EPIDEMIOLOGICAL INVESTIGATIONS OF RIFT VALLEY FEVER IN LIVESTOCK IN THREE ECOLOGICAL ZONES OF MALAWI

By

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A thesis submitted to the University of Zambia in fulfillment for the requirements of the award of the degree of Doctor of Philosophy in Epidemiology and Public health.

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LUSAKA

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DECLARATION

I, Henson Kainga, do hereby declare that the contents of the thesis being submitted herein are my original work and they have not been previously submitted to any other university for the award of a degree or any other qualification.

Signature…… Date…….

APPROVAL

This thesis submitted by HENSON KAINGA is approved as fulfilling requirement for the award of the degree of DOCTOR OF PHILOSOPHY IN EPIDEMIOLOGY AND PUBLIC HEALTH of the University of Zambia.

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ABSTRACT

Due to uncertainty on the status of Rift Valley fever (RVF) in Malawi, limited knowledge about its epidemiology, and debates on whether it is a real or perceived threat for the livestock and human population in the country, a study was formulated to investigate the epidemiology of RVF. The study was carried out between January, 2020 and July, 2020 in 8 districts of Malawi, namely Chitipa (CP), Karonga (KA), Salima (SA), Mangochi (MH), Chiradzulu (CZ), Thyolo (TO), Chikwawa (CK) and Nsanje (NE). These districts were purposively selected from three ecological zones (EZ). Across-section survey using semi-structured questionnaire (n=400), was conducted to capture knowledge, attitude and management practices (KAP) information towards RVF. Average KAP score was calculated from total scores for knowledge, attitude and practices and assessed. In addition, 1,523 whole blood samples of cattle, goat and sheep were collected and 361 livestock farmers participated in questionnaire administration to capture information on potential risk factors. Indirect competition ELISA (cELISA) and IgM Antibody Capture Enzyme-Linked Immunoassay (MAC-ELISA) were used to detect presence of Rift Valley fever virus (RVFV) antibodies in serum. Questionnaire survey and the sero-positivity data, were analyzed for quantitative results using descriptive statistics. Bivariate analysis for association was conducted using Pearson chi-square and univariate linear regression, followed by multivariate analysis using stepwise binary logistic regression to determine the predictors of knowledge and risk factors for RVFV sero-positivity. Further, molecular techniques were used to isolate and identify RVFV genome from serum that tested positive to IgM ELISA test.

Participants had overall poor knowledge (score= 17.94%), negative attitude (score= 9.40%), and poor management practices (score= 41.23%) towards RVF. Only 8.25% participants had sufficient knowledge on RVF. The crude seroprevalence was 17.14%, (95% CI= 15.33-19.11) at individual livestock level. The seroprevalence across the ecological zones (EZ) were 20.34%, 11.78% and 14.55% for EZ1, EZ2 and EZ3, respectively, while seroprevalence for species were 21.35%, 7.72% and 25.68% for cattle, goat and sheep, respectively. The overall herd seroprevalence was 33.24%, (95% CI= 28.18-38.11). Sheep herd registered seroprevalence of 100% (95% CI= 73.23–1.00) higher than cattle and goat (p=0.019), consisted of 64.29% for IgG seroprevalence and 35.71% for IgM seroprevalence herds. Further, the herd seroprevalence for EZ2 was comparatively

higher at 36.36%, (95% CI= 28.6-44.55) than EZ1 34.12%, (95% CI= 24.40-45.88) and EZ3 29.32%, (95% CI= 21.92–37.95), at (p=0.047). Risk factors for RVFV seropositivity at individual livestock was sex of livestock and risk factors at herd level were areas receiving rainfall amount of <1,000mm and mixed species herds. The study demonstrated the presence of RVFV through detection of RVFV genome from three samples of livestock.

Results of this study provided epidemiological information of RVF in livestock in Malawi and the available gaps in control and prevention of the disease. Therefore, the study recommends community sensitization on RVF and investigation of RVFV seroprevalence in humans working in the risky areas for better clarification of impact of predisposing factors and risky management practices observed in the study area. Further, recommends study on molecular epidemiology of RVFV in livestock, humans and mosquitoes to effectively describe the RVFV in circulation.

