., 2003).

Rift Valley Fever has become one of the most important zoonoses of sub-Saharan Africa over the last century, causing devastating health and economic impacts on domestic ruminants and humans (Redding et al., 2017). Social-economic impact of RVF was seen in 2006-2007, when Tanzania experienced disruption in the livestock market value chains, human and livestock deaths (Sindato *et al.*, 2014). Apart from that, high levels of malnutrition were recorded due to inability of people to obtain protein, including individual and national monetary losses that were experienced (Chengula *et al,* 2013) **.** Outbreaks have also been reported in Zambia and Zimbabwe resulting in numerous losses in livestock (Paweska, 2015; Javelle *et al.*, 2020). In Zambia, sera that was collected in 1978 from 15 different farms showed that cattle and sheep were sero-positive for RVFV, indicating a RVF epizootic in Chisamba, Ndola, Lusaka and Mazabuka districts (Davies *et al.*, 1992). In 1985 and 1986 , a sero-prevalence of 22% in animals of Namwala district and Lutale area of Mumbwa district was observed (Davies et al., 1992)**.** A prevalence of 9.4% of RVF antibodies was recorded in Lusaka abattoir workers in close contact with cattle, while in Mazabuka district 19 /167 tested sero-positive of RVFV antibodies and of these only 7 were in previous contact with cattle, for the rest transmission was assumed to be by mosquito bite (Morita, 1988). A compiled meta-analysis report of 17 studies (from countries generally from the horn of Africa, Kenya, Tanzania, Somalia, Yemen, Saudi Arabia and Sudan) shows the high economic impacts ranging from $5 to $470 million USD worth of losses due to RVFV infection (Peyre et al., 2015). In Niger in 2016, 28/105 deaths of suspected cases of

RVF were recorded (Javelle *et al.*, 2020). In 2006-2007, Kenya reported 700 cases including 158 deaths, while during the same period Tanzania reported 264 cases with 109 deaths, whereas in 2009-2011 South Africa and Namibia reported more than 250 RVF cases with 26 deaths. Zambia has not recorded any outbreak of RVF in the recent past, and as such little is known about the current sero-prevalence and risk factors associated with RVFV in human populations in the country, hence the need to carry out this research (Dar *et al.*, 2013; Baba *et al.*, 2016). In South Africa , high risk occupations such as butchering/slaughtering and processing of infected animal meat for consumption and exposure to mosquito bites were identified as exposure factors to RVF (Msimang *et al.*, 2019) The movement of animals with high vireamia have been reported to lead to the spread of the virus to other disease free areas due to the wide range of vectors capable of transmitting the virus (Gerdes, 2004)

1.2 PROBLEM STATEMENT

Rift Valley fever (RVF) is a re-emerging viral diseases that is of public health and veterinary importance in Africa (Dautu *et al.*, 2012). RVF outbreaks have previously been recorded in Zambia but information on its sero-prevalence in humans is scanty (Dautu *et al.*, 2012). There is also no information on levels of exposure to humans and the risk factors associated with the exposure to RVFV. Lack of knowledge of such factors can lead to inadequate prevention, control and management of the disease should it occur in the country. A study done in Tanzania showed that RVF outbreaks are a great threat to not only livestock and humans, but also has a negative impact on the social and economic status of the country (Chengula *et al*, 2013). High levels of morbidity and mortality in humans and livestock have previously been caused by several RFV outbreaks, leading to significant economic loss in the affected countries (Sindato *et al,* 2011). Recent studies suggest that RVFV is still circulating in livestock populations in Zambia, despite the country having not experienced an outbreak of the disease for several decades (Nyimbili, 2014). Those that live in close proximity to livestock and carry out slaughtering and processing of meat in likely infected areas are at high risk of RVF infection (Liu *et al.*, 2017). Previous studies have shown that pastoralist nomadic communities fall in the high and medium risk of RFV transmission (Alhaji *et al.*, 2018)

1.3 STUDY JUSTIFICATION

This study aimed to determine whether humans are exposed to RVFV in areas where the causative virus has previously been reported to circulate in animal populations in Zambia

(selected areas of central and western province) and also to determine factors that could be associated with such exposure. No study has been done to determine whether direct contact with animals, meat and other animal products with sub-clinical disease can lead to human infection in Zambia (Dautu *et al.*, 2012). Determining the risk factors that lead to RVFV infection can provide information on how best to protect those that are at risk should an outbreak of the disease occur. Individuals exposed to RVFV experience psychological distress and knowledge gathered can help with implementation of measure to prevent, manage or control the disease (Davies, 2010; Chengula *et al*, 2013)

1.4 Research Question

This research intended to answer the following research questions:

- What is seroprevalence of RVFV in humans in selected districts of Central and Western Provinces of Zambia?
- What factors are associated with exposure to this virus in humans in these selected districts?

1.5 Hypothesis

We hypothesis that RVFV is not prevalent in humans and no risk factors are associated with the exposure to the virus in selected districts of Central and Western Zambia

1.6 OBJECTIVES

1.6.1 General objective

The aim of this study was to determine the seroprevalence of RVFV in humans and to identify the associated risk factors associated with this exposure to the virus in selected districts of Central and Western Zambia

1.6.2 Specific objectives

- To determine the sero-prevalence of RVFV in humans in the selected districts of Central and Western Zambia
- To determine risk factors associated with seropositivity to RVFV in humans in the study areas.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Definition of the disease

Rift Valley fever (RVF) is a mosquito-borne zoonotic viral disease affecting domestic and wild ruminants, camels and humans (Paweska, 2015). Its transmission to human is via exposure to infected animal products and maybe self-limiting illness or maybe take a severe form of haemorrhagic disease(Wright *et al.*, 2019)

2.1 Aetiology of rift valley fever

Rift Valley Fever is a zoonotic disease caused by the RVF virus (RVFV), an ribonucleic acid (RNA) virus of genus *Phlebovirus*, family *Phenuiviridae* (Halawi *et al.*, 2019). The family *phenuviridae* also comprises of *banyangviruses* apart from *phleboviruses* (Kapuscinski *et al.*, 2021). RVFV has a single RNA genome that is divided into three segments L, M and S as shown in Figure 2.1. The L segments encodes for the RNA polymerase responsible for replication as well as mRNA transcription. The M segments encodes for the amino terminal glycoprotein (Gn) and carboxyterminal glycoprotein (Gc) which facilitate virus attachment and cell entry and helps in the viral assembly process (Faburay *et al.*, 2013). The M segment also encodes for two non-structural (NS) proteins NSm1 and NSm2 that function in suppressing the virus-induced apoptosis. The S segment encodes for the nucleoprotein also called the N protein which induces humoral and T-cell immunity response and also for the non-structural(NS) proteins NSs which down regulates the activated form of Interferon III of the human immune system (Street, 1979; Giorgi *et al.*, 1991; Billecocq *et al.*, 2004; Boshra *et al.*, 2011).

The RVFV appears as a lipid bi-layered enveloped virion measuring 90-110mm in diameter with the Gn and Gc heterodimers forming an icosahedral shell of 122 capsomers (Street, 1979; Huiskonen *et al.*, 2009). It infects the cells by receptor-mediated endocytosis and release of viral particles in the host cell cytoplasm where transcription, translation and replication of genetic material occurs (Anderson and Smith, 1987)

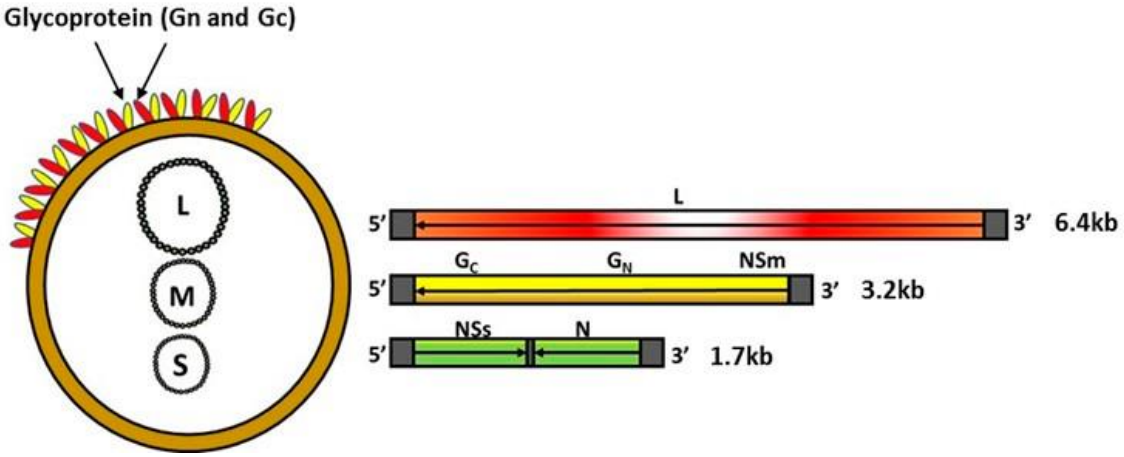

Figure 2.1: Schematic diagram of RVFV genome (Faburay et al., 2017).

Key: On the left is the viron containing small (S), medium (M) and large (L) RNA genome segments. On the right shows the representation of the coding strategy of the RNA genome segments; L = L protein; NSm= non-structural protein M; glycoproteins Gn and Gc; N= nucleoprotein; NSs= non-structural protein S (Faburay *et al.*, 2017)

2.2 Geographical distribution and major outbreaks

RVF was first discovered in Kenya in 1931 and is now endemic throughout multiple African countries and the Arabian Peninsula (Wright *et al.*, 2019). It is presumed global warming may lead to a spread of the vectors and of the RVFV out of their African habitats toward more polar regions, including Europe and North America, and into new host species such as camels, alpacas, and white-tailed deer (Ulrich, 2019).

This zoonotic viral disease of livestock and humans is found in Africa and the Middle East and is closely associated with high-rainfall conditions which are likely to favour the growth of RVFV mosquito vectors (Linthicum *et al.*, 2016). In 2016, Niger recorded 33/348 human deaths of reported cases of RVFV (Javelle *et al.*, 2020). Between 2006-2007, Kenya reported 700 human cases including 158 human deaths, while during the same period Tanzania reported 264 human cases with 109 deaths, whereas between 2009-2011 South Africa and Namibia reported more than 250 RVF human cases, with 26 deaths. Outbreaks have also been reported in Zambia and Zimbabwe resulting in numerous losses in livestock (Paweska, 2015; Javelle *et al.*, 2020). Repeated RVFV epizootics and epidemics have occurred in eastern and southern Africa (Namibia, Zimbabwe, Kenya, Nigeria, South Africa, Malawi,

Mozambique, Sudan, and Zambia) during years with exceptional rainfall (Linthicum *et al*, 2016).

In 2000-2001 the virus spread to the Arabian peninsula likely through importation of viraemic animals (Jupp *et al.*, 2002). Between 2006 and 2011 re-emergence was reported in East Africa, Madagascar and South Africa (Andriamandimby *et al.*, 2010; Mohamed *et al.*, 2010; Shieh *et al.*, 2010; Archer *et al.*, 2013). Between the years 1997- 2010, seven out of the nine RVFV outbreaks that occurred involved human cases and approximately 339,000 human infections in five of these outbreaks are believed to have occurred (Dar *et al.*, 2013). In 2016 an outbreak of RVF occurred on the western Niger border with Mali, at the time of diagnosis some of the infected cases had already returned from Mali to Europe (Tong *et al.*, 2019). Two of the three European cases were suspected to have been linked to direct contact with ruminants during slaughter or to mosquito bites, while the other case could have been due to a mosquito bite (Tong *et al.*, 2019). In 2016, a case of RVF was reported in a Chinese national who had been working in Angola and upon returning to China was admitted and tested positive for RVF (Liu *et al.*, 2017). The patient had no history of being in contact with livestock or any humans with fever but was frequently bitten by mosquitos (Liu *et al.*, 2017). In Zambia, of the sera that was collected in 1978 from 15 different farms of which 201 were from cattle and 186 were from sheep, 334 were sero-positive for RVFV, indicating a RVF was epizootic in Chisamba, Ndola, Lusaka and Mazabuka districts. A sero-prevalence of 22% in animals of Namwala and Lutale districts was observed between 1985 and 1986 (Davies *et al.*, 1992). A prevalence of 9.4% of RVF antibodies was recorded in Lusaka abattoir workers dealing with cattle and none out of the 40 that dealt with pigs were sero-positive, while in Mazabuka district 18.6% tested sero-positive of RVF antibodies having an overall sero-prevalence of 9.2% for both towns (Lusaka and Mazabuka) (Morita, 1988). Distribution of the virus is presumed to be country wide and is influenced by the presence and proximity of flooded dambo-type grassland depressions to cattle-producing areas (Mweene, 1997).

2.3 Epidemiological distribution of RVFV

In Madagascar, a study done by Lancelot et al (2017) showed that anthropogenic activities such as ruminant trade and cattle movement led to spread of RVFV to humans. This was deemed so because the epidemic of 1990 had lower rainfall than usual, thus suggesting that anthropogenic factors in this case were the main drivers of RVF than environmental

conditions (Lancelot *et al.*, 2017). Severe re-emergence of RVF have continued in historically endemic areas, resulting in negative effects on the economy and physical dependency on animal food threatens the public health and veterinary sectors (Paweska, 2015). Due to scepticism over the animal vaccines that are on the market, farmers are reluctant to vaccinate their animals, due to vaccines lacking safety, have been reported to cause abortions and fatal malfunctions (live attenuated Smithburn and Clonel3 vaccines), need multiple vaccination to provide protection (Formalin-Inactivated vaccine) and lack of differentiation between infected and vaccinated animal (DIVA) (Alhaj M. AO - Alhaj, 2016). Additionally occurrence of irregular outbreaks of RVFV are most common following exceptionally heavy rainfall (Alhaj M. AO - Alhaj, 2016). A compiled meta-analysis report of 17 studies (from countries generally from the horn of Africa, Kenya, Tanzania, Somalia, Yemen, Saudi Arabia and Sudan) shows the high impacts ranging from $5 to $470 million USD worth of losses due to RVFV infection (Peyre et al., 2015). Introduction of RVFV into disease free countries by importation of infected animals and infected mosquitos have resulted into negative socio-economic impact for both national and local economies due to restriction of access to exporting markets (Peyre *et al.*, 2015)

During RVF outbreaks, infection in livestock leads to increased occupational risk for humans (farmers and farm workers, veterinary professionals and those employed in the animal processing industry) exposed to tissues/ aborted foetuses and fluids of infected animals (Alhaji *et al.*, 2018; Msimang *et al.*, 2019). Human seropositivity correlates with animal seropositivity, suggesting that animal to human transmission may be the predominant mode of virus spread. Butchers and raw meat handlers have been shown to be more at risk of RVFV (Nyakarahuka *et al.*, 2018). Aerosolization of blood at slaughter is a means for transmission of RVFV and the slaughter man who is directly responsible for slitting the animal's throat has previously been reported to be at high risk for RVFV seropositivity in regions where epizootics occur (Cook *et al.*, 2017). Even though humans can be infected via mosquitoes, factors such as older age, being male, preparing of raw meat, working as a butcher, with a history of slaughtering or butchering or skinning animals have been significantly associated with RVFV seropositivity (Nicholas *et al*, 2014; LaBeaud *et al.*, 2015; Nyakarahuka *et al.*, 2018).

Raw milk of animal origin can too be a mode of transmission. Intake of raw milk and milking an infected animal has also been associated with increased risk of getting infected. This is because the RVFV can be shed in milk during high viraemic stages (Pepin *et al.*,

2010; Grossi-Soyster *et al.*, 2018). However the presence of the virus in faeces and urine has not been demonstrated to be infectious (Pepin *et al.*, 2010). These risk factors can be prevented or reduced by use of personal protective clothing and pasteurization of milk can help reduce transmission of the RVFV (Nicholas *et al*, 2014). In Zambia, wildlife has been suspected to play a role in RVFV maintenance during the inter-epizootic period due to evidence of RVFV silent circulation and disease emergence risk in some parts of the country. Above normal rainfall has shown to be a positive correlation with RVF infection in wild and domestic animals (Chambaro *et al.*, 2022)

2.4 Hosts and vectors of rift valley fever

There are a wide range of hosts for RVFV, be it domestic or wild ruminants. The hosts ranges from cattle, sheep, goats, pigs, camels, wild ruminants to humans and bats. However, the primary amplifying hosts are domestic ruminants (Pepin *et al.*, 2010; Gregor *et al.*, 2021; Omar Sayed Saeed *et al.*, 2021)

It's been assumed that wildlife are reservoir hosts of RVFV. However due to little published data on RVFV pathogenicity on various wildlife species, validation of diagnostic tests for wildlife exposure to RVFV and understanding the virus dynamics during the endemic periods in wild ruminants has not been carried out (Rosta *et al.*, 2017).

Vectors of rift valley fever include mosquitoes of the *Culex* and *Aedes* species. It has been reported that 53 species (from eight genera within the *Culicidae* family) caught in the field were positive for RVFV, while more than 65 species are described as potential vectors, the vast majority are from *Aedes* and *Culex* species. *Aedes* species are the primary reservoir vectors, while the others such as *Culex, Anopheles* and *Mansonia* species are secondary vectors important in the amplification of the infection by feeding on animals or humans (Linthicum *et al*, 2016; Hartman, 2017). *Culex* are associated with more permanent fresh water bodies while *Aedes species* are associated with freshly flooded temporary or semi-permanent fresh-water bodies (Pepin *et al.*, 2010; Pachka *et al.*, 2016). The population of these two species is highly dependent on the environmental factors at play in an area, where conditions such as unavailability of permanent water can prevent the desiccation of eggs hatching in the case of the *Aedes* spp (Pachka *et al.*, 2016). Infected *Aedes* spp lay eggs/progeny that contain the RVFV (transovarial transmission of the virus) therefore enabling survival of the virus during periods of drought or the inter-epidemic period and

infected eggs can hatch upon flooding , therefore associating RVF to heavy rains or irrigation (GARGAN *et al,* 1988).

Other biting arthropods, such as midges (Culicoids), ticks and sandflies can become infected with the virus and could potentially act as mechanical vectors (Lee, 1979; Davies and Highton, 1980; Linthicum *et al*, 2016; Klimentov *et al.*, 2020).

2.5 Transmission of RVFV

There are two main modes of RVFV transmission as shown in Figure 2.2. Mosquito-borne infections is the route of transmission in animals and while direct contact of transmission occurs when humans come into contact with infected animal tissues or bodily fluids, large numbers of virus particles are found in aborted animal foetal materials and can contaminate the local environment directly or infect humans or animals close-by. (Pepin *et al.*, 2010).