DEDICATION

I dedicate this work to my family, my wife Carolyn and the two sons, Joshua and Joel.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
AVO	Assistant Veterinary Officer
AU-CTTBD	African Union Center for Tick and Tickborne Diseases
BI	Bayesian inference
Bioedit	Biological sequence alignment editor
BLADD	Blantyre Agriculture Development Division
BLAST	Basic Local Alignment Search Tool
BHQ	Black Hole Quencher
CHSU	Community Health Sciences Unit
CVL	Central Veterinary Laboratory
cELISA	Competitive Enzyme-Linked Immunosorbent Assay
cRNA	Complement Ribonucleic Acid
DAHLD	Director of Animal Health and Livestock Development
DAO	District Agriculture Office
DIVA	Differentiation of Infected from Vaccinated Animals
DNA	Deoxyribonucleic Acid
ECDC	European Centre for Disease and Control
ECF	East Coast Fever
EDTA	Ethylene-Diamine-Tetra-Acetic acid
EID	Emerging Infectious Disease
FGD	Focus Group Discussion
G	Glycoproteins
GDP	Gross domestic product
GPS	Geographical Positioning System
IFA	Immunofluorescence Antibody Assay
JAICA	Japanese International Cooperation Agency
KAP	Knowledge, Attitude and Practices
KII	Key Informants Interviews

L	Large
M	Medium
MAC-ELISA	IgM Antibody **C**apture – Enzyme-Linked Immunoassay
MCM	Medical Council of Malawi
MEGA6	Molecular Evolutionary Genetics Analysis version 6.0
ML	Maximum Likelihood
MoAIWD	Ministry of Agriculture, Irrigation and Water Development
MUSCLE	Multiple Sequence Comparison by Log-Expectation
NCBI	National Centre for Biotechnology Information
N	Nucleotides
Net OD_{pc}	Net Optical Densities for Positive Control
Net OD_{nc}	Net Optical Densities for Negative Control
NSs	Non-Structural protein S
NP	Nucleoprotein
NP-HRP	Nucleoprotein-Horseradish Peroxidase
PCR	Polymerase chain reaction
RNA	Ribonucleic Acid
rNP	Recombinant nucleocapsid protein
RVF	Rift Valley fever
RVFV	Rift Valley fever virus
S	Small
SHMPA	Shire Highlands Milk Producers Association
SHVADD	Shire Valley Agriculture Development Division
SISPA	Sequence Independent Single Primer Amplification
SPSS	Statistical Package for the Social Sciences
USA	United States of America
USD	United States Dollar
USDA	United States Department of Agriculture
UNZABREC	University of Zambia Biomedical Research Ethics Committee
WHO	World Health Organization

WVI	World Vision International
aOR	Adjusted Odds Ratio
OR	Odds Ratio
RdRp	RNA-dependent RNA polymerase
P	Probability (p-value)
pH	Power of hydrogen
RNA	Ribonucleic Acid
RT-qPCR	Reverse Transcription - Real-Time Polymerase Chain Reaction
VIF	Variance Inflation Factors
VNT	Virus Neutralization Test

PUBLICATIONS AND PRESENTATION

Publications during the thesis period

1. Kainga, H., Mponela, J., Basikolo, L., Phonera, M.C., Mpundu, P., Munyeme, M., Simulundu, E., Saasa, N. 2022. "Assessment of Knowledge, Attitudes and Practices towards Rift Valley Fever Among Livestock Farmers in Selected Districts of Malawi. *Trop. Med. Infect.* Dis.7,167. https:// doi.org/10.3390/tropicmed7080167

2. Kainga, H., Phonera, M.C., Chatanga, E., Kallu, S.A., Mpundu, P., Samutela, M., Chambaro, H.M., Kajihara, M., Shempela, D.M., Sikalima, J., Muleya, W., Shawa, M., Chulu, J., Njunga, G., Simuunza, M., Takada, A., Sawa, H., Simulundu, E., Saasa, N. 2022. "Seroprevalence and Associated Risk Factors of Rift Valley Fever in Livestock from Three Ecological Zones of Malawi" *Pathogens* 2022, *11*, 1349. https://doi.org/10.3390/pathogens11111349.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Rift Valley fever virus (RVFV), a member of the genus *Phlebovirus* of the family *Phenuiviridae,* is the causative agent of Rift Valley fever (RVF) a mosquito-borne viral disease that is a global threat to both animal and human health (Adams et al., 2017). It is considered an important emerging infectious disease (EID) because of its socio-economic and public health impact on people's livelihoods, on local and international trade of animals and animal products, and on food security in regions where ruminants are the primary source of proteins (Davies, 2006; EFSA, 2013).

In animals, RVF causes high mortality in young animals and/ or abortions in pregnant animals (El Mamy et al., 2011; Fafetine et al., 2016). The clinical signs in humans range from asymptomatic to severe neurological or hemorrhagic syndrome of public health concern (Hassan et al., 2011). The RVFV was first isolated in Kenya in 1931 (Daubney et al., 1931) and it has since spread across the African continent. About 12 countries have reported severe epidemics, while 16 countries demonstrated serological evidence of the disease in livestock and humans for the last three decades (Heinrich et al., 2012).