However, the main transmission to humans is through contact with infected animals or animal tissues and fluids (Pepin *et al.*, 2010). The human genome has coding for low-density lipoprotein receptor-related protein 1 (LRP1), heat shock protein (Grp94), and receptor-associated protein(RAP) which are essential host factors for RVFV infection (Ganaie SS *et al, 2021)*. Transmission of RVFV from infected mosquitoes to humans was never directly reported but probably more than one species of the *Ae. tarsalis* group and 78 mosquito species from eight genera have been associated with RVFV (Tantely *et al*. However, mosquitoes have been suspected to also cause human

RVF infection in some cases (Tong *et al.*, 2019)

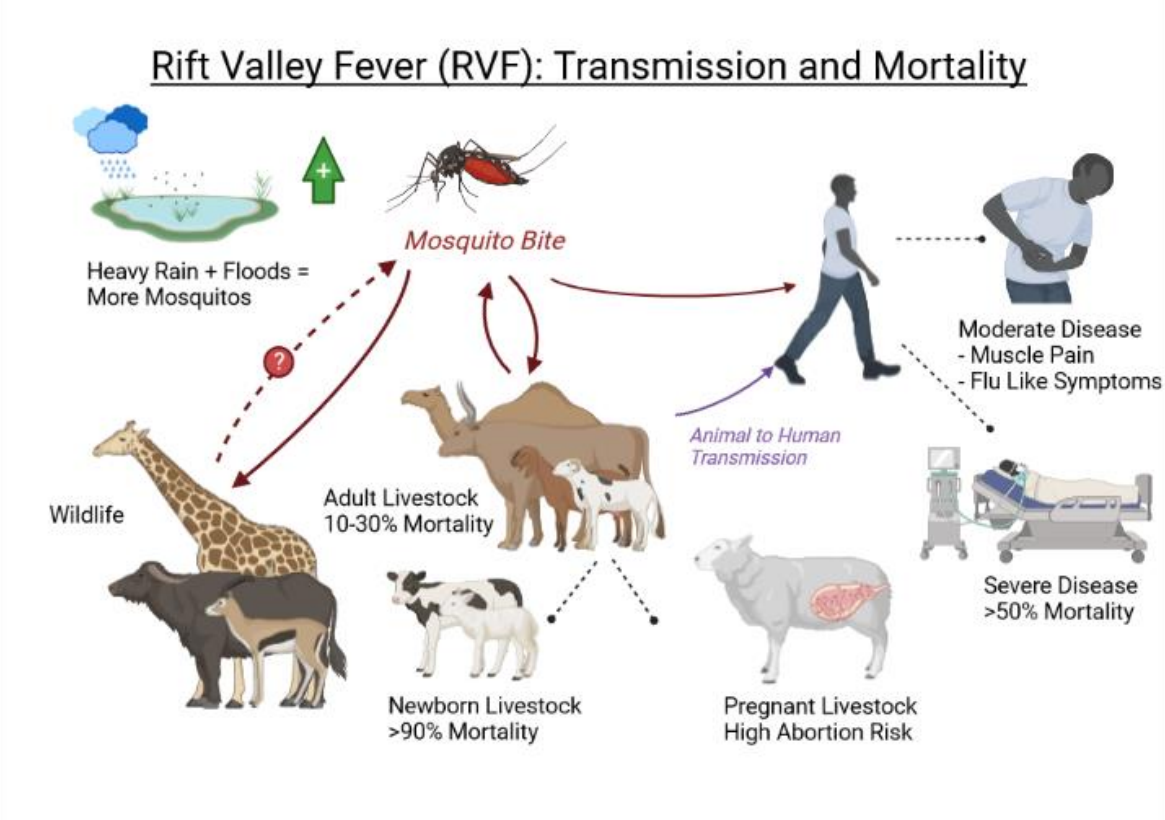

Figure 2.5: The transmission cycle and motility of RVFV in both animals and humans (Rift Valley Fever (RVF) — The Jenner Institute, no date).

2.6 Clinical manifestations of Rift Valley fever in humans

RVF transmission via mosquitoes and its viral replication in ruminants causes a high rate of animal mortalities and abortions, whereas in humans it mostly presents as a self-limiting, acute or febrile illness (Ikegami and Makino, 2011). In a study done by Pepin et al, (2010), most human cases of RVF infection were asymptomatic and a small proportion presented with flu-like symptoms, while severe RVF remained variable. In some individuals the flu-like fever presents for three days, a day or two of remission followed by another two to three days of flu-like fever (Madani *et al.*, 2003; Hartman, 2017). Symptoms that accompany the fever include weakness, headache, joint and muscle pain. Jaundice, diarrhoea, vomiting insomnia and painful eye follow later (Fawzy and Helmy, 2019) as shown in Figure 2.6 below.

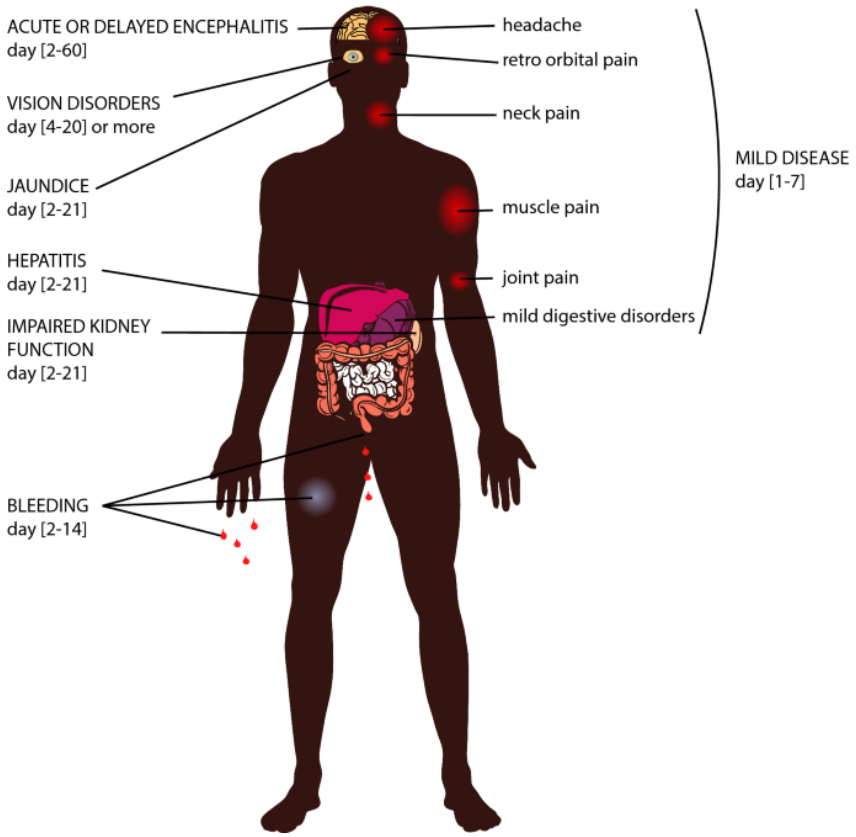

Figure 2.6 : Clinical manifestation of rift valley fever during acute or febrile illness in humans (Javelle *et al.*, 2020)

Furthermore, human clinical signs may include hepatitis, rhinitis, delayed- onset encephalitis and in most severe cases, haemorrhagic disease, with a case fatality rate of 10-20 % (Pepin *et al.*, 2010; Dodd *et al.*, 2012). Patients can present reduced vision or blurred vision due to inflammation of the retina and eye vessels may show retinal haemorrhage upon examination (Hartman, 2017). Individuals that present with high viral load were found to be associated with fatal outcomes. (Pepin *et al.*, 2010). In 2004 a bioterror incident occurred in California were RVFV was released by aerosolization and approximately 30 humans were infected at that site (Mandell and Flick, 2010).

2.6.1 Pathology of Rift Valley fever in humans

The RVFV is primarily hepatotropic, therefore the primary site of the virus induced lesions is in the liver leading to severe hepatic damage, especially in the absence of efficient innate response due to blocking of host interferon by the virus (Pepin *et al.*, 2010). The adaptive immune system kicks in after day 4-8 post-infection and anti-bodies (Abs), immunoglobulin IgG and IgM are produced against the viral nucleoprotein N and the non-structural protein NSs (Pepin *et al.*, 2010). Cases of encephalitis are seen in patients presenting with headache,

neck rigidity, confusion and temporal vision loss (Ikegami and Makino, 2011). Figure 2.6. shows more of the pathological forms of RVF in humans.

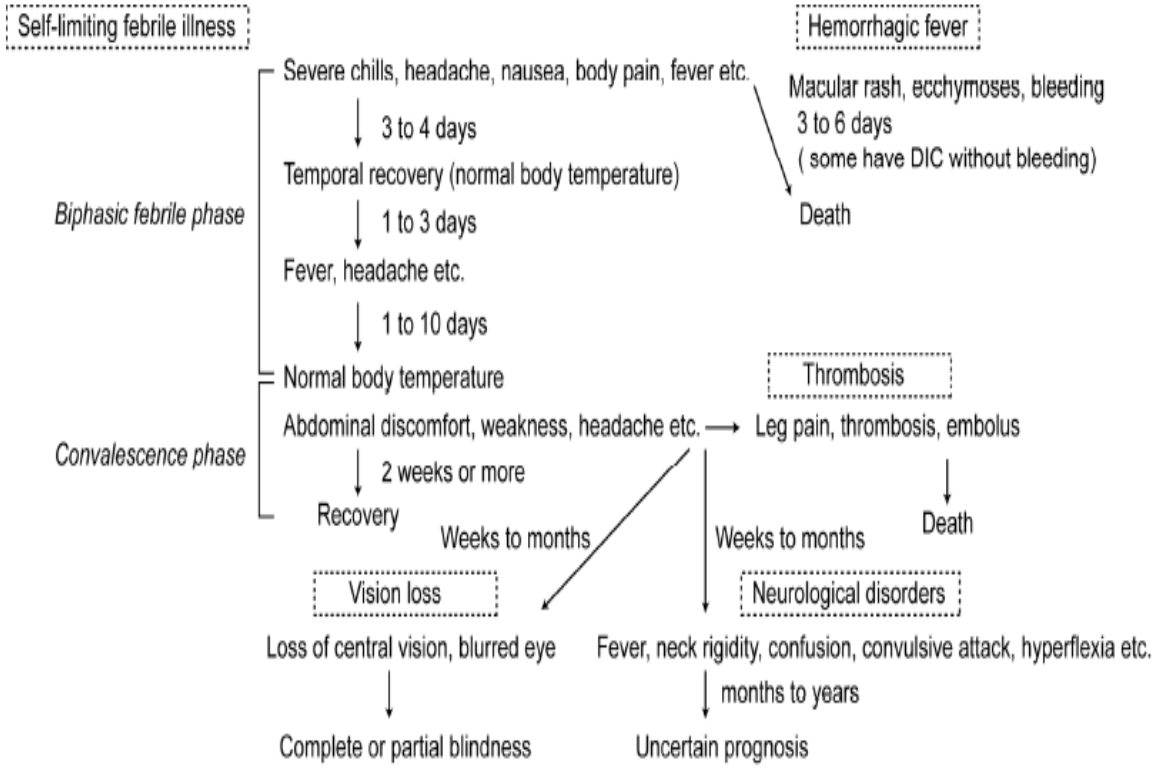

Figure 2.6.1: Pathophysiology forms of RVF in humans (Ikegami and Makino, 2011)

2.7 Diagnosis of Rift Valley fever in humans

Blood samples can be tested for the virus by use of reverse transcriptase polymerase chain reaction (RT-PCR), antigen-detection enzyme-linked immunosorbent assay (ELISA) or live virus isolation. Antibodies IgM and IgG can be detected using multi-species competitive ELISA for detection of both antigens (Matiko *et al.*, 2018). However this assay does not distinguish which of the two Igs are present thus the need to use the IgM- antibody captive ELISA for detection of IgM antibodies from RVFV antibody positive samples (Matiko *et al.*, 2018). The IgM- antibody captive ELISA can detect the IgM antibodies only. All samples that test positive for total RVFV antibodies but negative for IgM will be assumed to be positive for anti- RVF IgG antibodies (Mohamed *et al.*, 2014).

Innovative Diagnostics (ID) Screen Rift valley fever Multi-species competitive ELISA (cELISA) and IgM- antibody captive ELISA are low cost and easy surveillance tools with good overall accuracy (de Bronsvoort *et al.*, 2019). Detection of IgM indicates patients that

have been newly infected, while IgG antibodies are detected in recovered patients or older cases (Hartman, 2017).

The Lateral Flow Test (LFT) can be used under non-laboratory settings and does not require biosafety containment. However it has a lower specificity and sensitivity as compared to other tests (Cêtre-Sossah *et al.*, 2019). Other tests that can be used include immunohistochemistry, immunofluorescence, radioimmunoassay, agar gel, hemagglutinin inhibition and complete inhibition test. However, the complete inhibition test have a level of cross detection with other phleboviruses apart from RVFV (Saasa *et al.*, 2018; Odendaal L, Davis AS, 2021). ELISA methods are reliable in detection of immune antibodies against the RVFV in low income countries as compared to molecular and cell culture methods (Fafetine *et al.*, 2013)

2.8 Prevention and control of Rift Valley fever

There are no approved treatments for RVF. However over the counter medications can be useful in treatment of symptoms (fever and body pain), while hospitalized patients can receive supportive care such as fluid replacement (Hartman, 2017). Treatment and prevention is by use of potential anti-haemorrhagic drugs and vaccines, respectively. Drugs such as ribavirin has historically shown to have potential in the treatment of haemorrhagic fevers and potentially reduces mortality in Lassa fever cases. It has also shown potential in treatment of RVFV infected mice (Huggins, 1989). Favipiravir has also shown high potential against several haemorrhagic fever viruses in small animal models (Madelain *et al.*, 2017). Developments of drugs such as favipravir T-705, 2'-fluoro-2'-deoxycytidine (2'-FdC) and benzavir-2 are underway (Scharton *et al.*, 2014; Islam *et al.*, 2018; Smee *et al.*, 2018).

The RVFV genome is highly conserved, suggesting that a recombinant vaccine or a live-attenuated constructed one would have potential of giving protective immunity against all RVF virus lineages (Anderson and Smith, 1987). Use of vaccines against RVFV in livestock can be key in breaking the chain of human epidemic leading to control of a significant public health threat. Vaccines such as RVF virus replicon particles (VRPRVF), a live-attenuated vaccine shows high potential of giving high efficacy and elicited a robust immune response in distinct tissues within hours of immunization. This was shown in mice and cell culture (Dodd *et al.*, 2012). A vaccine based on the RVFV Gn and Gc glycoproteins showed development of neutralizing antibodies above the threshold in all the animals vaccinated (Bird and Nichol, 2012). The Formalin-inactivated RVF vaccine (TSI-GSD-200) is the

current vaccine being used and has shown to be safe and effective at protecting those at high risk. However a single dose had low immune response and a booster dose was required especially for those that did not respond to the primary dose (Dungu *et al*, 2018).

Apart from massive vaccination of animals during non-endemic periods, restricted movement of susceptible livestock to endemic areas can also help in reduction of RVF outbreaks (Bird and Nichol, 2012) . During an outbreak individuals must avoid close contact with body fluids of infected animals, practicing proper hand washing, wearing gloves to control animal-to-human transmission, avoid consumption of infected raw milk or animal tissues, use of protective measures to avoid mosquito bites by using mosquito nets, insect repellent, light, and wearing of coloured clothing (long-sleeved shirts and trousers), and avoid outdoor activity such as camping at the peak biting times to reduce human infection (Fawzy and Helmy, 2019).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was carried out among livestock keepers and workers in slaughterhouses or abattoirs in select districts of Western and Central provinces of Zambia. The districts were purposively selected based on their having been reported to have livestock exposed to RVFV (Saasa *et al.*, 2018). The districts purposively selected included Chisamba (Central province) Mulobezi, Mwandi and Sesheke (Western province) as shown in figure 3.1.

Chisamba is located in Central province of Zambia on lattude13º and 15º south with longitude of 27º and 29º East, with a human population of 160,828. Chisamba receives rainfall ranging from 160 - 200mm from November to March (NDJFM) (Aregheore, 2009;Zhang, 2013). Chisamba is well known for agricultural activities with over 32000 small scale farmers and 256 commercial farmers (Chilufya, 2016)

Mwandi and Sesheke districts are adjacent to each other in Western province of Zambia. These two districts have the lowest agricultural potential with less than 800mm per annum and a medium to high risk of drought (Turpie *et al*, 2015). The people in the area rely on livestock for income and livelihood. The flood plains and river banks offer a conducive grazing area for the cattle. Apart from cattle, chickens, goats, pigs and donkeys are kept as livestock (Saasa *et al.*, 2015). Sesheke has a human population of 72,655 and receives between 100-140mm in NDJFM (Zhang, 2013; Zambia Central Statistical Office, 2022). Mwandi district is located on latitude -16º and longitude 24.6º. Mwandi has more cattle than cattle owners (2,338/350000) showing that livestock keeping is the main occupation of the people living in the area (Saasa *et al.*, 2015). Currently, the human population of Mwandi stands at 40,418. In NDJFM, Mwandi experiences 100-120mm of rain.

Mulobezi district is also located in western province of Zambia with latitude -16.8º, longitude 25.2º. It has a human population of 45,326 and receives 120-140 mm of rainfall per year (Zambia Central Statistical Office, 2022). Mulobezi district shares boundaries with Sesheke, Lumpa , Kazungula, Senanga and Mwandi districts. It is called the Land of Timber and cattle and it is known for timber production and cattle rearing. The main activities are food production , fibre crops and livestock rearing (Hanson, 2016)

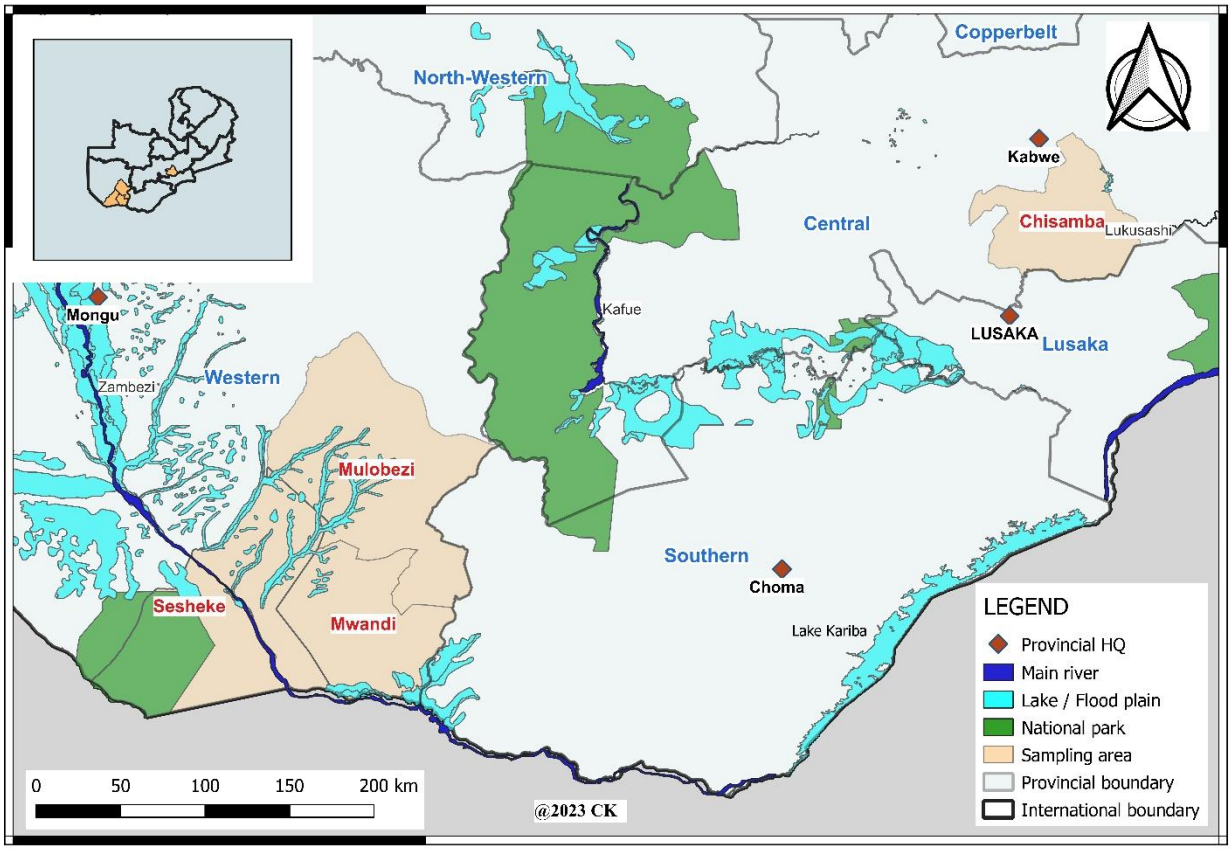

Figure 3.1: Map showing the study areas

3.2 Study design and sample size calculation

This was a cross-sectional study targeting livestock farmers and those who work in abattoirs, slaughter houses and meat processing plants. The sample size was calculated using the following formula for proportions (Dohoo *et al*., 2003):

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

Where:

n = required sample size

Z= 1.96 (critical value of confidence level at 95%)

P = prior prevalence of disease (Estimated to be 9.4%)(Morita, 1988)

d = 0.05 (maximum allowable error at 5%)

From this, the sample size was calculated to be a minimum of 124

Based on the 2010 Census (CSO, 2010), these were to be proportionally allocated to each district as follows: Chisamba 63, Sesheke 27, Mulobezi 19, Mwandi 15.