It is estimated that it takes between 4 to 15 years to have the epidemic form of the disease, which is associated with massive abortions and death of young animals (Thonnon et al., 1999). Mosquitoes are known to play a major role in maintaining and transmitting RVFV (Turell et al., 2008; Sang et al., 2010; Mwaengo et al., 2012). The periodic dormancy stage of RVFV in *Aedes* eggs found in dambos accounts for long intervals of RVF disease outbreaks. Vectors of RVF are classified into "reservoir/maintenance" vectors and "epidemic/amplifying" vectors. Reservoir/maintenance vectors include certain *Aedes* species mosquitoes (Diptera; *Culicidae*) associated with freshly flooded temporary or semi-permanent fresh-water bodies while "epidemic/amplifying" vectors, consist of *Culex* spp. associated with more permanent fresh-water bodies (Cornel et al., 2018; Braack et al., 2018).

Aedes mcintoshi is the most important maintenance vector of RVFV in Eastern and Southern Africa (Turell et al., 2007). It has all attributes of a competent vector such as abundance, longevity, wide distribution and adaptive feeding ability (FAO, 2014; Braack et al., 2018; Oyas et al., 2018). The accepted transmission paradigm involves survival of RVFV in mosquito eggs through transovarial transmission from parous *A. mcintoshi* and *A.* (*neomelaniconion*) *circumluteolus* females to their progeny during dry periods. These *A. mcintoshi* and *A.*(*neomelaniconion*) *circumluteolus* oviposit on the soils surrounding the stagnant water. The eggs require a period of dehydration before they hatch thus potentially making *A. mcintoshi* and *A.* (*neomelaniconion*) *circumluteolus* the ideal vehicle for survival of RVFV over long periods of time in floodwater (Pepin et al., 2010; Dar et al., 2013; Diop, 2015).

Epidemics of RVF in animals have been economically disastrous to the livestock industry (Peyre et al., 2015). South Africa reported a major outbreak in 2006-2011 (van Vuren et al., 2018). During the same period of 2006-2011, Tanzania, Kenya, Swaziland and Somalia reported RVF outbreaks that negatively affected livestock populations especially cattle and goats as well as the economic status of the countries and farmers (Pepin et al., 2010; Diop 2015; van Vuren et al., 2018).

In Malawi, the last confirmed RVF outbreak occurred in 1990 (Edelsten, 1990). A sero-epidemiological study confirmed the presence of RVFV antibodies in cattle (Bryony, 1992). During the 2006-2007 rainy season, Malawi experienced a suspected outbreak of RVF, but was not confirmed by laboratory tests due to lack of diagnostic capacity. The disease caused massive cases of abortion among dairy cattle and goats in southern region mostly within the milk catchment area of Shire Highlands Milk Producers Association (SHMPA). Epidemiologically, the disease appears to have been sparked by movement of animals from RVF infected regions of Tanzania where the animals were sourced by World Vision International Malawi (WVI) (GoM, 2008). The clinical signs were noticed in both livestock and humans. The morbidity and mortality from suspected RVF in both animals and humans led to economic crisis to the farmers and the country. Rift Valley fever as a zoonotic and occupation hazard possess a great threat to human health and sustainable livestock productivity. Livestock industry has many people likely working with potentially infected livestock. Therefore, the current study attempted to elucidate the epidemiology of RVF in Malawi.

1.2 Problem Statement

Rift Valley fever outbreaks have severe impact on the health of people and livestock with negative socio-economic outcomes due to loss of man power and death or abortion in animals. In humans, RVF usually causes mild to an acute undifferentiated fever, but in severe cases hemorrhagic fever, neurological disorders, or blindness and death can occur (Chevalier et al., 2010). The most severe outbreaks during 1997–1998 and 2006–2007 seasons in Tanzania, Kenya and Somalia caused 478 and 309 human deaths, respectively. The outbreak that occurred in 2000 resulted in 124 human deaths in Saudi Arabia and 166 human deaths in Yemen. In Sudan, the 2007 outbreak resulted in 222 human deaths (Sindato et al., 2011; Sindato et al., 2012; Himeidan et al., 2014). Further, RVFV is also considered as a potential bioterrorism tool that could have direct and indirect impact in countries that are free from the virus. Peyre and colleagues reported that from 2006 to 2007, an epidemic in Kenya and Tanzania resulted in more than 30,000 animal and 1,000 human cases (Peyre et al., 2015). The estimated economic losses due to the outbreak were high, ranging from 50 to 470 million USD (Peyre et al., 2015). According to Sindato et al., (2012), the 2007 outbreak in Tanzania had dramatic impact on the international animal trade in which there was a 54 % decline in exports equivalent to a loss of $352,750.00. The estimate of loss as a result of cattle mortalities was $4,243,250.00, whereas that of goats and sheep was $2,202,467.00. In Malawi, there have been suspected outbreaks of RVF since 1990 affecting livestock (Edelsten, 1990; Bryony, 1992).