3.3 Study population

Individuals at high risk of being exposed to RVFV and are 18 years and above.

3.4 Inclusion Criteria

Participants 18 years or older in close proximity to ruminates and/or involved in slaughtering or preparation of meat products

3.5 Exclusion criteria

All who are not in close contact with animals or animal products.

3.6 Blood sample collection

After consent of the participants was obtained, blood samples were collected from the participants' cephalic vein (5 mls) by a qualified biomedical technologist into a plain vacutainer tubes. Sample collection was done between March 2022 August 2022 . The blood samples collected were individually labelled with unique identification codes according to the districts from which they were collected. Samples were stored on ice in cooler boxes until taken to the laboratory, at the university of Zambia, School of Veterinary Medicine. In the laboratory, each sample tube was centrifuged at 2500 rpm for 15 minutes and then the serum was aliquoted into appropriately labelled cryotubes and stored at -80ºCs until sample analysis. GPS coordinates of the sampling sites were recorded.

3.7 Questionnaire survey

A pre-tested semi-structured questionnaire (Appendix 8.1) was administered to each sampled person to capture information on potential risk factors for exposure to RVFV. The questionnaire was composed of two (2) sections. Section one were the personal details of the participant with only three (3) questions, Section two (2) was for the clinical details of the participants with 14 questions. Only respondents above the age of 18 were interviewed and sampled. Those below the age of 18 were considered minors and no ethical clearance was obtained to sample them as a result they were excluded from this study. The interviews focused on collection of information on socio-demographics of the respondents, age and sex, exposure to mosquitoes, any symptoms related to RVFV, attitude, practices and frequency of contact between domestic and the presence wildlife species, work exposure and use of personal protective equipment when handling the animals or meat.

3.8 Laboratory analysis of the samples

3.8.1 Rift valley fever competitive Multi-species ELISA

Laboratory analysis was carried out at the Public Health Laboratory in the Department of Disease Control, Samora Machel School of Veterinary Medicine, University of Zambia in Lusaka. The ID competitive multi-species ELISA kit that detects anti bodies against the RVFV nucleoprotein (NP) in serum or plasma was used.Presence of these anti-nucleoprotein antibodies by this ELISA method indicates exposure to the virus by natural infection or vaccination. The ID screen RVF Competition Multi-species ELISA or competitive ELISA (cELISA) targets both IgG and IgM in serum or plasma(Chambaro *et al.*, 2022). This ELISA method has been proven to be a low cost surveillance tool for the African context with a better accuracy than other ELISA methods, with a specificity of 0.997 and a sensitivity of 0.96 (Paweska *et al.*, 2005; de Bronsvoort *et al.*, 2019)

The cELISA was carried out according to the manufactures instructions (innovative Diagnostics, Grabels, France). All reagents were allowed to come to room temperature before use and were homogenized by inversion. A volume of 50µl of the dilution buffer 19 was added to each well coated with a recombinant RVF nucleoprotein. About 50µl of the positive and negative controls were added to wells A1, B1 and C1, D1 respectively as shown in figure 3.8a below. In the remaining wells 50µl of each serum sample was then added and incubated for one hour at 37ºC.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	**PC**	S5	S13	S21	S29	S37	S41	S49	S57	S65	S74	S82
B	**PC**	S6	S14	S22	S30	S38	S42	S50	S58	S66	S75	S83
C	**NC**	S7	S15	S23	S31	S39	S43	S51	S59	S67	S76	S84
D	**NC**	S8	S16	S24	S32	S40	S44	S52	S60	S68	S77	S85
E	S1	S9	S17	S25	S33	S37	S45	S53	S61	S69	S78	S86
F	S2	S10	S18	S26	S34	S38	S46	S54	S62	S70	S79	S87
G	S3	S11	S19	S27	S35	S39	S47	S55	S63	S72	S80	S88
H	S4	S12	S20	S28	S36	S40	S48	S56	S64	S73	S81	S89

Figure 3.7a. Layout of the cELISA plate

Plate layout

PC	Positive control
NC	Negative control serum
S1-S56	Human Test sera

After incubation, the wells were emptied and washed three times with 300µl of the wash solution. The Anti-RVF nucleoprotein conjugate (Anti-RVF-NP Conjugate) 1X was prepared by diluting the Anti-RVF-NP-Po Conjugate 10X to 1/10 in dilution buffer 19. A 100µl of the conjugate 1X was then added to each well and incubated for 30mins at room temperature (21ºC ± 5ºC). The wells were emptied and washed three times with 300µl of the Wash Solution. A 100µl of Substrate Solution was added to each well and incubated in the dark for 15 minutes at room temperature. Then 100µl of the stop solution was added to each well to stop the reaction. The resulting discolouration depended on the quantity of the specific anti-bodies present in the sample tested. Where there was absence of anti-bodies, a blue solution appeared and turned yellow upon addition of the stop solution while in the presence of anti-bodies no coloration occurred. The microplates were then read at 450nm Optical Density (O.D) with a microplate immunoskan reader (Biological Diagnostic Supplies Limited, Scotland, United Kingdom) with an inference filter of 405 nm.

3.8.2 *Rift valley fever IgM Capture ELISA*

The IgM-capture ELISA is designed to detect IgM anti-bodies directed against RVFV NP in serum or plasma and their presence indicates recent infection. From a validation study done in 2005, it showed a sensitivity and specificity of 96.47 and 99.44%, respectively (Paweska *et al,* 2005). RVF capture ELISA is proven to be 100% sensitive 5-42 days post-infection and 12.5% sensitive three weeks later, indicating that the stage of the disease may affect the outcome of the assay results and interpretation especially in epidemic situations (Paweska *et al.*, 2003)**.**

The capture-ELISA was carried out according to the manufactures instructions (innovative Diagnostics, Grabels, France). All reagents were allowed to come to room temperature before use and were homogenized by inversion. A volume of 40µl of the Dilution Buffer 14 was added to each well. Each serum sample/control was deposited twice (adjacent in even and odd-numbered wells, as shown in Figure 3.8b. A volume of 10µl of the negative and positive controls were added too wells A1, B1, A2, B2 and C1, D1, C2, D2 respectively.

	D14	RVFV -NP	D14	RVFV -NP	D14	RVFV -NP	D14	RVFV -NP	D14	RVFV -NP	D14	RVFV -NP
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	**NC**	**NC**	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	**NC**	**NC**	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	**PC**	**PC**	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	**PC**	**PC**	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S37	S37
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S38	S38
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S39	S39
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S40	S40

Figure 3.8b: Layout of the capture-ELISA plate

Plate layout

PC	Positive control
NC	Negative control serum
S1-S40	Human Test sera

Thereafter, 10µl of each sample was added in duplicate to the remaining wells as shown in figure 3.5. The microplates were covered and incubated for 60 min at 37ºC. The RVFV Nucleoprotein 1x was prepared by diluting the RVFV Nucleoprotein 10X to 1:10 in Dilution Buffer 14.

After incubation the wells were emptied and washed three times with 300µl of Wash Solution. A volume of 50µl of the prepared RVFV nucleoprotein 1X was added to the even-numbered columns only and 50µl of the Dilution Buffer 14 was added to the odd-numbered columns.

The plates were covered and incubated for 60min at 37ºC. Conjugate 1X was prepared by diluting the Conjugate 10X to 1:10 in the Dilution Buffer 14. The wells were emptied and washed three times with 300µl of the Wash Solution. A volume of 50µl of the prepared Conjugate 1X was added to each well, the microplates were covered and incubated for 60 mins at 37ºC.

The wells were emptied and washed three times with 300µl of Wash Buffer, thereafter, a volume of 100µl of the Substrate Solution was added to each well. The microplates were covered and incubated in the dark for 15 minutes at room temperature, after which a 100µl of the substrate solution was added to each well and the plate was covered and incubated for 15minutes in the dark at room temperature. A volume of 100µl of the Stop Solution was added to each well to stop the reaction. The resulting colouration depended on the quality of

specific antibodies present in the sample tested. In the presence of antibodies, a blue colouration appeared which became yellow after addition of the stop solution and in the absence of antibodies, no colouration appeared. The microplates were then read at 450nm Optical Density (O.D) with a microplate immunoskan reader (Biological Diagnostic Supplies Limited, Scotland, United Kingdom), with an inference filter of 405 nm.

3.8.2 Determination of test sera positivity

Rift Valley Fever Competition Multi-species assay

Assay validation

The results were analysed in Microsoft Excel 2016. The test was valid if the mean of the OD_{NC} was greater than 0.7. The mean was calculated by adding the ODs of the duplicated negative controls and dividing by 2.

$$\mathbf{OD_{NC} > 0.7 \ldots\ldots\ldots\ldots\ldots\ldots Equation\ 1}$$

and if the mean value of the positive control (OD_{PC}) is less than 30% of the $OD_{NC.}$

$$\mathbf{OD_{PC} / OD_{NC} < 0.3 \ldots\ldots\ldots\ldots\ Equation\ 2}$$

Interpretation

For each sample the competition percentage (S/N%) was calculated by dividing the sample optical density (OD_{sample}) over the negative control OD (OD_{NC}) and the result was converted to percentage, the formula was as follows:

$$\mathbf{S/N\% = (OD_{sample} / OD_{NC}) \ X \ 100 \ldots\ldots\ldots\ldots\ldots\ Equation\ 3}$$

In this study, the sera with S/N% ≤ 40 were positive, 40< SN% ≤50 were doubtful and those >50 were negative.

Rift Valley Fever IgM Capture assay

Assay validation

The results were analysed using Microsoft Excel 2016. For the validation steps calculation of the net OD results was as follows:

$$\mathbf{net\ OD = OD_{even\ well} - OD_{odd\ well} \ldots\ldots\ldots\ldots\ldots\ Equation\ 4}$$

The test was valid if the mean value of the net positive control OD (net OD_{PC}) was greater than 0.350.

$$\mathbf{net\ OD_{PC} > 0.350 \ldots\ldots\ldots\ldots\ Equation\ 5}$$

and it was also valid if the ratio of the mean values of the net positive and negative control ODs (net OD_{PC} and net OD_{NC}) was greater than 3.

net OD_{PC}/I net OD_{NC} I >3 ……..Equation 6

Interpretation

For each sample the S/P% was calculated using the formulae:

S/P%= (net OD $_{sample}$ / net $OD_{PC)}$ X 100 …… Equation 7

In this study, the sera with S/N% ≤ 40% were negative, 40< S/P% <50 were doubtful and those greater than 50% were positive.

3.9 Data analysis

The data generated was stored and cleaned in Microsoft Excel 2016® before being transferred to SPSS statistical package version 21(IBM, USA) for descriptive and inferential statistical analysis. Bivariate analysis of association between possible risk factors and occurrence of RVFV antibodies was compared using the Chi-square test or the Fishers Exact test, which ever was applicable. A step-wise binary logistic regression model was used to determine risk factors that were associated with people being seropositive to RVF (Hosmer and Lemeshow, 2000). All variables with p-values less than 0.250 in the bivariate analysis were included in the model.

The Logit link function returned the coefficient, p-value, odds ratio (OR) and 95% lower and upper confidence interval values for the OR. Criteria used in determining whether each of the constructed models adequately fitted the data were, a non-significant Hosmer and Lemeshow Test ($p > 0.05$) and a significant Omnibus Test of Model Coefficients ($p < 0.05$). All statistics tests were considered significant at $p \leq 0.05$.

3.10 Ethical consideration

Participants (18 years and above) signed a written consent to participate in the research and all the information concerning the research was availed to them. Only respondents who were eligible to participate and signed the consent form were interviewed and sampled. The study's ethics approval was obtained from the University of Zambia Biomedical Research Ethics Committee (UNZA-BREC) REF.NO.1294-2020. Approval to carry out research was also obtained from National Health Research Authority (NHRA) and Ministry of Health (MOH)

CHAPTER FOUR

4.0 RESULTS

4.1 Descriptive results

Two hundred and two (202) samples were collected from the study districts. Over sampling from 124 to 202 was done in order to reduce the sampling error due to having a small sample size. The distributions of the descriptive statistics of the participants are shown in Table 4.1. From the total 202 participants there were 159 males and 43 females. Majority of the participants (34.16%) were below 25 years of age, with the least (9.41%) being above or equal to 56 years old. As shown in table 4.1, Sesheke district had the largest percentage of participants followed by Chisamba, Mulobezi and Mwandi respectively.

Table 4.1: Descriptive statistics of the study participants and their exposure variables

Variable	Categories	n	Percentage (95% CI)
Gender	Male	159	78.7 (72.42-84.15)
	Female	43	21.29(15.85-27.58)
Age	18≤25 years	69	34.16(27.65-41.14)
	26-35	46	22.77(17.18-29.18)
	36-45	40	19.18(14.54-25.97)
	46-55	28	13.86(9.41-19.41)
	≥56 years	19	9.41(5.76-14.3)
Study site	Sesheke	78	38.61(31.86-45.7)
	Chisamba	71	35.15(28.58-42.16)
	Mulobezi	28	13.86(9.41-19.41)
	Mwandi	25	12.38(8.17-17.73)
Occupation	Livestock farmer	111	54.95(47.81-61.94)
	Student	31	15.35(10.67-21.07)
	Slaughter man	24	11.88(7.76-16)
	Butcher	20	9.9(6.15-14.88)
	Farm worker	16	7.92(4.6-12.54)

Mosquito exposure	Yes	164	81.19(75.11-86.33)
	No	38	18.81(13.67-24.89)
Use of Mosquito net/Repellent	Yes	124	61.39(54.3-68.14)
	No	78	38.61(31.86-45.70)
Flu-like fever	Yes	103	50.99(43.88-58.07)
	No	99	49.01(41.93-56.12)
Headache	Yes	118	58.42(51.29-65.29)
	No	82	40.59(33.76-47.71)
Neck stiffness	Yes	21	10.40(6.55-15.45)
	No	181	89.6(84.55-93.45)
Vomiting	Yes	24	11.88(7.76-17.16)
	No	178	88.12(82.84-92.24)
Loss of appetite	Yes	61	30.2(23.95-37.04)
	No	141	69.8(62.96-76.05)
General aches and pains	Yes	77	38.12(31.39-45.2)
	No	123	60.89(53.79-67.66)
Dizziness	Yes	52	25.74(19.86-32.35)
	No	150	74.26(67.65-80.14)
Blurred or decreased vision	Yes	24	11.88(7.76-17.16)
	No	178	88.12(82.84-92.24)
Live near dambo/swamps	Yes	116	56.43(50.29-64.34)
	No	86	42.57(35.66-49.71)
Keep any livestock	Yes	171	84.65(78.93-89.33)
	No	31	15.35(10.67-21.07)
Type of livestock kept	Cattle & goats	60	34.48(27.45-42.05)
	Cattle	56	32.18(25.31-39.67)
	Cattle, Goats & Pigs	31	17.82(12.44-24.32)
	Cattle, Goats & Sheep	18	10.34(6.25-15.86)

	Goats	9	5.17(2.39-9.59)
Animals move for pasture and water	Yes	107	61.49(53.83-68.76)
	No	67	38.51(31.24-46.17)
Graze with wild animals	Yes	132	75.86(68.81-82.02)
	No	42	24.14(17.98-31.19)
Interaction with the wild animals <30 days	Yes	142	81.14(74.55-86.65)
	No	33	18.86(13.35-25.45)
Raw milk intake	Yes	132	65.35(58.35-71.89)
	No	70	34.65(28.11-41.65)
Milk from	Cow	124	93.94(88.41-97.35)
	Cow & Goat	8	6.06(2.65-11.59)
Preparation of raw meat	Yes	120	59.41(52.29-66.24)
	No	82	40.69(33.76-47.71)
PPE	Yes	55	34.38(27.06-42.28)
	No	105	65.63(57.72-72.94)

n = number, CI = confidence interval, PPE = personal protective clothing

Most of the respondents (54.95%) were livestock farmers and 81.19% of all the participants reported having been exposed to mosquito bites. Mosquito nets and repellents were only used by 61.39% of the participants. Majority of the respondents reported having experienced flu-like symptoms (50.99%) and headaches (58.42%) during the previous one year. Among those that reported keeping livestock, 34.48% kept both cattle and goats, followed by 32.18% that only kept cattle while those that only kept goats were the fewest (5.17%). The majority of the farmers (61.49%) reported that they moved their animals in search of pastures and water. In the past 30 days, 81.14% of the livestock owners left their livestock to interact with wildlife as they were out grazing in the fields. Most of the participants consumed raw milk (65.35%) from local cows and goats and were involved in the preparation or processing of raw meat (59.41%) see Table 4.1.

4.2 Prevalence of RVF

In this study, out of 202 participants 20 were seropositive giving an overall seroprevalence of 9.9% (95% CI = 6.15 – 14.88%). All the 20 seropositive study participants were positive for IgG only and all were male. No IgM seropositive were found in all samples tested.

Males had a significantly higher seroprevalence of RVF than females ($p = 0.014$). There was also a significant difference in seroprevalence of RVF with age, with the prevalence being higher among those who were older than 56 years of age. The seroprevalence was significantly higher in Sesheke ($p = 0.003$) than in other districts sampled. Only livestock farmers and slaughter men were found positive, with the seroprevalence being significantly higher among the former than the latter ($p = 0.025$). There was no significant difference in seroprevalence among those who reported being exposed to mosquitoes and those not exposed ($p = 0.646$) nor was there any significant difference in prevalence among those that used mosquito nets or repellents ($p = 0.893$) as shown in Table 4.2a.

Table 4.2a Prevalence of RVF according to Demographic, study site and occupational characteristics of respondents

Variable	Categories	n	Number of positives IgG	Prevalence (95% C.I)	p-value
Gender	Male	159	20	12.58 (7.86-18.76)	**0.014**
	Female	43	0	0.0	
Age	≤25 years	69	0	0.0	**0.009**
	26-35	46	5	10.87 (3.62-23.57)	
	36-45	40	6	15 (5.71-29.84)	
	46-55	28	5	17.86 (6.06-36.89)	
	≥56 years	19	4	21.05 (6.05-45.57)	
Study site	Sesheke	78	15	19.23 (11.18-28.73)	**0.003**
	Chisamba	71	1	1.41 (0.04-7.6)	
	Mulobezi	28	2	7.14 (0.88-23.50)	
	Mwandi	25	2	8 (0.9826.03)	
Occupation	Livestock farmer	111	16	14.41 (8.47-22.35)	**0.025**
	Student	31	0	0.0	
	Slaughter man	24	4	16.67 (4.74-37.38)	
	Butchery	20	0	0.0	
	Farm worker	17	0	0.0	
Mosquito exposure	Yes	164	17	10.37 (6.16-16.08)	0.646
	No	38	3	7.89 (1.66-21.38)	
Use of Mosquito net/Repellent	Yes	125	12	9.6 (5.06-16.17)	0.893
	No	78	8	10.26 (4.53-19.21)	

Study participants reported having experienced a number of symptom during the year as listed in Table 4.2b. However, none of these symptoms were significantly associated with

being positive to RVF antibodies except for dizziness, where the prevalence was significantly higher among those that felt dizzy than those that did not (p = 0.038) (Table 4.2b). The other factors shown in the table were not significantly associated with participants being seropositive to RVFV.

Table 4.2b. Sero-prevalence of RVF according to Symptoms reported by the respondent

Variable	**Categories**	**n**	**Number of positives IgG**	**Prevalence (95% C.I)**	**p-value**
Flu-like fever	Yes	103	11	10.68 (5.45-18.31)	0.705
	No	99	9	9.09 (4.24-16.56)	
Headache	Yes	118	10	8.47 (4.14-15.03)	0.279
	No	84	10	11.9 (5.86-20.81)	
Neck stiffness	Yes	21	3	14.29 (3.05-36.34)	0.477
	No	181	17	9.39 (5.57-14.61)	
Vomiting	Yes	24	3	12.5 (2.66-32.36)	0.650
	No	178	17	9.55 (5.6-14.85)	
Loss of appetite	Yes	61	6	9.84 (3.7-20.19)	0.984
	No	141	14	9.93 (5.54-16.1)	
General aches and pains	Yes	77	9	11.69 (5.49-21.03)	0.116
	No	123	11	8.94(4.55-15.44)	
Dizziness	Yes	52	9	17.31 (8.23-30.33)	**0.038**
	No	150	11	7.33(3.72-12.74)	
Blurred or decreased vision	Yes	24	2	8.33 (1.03-27)	0.784
	No	178	18	10.11 6.1-15.51)	

However, participants that experienced dizziness showed a significantly high sero-prevalence of 17.31, 95% CI= 8.23-30.33 compared to those that did not experience dizziness 7.33,95% CI= 3.72-12.74.

The results of the association between being seropositive to RVFV and types of livestock kept, livestock management and behavioural risk factors are shown in Table 4.4. From these results obtained, those that reported that their cattle were moved in search of pasture and water and grazed with wildlife were associated with RVFV. Furthermore, the participants who reported drinking raw milk from cattle were also associated with being seropositive to RVFV (Table 4.2c). All the other factors shown in the table were not significantly associated with participants being seropositive to RVFV.

Table 4.2c Sero-prevalence of RVFV according to type of livestock, management and behavioural risk factors of respondents

Variable	**Categories**	**n**	**Number of positives IgG**	**Prevalence (95% C.I)**	**p-value**
Live near dambo/swamps	Yes	116	15	12.93 (7.42-20.43)	0.094
	No	86	5	5.81 (1.91-13.05)	
Keep any livestock	Yes	171	17	9.94 (5.9-15.44)	0.964
	No	31	3	9.68 (2.04-25.75)	
Type of livestock kept	Cattle & goats	60	10	16.67 (8.29-28.52)	0.092
	Cattle	56	6	10.71 (4.03-21.88)	
	Cattle, Goats & Pigs	31	0	0.0	
	Cattle, Goats & Sheep	18	1	5.56 (0.14-27.29)	
	Goats	9	0	0	
Animals move for pasture and water	Yes	107	16	14.95 (8.8-23.14)	**0.004**
	No	67	1	1.49 (0.04-8.04)	
Graze with wild animals	Yes	132	17	12.88 (7.68-19.82)	**0.014**
	No	44	0	0.0	
Interaction with the wild animals <30days	Yes	142	14	9.86 (5.5-15.99)	0.893
	No	33	3	9.09 (1.92-24.33)	
Raw milk intake	Yes	132	19	14.39 (8.89-21.56)	**0.003**
	No	70	1	1.43 (0.04-7.7)	
Milk from	Cow	124	18	14.52 (8.84-21.97)	0.875
	Cow & Goat	8	1	12.50 (0.32-52.65)	
Preparation of raw meat	Yes	120	13	10.83 (5.9-17.81)	0.591
	No	82	7	8.54 (3.5-16.8)	
PPE	Yes	55	2	3.64 (0.44-12.53)	0.251
	No	105	12	11.43 (6.05-19.11)	

One Hundred and Seven (107) of the Participants let their animals move for pasture and water 14.9, 95% CI =8.8-23. Fourteen (14) were seropositive while of the 70 that did not allow their animals to do this only 1.49, 95% CI = 0.04-8.04 were positive. The livestock farmers that let their livestock to go out and graze with other wild animals 12.88, 95% CI = 7.68-19.82, p=0.014 had a significant seropositivity while the participants that did not allow their livestock to interact with wild animals showed no seropositivity.

Ingestion of raw milk from cows and goats was very common by the local people and livestock farmers, of the 132 that consumed raw milk 19 were seropositive for RVF while only one of the 70 participants that did not consume raw milk was seropositive. There was a statistical difference between those that consumed raw milk and those that did not (p=0.003). Of the 124 that consumed raw milk from cows only 14.52% were positive, while eight participants consumed from both cow and Goat of which only one was seropositive with p=0.875 showing that there is no significant difference. There was no significant difference (p=0.591) between participants that were involved in preparation of meat and those that were not involved in meat preparation. Most of the participants that were involved in meat preparation mentioned that they did not use personal protective equipment (PPE-gloves, apron, gumboots and goggles) and out of 105 that did not use PPE 11.43% were seropositive while 55 of the participants that used PPE 3.64% were positive with a p-value of 0.251. Therefore, there was no significant difference between those that wore PPE and those that did not.

4.3 Maximum likelihood estimates of people being seropositive to RVF

A stepwise binary logistic regression model was used to determine predictors of people being seropositive to RVFV on ELISA. A non-significant Hosmer-Lemeshow goodness-of-fit statistic ($p = 0.902$) and a significant Omnibus Test of Model Coefficients ($p < 0.001$) were obtained, indicating that the model fitted the data. Table 4.3 shows that only the movement of animals from one pasture land to another in search for food and water was a significant factor associated with RVFV seropositivity.

Table 4.3: Maximum likelihood estimates of predictors of an individual being seropositive to RVFV

Variable	Categories	Odds Ratio	95% C.I.for Odds Ratio		p-value
			Lower	Upper	
Animals move for pasture and water	Yes	11.60	1.50	89.69	0.019
	No				
Constant		0.02			< 0.001

CHAPTER FIVE

5.0 DISCUSSION

The aim of this study was to determine whether people were being exposed to the RVFV in in selected districts of Western and Central provinces of Zambia and to identify potential risk factors associated with this exposure. This study revealed a prevalence of 9.9%, with Sesheke having the highest prevalence while Chisamba had the lowest prevalence. Sesheke district is found to be in western province which is considered to have a welfare status below rural areas while Chisamba (Central province) is considered to have a welfare status relatively better than the nation as a whole (Analysis, 2014). Previous studies done in Mazabuka and Lusaka indicated a prevalence of 9.2%. (Morita, 1988; Nyakarahuka *et al.*, 2018). Antibodies against RVF were detected in all the studied districts in both provinces, indicating that the disease maybe prevalent in a number of districts in the country.

In 2018, antibodies to RVFV were detected in Sesheke among cattle showing a circulation of the virus in that area (Saasa *et al.*, 2018). In 1974, Zambia had its first ever report of RVF in Chisamba and this study showed that the virus was in circulation in the district (Davies *et al.*, 1992). Mulobezi district has no record of having RVF in circulation but is adjacent to both Mwandi and Sesheke districts. Therefore, transmission of the virus to Mulobezi district is no exception.

Only IgG antibodies (past RVF infection) were detected in this study suggesting that there was no recent exposure to the virus. The levels of IgM antibodies are high between 6-22 days post infection (Paweska *et al*, 2005) and the possibility that samples are collected right after the disappearance of IgM in the samples is highly likely (Andriamandimby *et al.*, 2010). Furthermore , according to a study by Chambaro et al (2022), lack of detection of IgM in Zambia is suggestive of absence of active cases of RVF infections and low viral activity in RVF interepizootic period of more than three decades (Chambaro *et al.*, 2022). It is during the interepizootic period that the *Aedes spp* mosquitoes maintain the low levels of the disease by transmitting the virus to their eggs in unfavourable seasons (Chambaro *et al.*, 2022).

In this study, it was found that gender, age, study site, occupation, dizziness, movement of animals from one area to another in search for food and water, animals grazing together with wild animals and drinking of raw milk were factors that increased RVF seropositivity.

The current study showed a higher proportion of males compared to females in the study population and this might have increased the chances of males being seropositive to RVF. In previous studies it has been shown that being male is a significant risk factor to RVFV

with significant association (LaBeaud *et al.*, 2015). This is because males were more involved in the livestock keeping and heading of cattle and were more in contact with the animals than the female gender. A study done in Kenya agreed that males were three times more likely to be have antibodies to RVFV than the opposite sex (Woods *et al.*, 2002). However, a study on social culture in Africa showed that women were most likely to be more exposed to RVF due to frequently spending more time in animal care (Muga *et al.*, 2015). In sub-Saharan Africa women work more with animals and animal products like milking and slaughtering of small animal such as Goats and sheep (Muga *et al.*, 2015). Gender roles therefore do cause differential exposure to RVF in this case.

Additionally, there is a statistically significant variance in RVF seropositivity across different age groups, notably higher among individuals aged over 56 years while individuals under 25 years old tested negative for RVFV antibodies while older individuals showed IgG positive results. Previous studies have associated RVF infection with older age, in that older people have had a longer time to be exposed to RVFV infected mosquitoes and livestock (LaBeaud *et al.*, 2015). Past studies suggest a significant step in RVFV infection risk as one gets older (LaBeaud *et al.*, 2008, 2011)

Movement of animals from pasture to pasture in search for food and water showed significant association to RVF seropositivity and in a previous study done in Tanzania showed that movement of animals plays a key role in the spread of the infection (Kifaro *et al.*, 2014). Disease free areas are therefore under threat due to increased national and transboundary nomadic and commercial animal movements (Durand *et al.*, 2020).

This study showed that interaction with wildlife (wild rabbits, wild pigs, hyenas, impala, gazelle, elephants, fox) as livestock are let out to look for pasture and water showed a significantly association with seropositivity. This suggested that interaction is a potential spill over of the disease into livestock especially during outbreak periods, however more research needs to be conducted to estimate the force of infection in livestock , wildlife and humans in understanding RVFV transmission dynamics (Clark *et al.*, 2018). In other countries wildlife such as African buffaloes have been suggested to help and play a role in virus amplification during the interepizootic period (Dautu *et al.*, 2012; Clark *et al.*, 2018).

The ingestion of raw milk in this study showed that it has potentially large consequences on public health, as drinking raw/ unpasteurised milk is common and significantly linked to RVFV seropositivity in the bivariate analysis. Some studies have suggested that RVFV can also be contracted through ingestion of raw milk from infected animals and ingestion of

unpasteurised milk was significantly associated with RVFV seropositivity (LaBeaud *et al.*, 2011; Youssouf *et al.,* 2020). A study done in 2011 showed that those that consumed raw milk were three times more likely to be seropositive to RVF (LaBeaud *et al.*, 2011).

A Multi-variate analysis of this study results showed an overall significance that movement of animals from pasture to pasture in search of food and water was the only factor that was significantly associated with RVFV seropositivity. Therefore, it can be concluded that those that were in close contact with animals that were left out to move from pasture to pasture were 11.6 times more likely to be seropositive to RVFV than those that did not. Previous studies have shown that human rift valley infection was associated with the proximity of infected animals to humans and animal movements showing high risk to RVFV maintenance and circulation (Kim *et al.*, 2021). Movement of infected animals from enzootic areas has been shown to have triggered outbreaks in other countries due to uncontrolled animal movements (Sindato *et al*, 2011; Ikegami, 2012; Jäckel *et al.*, 2013). In this study, other RVFV risk factors that were associated with seropositivity were not significant in the multivariate analysis. Multi- variate analysis of previous studies have shown that factors such as gender, age , contact with domestic animals and drinking milk were not associated with RVF seropositivity (LaBeaud *et al.*, 2015; Msimang *et al.*, 2019)..

5.1 Limitations

The seroprevalence estimate was based on selected districts with recored of RVFV prevalence in animals and is not representative of the country as a whole. The study targeted at-risk occupational groups in a high-risk area for RVFV exposure in order to find sufficient seropositivity to power a risk factor analysis. Future research should also include other areas of Zambia, including those that are at low risk of outbreaks, to get a better understanding of how RVFV epidemiology varies geographically. Our calculations were based on information provided by the participants and may have been

affected by recall bias and false self-reporting, e.g., it is much easier for a person to recall whether he/she had contact with animals than a mosquito bite exposure. Despite this , we were able to draw some conclusions about factors associated with RVFV seropositivity which should be interpreted with caution.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The prevalence in this study (9.9%) is not that much different from the prevalence in the previous study (9.2%) done in Mazabuka and Lusaka in 1987. However, the level of IgG seropositivity indicates that RVFV has been circulating in central and western provinces of Zambia for some time and further studies are needed to identify when this introduction may have occurred. Therefore, it can be concluded that no acute RVFV infection was detected and only evidence of previous infection was captured due to lack of IgM detection. Seropositivity was amongst livestock owners and those that worked in slaughter houses indicating that these two groups are at a higher risk of being infected by RVF. The risk factors that were significantly associated with being seropositive to RVFV in this study included gender age, occupation, animals moving in search for pasture and water, animals grazing with wild animals and human ingestion of raw/unpasteurized milk.

Movement of animals in search for pasture and water plays a key role in the disease spread as it was the only factor that showed significant association with RVFV at multivariate analysis. Therefore, we assume that seropositive humans were most likely to be exposed to RVFV from contact with livestock that has been let out to feed from pasture to pasture with high possibility of being in contact with wild animals that may harbour the disease. Due to this, it puts other disease free areas at risk of having the RVFV spread there.

6.2 Recommendations

A similar and more comprehensive study with a lager sample size should be extended to other provinces to ascertain the distribution and sero-prevalence of RVF in Zambia.

More research studies are needed to better understand other ecosystems such as that of wildlife, domestic animals and mosquitoes. That knowledge can help in understanding their role in RVFV transmission, maintenance and epidemiology.

Ascertain the phylogeny of the RVFV strains circulating in the country and compare their genetic relationship with strains in other countries in the region using molecular biology can provide more information about the origin of the virus in Zambia. This strategy may also suggest other patterns of transmission which may have been overlooked in previous studies.

Public education of livestock farmers, abattoir workers, communities, livestock and veterinary staff needs to be enhanced in order to increase their knowledge of RVF and its risk factors. This study and the information gathered will assist in decision making for developing strategies and resource allocation for surveillance and potential zoonotic disease control interventions

Most importantly, the ‘’One Health’’ approach should be encouraged between animal and human surveillance amongst veterinarians, environmentalists, social scientists, ecologists, public health professionals and medics. Its implementation will lead to increased understanding of RVF in Zambia and subsequently achieving the execution of effective control strategies.

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APPENDICES

8.1 Semi-structured questionnaire used in the study

Questionnaire
Enquiry possible exposure to Rift Valley Fever

Study Title: **SEROPREVALENCE OF RIFT VALLEYIN HUMANS AND THE ASSOCIATED RISK FACTORS IN SOME SELECTED DISTRICTS OF CENTRAL AND WESTERN ZAMBIA**

Aim of the study: The aim of this study was to determine the extent of seroprevalence to RVFV in humans and to identify the associated risk factors associated with this exposure to the virus in selected districts of Central and Western Zambia

Specific objectives

1. To determine the sero-prevalence of RVFV in humans in the selected districts of Central and Western Zambia
2. To determine risk factors associated with seropositivity to RVFV in humans in the study areas.

This questionnaire should be completed by all individuals work in abattoirs and /or are livestock farmers

Interviewer's name _____

Date and time of interview _____ at _____
date *time*

Interview number /___/___/

Village/farm: _____

Geographical coordinates: Latitude: _____
Longitude: _____

Person interviewed: self ☐ other ☐ (please specify) _____

Section 1 – Personal details

1. Sex M □ F □
2. Age _____ years

3. Occupation *(describe what person actually does)*

_____

_____

Section 2 - Clinical details

4. For the past six months have you been exposed to mosquitos

Yes -1- No -2-

5. Do you use any mosquito repellent or mosquito net?

Yes -1- No -2-

6. . Have you had any of the following symptoms in the past one month?
(if symptoms still continuing code 9999)

	Yes	No	DK	Duration
Flu-like fever	1	2	9	_____
Headache	1	2	9	_____
Neck stiffness	1	2	9	_____
Vomiting	1	2	9	_____
Loss of appetite	1	2	9	_____
General aches and pains	1	2	9	_____
Dizziness	1	2	9	_____
Blurred or decreased vision	1	2	9	_____
Other symptoms (please describe)	1	2	9	_____

7. Do you stay near swamps/ damboos? Yes -1- No -2-

8. Do you keep any ruminant livestock? Yes -1- No -2-

   If 'Yes', how many? Cattle: _____ Sheep: _____ Goats: _____

9. Do you move the animals from one area to another during the year in search of pasture and/water? Yes -1- No -2-

10. Does your livestock share same grazing lands with wild animals? Yes -1- No -2-

11. Have you been in contact with any of these animals in the past 30 days?

    Yes -1- No -2- (**go to Q13)**

12. What animals are those_____

    _____

13. Have you ingested any raw milk from the animals in the past 30 days?

    Yes -1- No -2- (**go to Q15)**

14. From which animal/s was that milk from? _____

15. Have you been involved in preparation of raw meat in the past 30 days? Yes -1- No -2- (end of interview)

16. Do you use any type of personal protective clothing when handling the meat? Yes -1- No -2- (end of interview)

17. What type of PPE do you use? _____

This completes the interview. Thank you very much for your cooperation

8.2 Letters of approval used for the research

NATIONAL HEALTH RESEARCH AUTHORITY
Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@gmail.com | www.nhra.org.zm

Ref No: NHRA00005/4/03/2021 **Date: 4th March, 2021**

The Principal Investigator,
Ms. Chilufya Chola Kasongamulilo,
University of Zambia,
Department of Disease Control,
P.O Box 50110,
Lusaka.

Dear Ms. Kasongamulilo,

Re: Request for Authority to Conduct Research

The National Health Research Authority is in receipt of your request for authority to conduct research titled **"EVIDENCE OF HUMAN EXPOSURE AND RISK FACTORS OF RIFT VALLEY FEVER IN ZAMBIA."** I wish to inform you that following submission of your request to the Authority, our review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
2. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
3. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
4. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, University leadership, and all key respondents.

Yours sincerely,

Prof. Godfrey Biemba
Director/CEO
National Health Research Authority

All correspondences should be addressed to the Director/CEO National Health Research Authority

UNIVERSITY OF ZAMBIA
BIOMEDICAL RESEARCH ETHICS COMMITTEE

Telephone: 260-1-256067
Telegrams: UNZA, LUSAKA
Telex: UNZALU ZA 44370
Fax: + 260-1-250753
Federal Assurance No. FWA00000338

Ridgeway Campus
P.O. Box 50110
Lusaka, Zambia
E-mail: unzarec@unza.zm
IRB00001131 of IORG0000774

1st March, 2022.

Your Ref: 1294-2020.

Ms Chilufya Kasongamililo
University Teaching Hospitals,
Blood Bank,
P/Bag RW 1X,
Lusaka.

Dear Ms Kasongamulilo,

RE: REQUEST FOR RENEWAL FOR THE STUDY TITLED: "EVIDENCE OF HUMAN EXPOSURE AND RISK FACTORS OF RIFT VALLEY FEVER IN ZAMBIA" (REF. NO. 1294-2020)

We acknowledge receipt of your request for Renewal and enclosed Progress Report therewith.

Renewal is hereby granted for a period of one year from 4th February 2022 to 3rd February 2023.

Yours sincerely,

Sody Mweetwa Munsaka, BSc., MSc., PhD
CHAIRPERSON
Tel: +260977925304
E-mail: s.munsaka@unza.zm

All Correspondence should be addressed to the
Permanent Secretary
Telephone: +260 211 253040/5
Fax: +260 211 253344

In reply please quote:

No.....

MH/101/23/10/1

REPUBLIC OF ZAMBIA

MINISTRY OF HEALTH

NDEKE HOUSE
P. O. BOX 30205
LUSAKA

25 March, 2021

Ms. Chilufya Chola Kasongamulilo
Private Bag 1X
LUSAKA.

RE: APPLICATION FOR PERMISSION TO CONDUCT RESEARCH

Reference is made to the above stated subject matter.

The Ministry wishes to acknowledge receipt of your letter requesting for permission to conduct research titled "Evidence of Human exposure and risk factors of Rift valley fever in Zambia". The Research is expected to be conducted in Chisamba, Shibuyunji, Sesheke and Mwandi Districts of Zambia.

Following the review of the attached documents, the Ministry has no objection to the application and you are further advised to submit your research protocol to the Ministry. Once in the respective districts of your study, ensure you engage the District Health Offices for further guidance

Mr. Emmanuel Ngulube
Permanent Secretary (A)
MINISTRY OF HEALTH

CC: PHD- Central Province
CC: PHD – Western Province

13 SEP 2021
OFFICE OF THE DISTRICT HEALTH
DIRECTOR
P.O. BOX 820010, CHISAMBA