However, no recent study has been done to confirm the existence of RVF in Malawian livestock. Moreover, the latest RVF suspected outbreak in Malawi occurred in March, 2006, following a period of heavier than usual rainfall and widespread flooding. The suspected outbreak spread to many districts in the Southern region in July 2007. Currently, all probable cases of RVF are thought to be other diseases such as East Coast Fever (ECF) and Brucellosis. In many instances however, the samples test negative for ECF and Brucellosis at Central Veterinary Laboratory (CVL), and was demonstrated by the work of (Kothowa et al., 2021). Failure to confirm cases of suspected RVF is mainly due to lack of diagnostic capacity in the country.

Furthermore, cases of suspected RVF lower productivity of most dairy cattle and beef establishments. As a result of numerous abortion cases, the livestock population growth at smallholder farmer level is constant or decelerating which reflect slow livestock population growth nationally (DAHLD, 2014; DAHLD, 2016). Similar RVF probable disease effects are experienced in small ruminant production. Low population growth of goats and sheep is more significant in many districts due to undiagnosed infectious diseases with epidemiological characteristics of RVF (DAHLD, 2016).

Lack of epidemiological knowledge of RVF in Malawi is considered as a risk to many livestock and humans to this zoonosis. For better and safe working environment in the livestock industry, there was need to determine the epidemiology and risk factors of RVFV in Malawi.

1.3 Justification and Significance of the study

Lack of epidemiological data on RVF in Malawi makes it difficult to prevent and control the disease. Therefore, there was a need to generate epidemiological data through a well-designed study. The availability of current molecular techniques that are highly sensitive, cost effective and rapid diagnostic kits provided a good opportunity to carry out this study. Furthermore, the scientific knowledge and epidemiological data generated from this study will be used to promote disease awareness for the farmers, to formulate better policy and foster multidisciplinary approach in the control of zoonotic and other emerging diseases. The study defined livestock and human populations at risk and provide basis for preventive intervention. Therefore, the current study has elucidated the epidemiology of RVF in Malawi. The study has also provided epidemiological information on RVF in Malawi, which can be used in designing effective control strategies for a sustainable livestock industry within the three regions of the country and at national level.

1.4 Research Questions

The study was set to answer the following questions

- **i.** What was the knowledge, attitude, and management practices of livestock farmers towards RVF?
- **ii.** What was the seroprevalence of RVFV in cattle, goat and sheep?

iii. What were the potential risk factors associated with seroprevalence of RVFV in livestock in Malawi?

iv. Can we detect the circulating RVFV among livestock in Malawi?

1.5 Objectives

1.5.1 General Objective

To determine the seroprevalence, risk factors and spatial distribution of Rift Valley fever in Malawi.

1.5.2 Specific Objectives

i) To determine the knowledge, attitude, and management practices of livestock farmers towards RVF.

ii) To assess the seroprevalence of RVFV in cattle, goats and sheep.

iii) To determine the risk factors associated with RVFV sero-positivity of individual livestock and herds.

iv) To identify the genome of the RVFV in livestock samples.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical background of Rift Valley fever

Rift Valley fever (RVF) is an emerging disease, affecting mainly ruminants, then transmitted to humans, which was first reported in 1931 on a farm in the Rift Valley of Kenya (Daubney et al., 1931). Since then, outbreaks have been reported largely in sub-Saharan Africa, West Africa, North Africa and in the year 2000, in the Arabian Peninsula (Bird et al., 2007a; Seufi and Galal, 2010). The disease is considered to be endemic in sub-Saharan African countries, with periodic major outbreaks, associated with incidents of heavy rainfall and flooding (Davies et al., 1985, Nderitu et al., 2010). Outside sub-Saharan Africa, RVF epidemics were confirmed in Egypt in 1977, Mauritania and Senegal in West Africa (El-Akkad, 1978; Fontenille et al., 1998; Diallo et al., 2005), Saudi Arabia and Yemen in the Arabian Peninsula (Jupp et al., 2002), and Madagascar and the Comoros islands in the Indian Ocean (Morvan et al., 1991; Sissoko et al., 2009). A recent human RVF outbreak in Africa, was reported in Kenya (WHO, 2021), and outside Africa was at Mayotte, France (Youssouf et al., 2020). The spread of RVFV beyond its endemic region raises concerns about threat of RVFV introductions to new geographical areas (Pepin et al., 2010). About 12 countries have reported severe epidemics, while 16 countries demonstrated serological evidence of the disease in livestock and humans for the last three decades in Africa (Figure 1-1) (Heinrich et al., 2012). The red circle in (Figure 1-1) shows the location of Malawi where there have been periodic isolations and serological evidence of RVF (Sumaye et al., 2019).

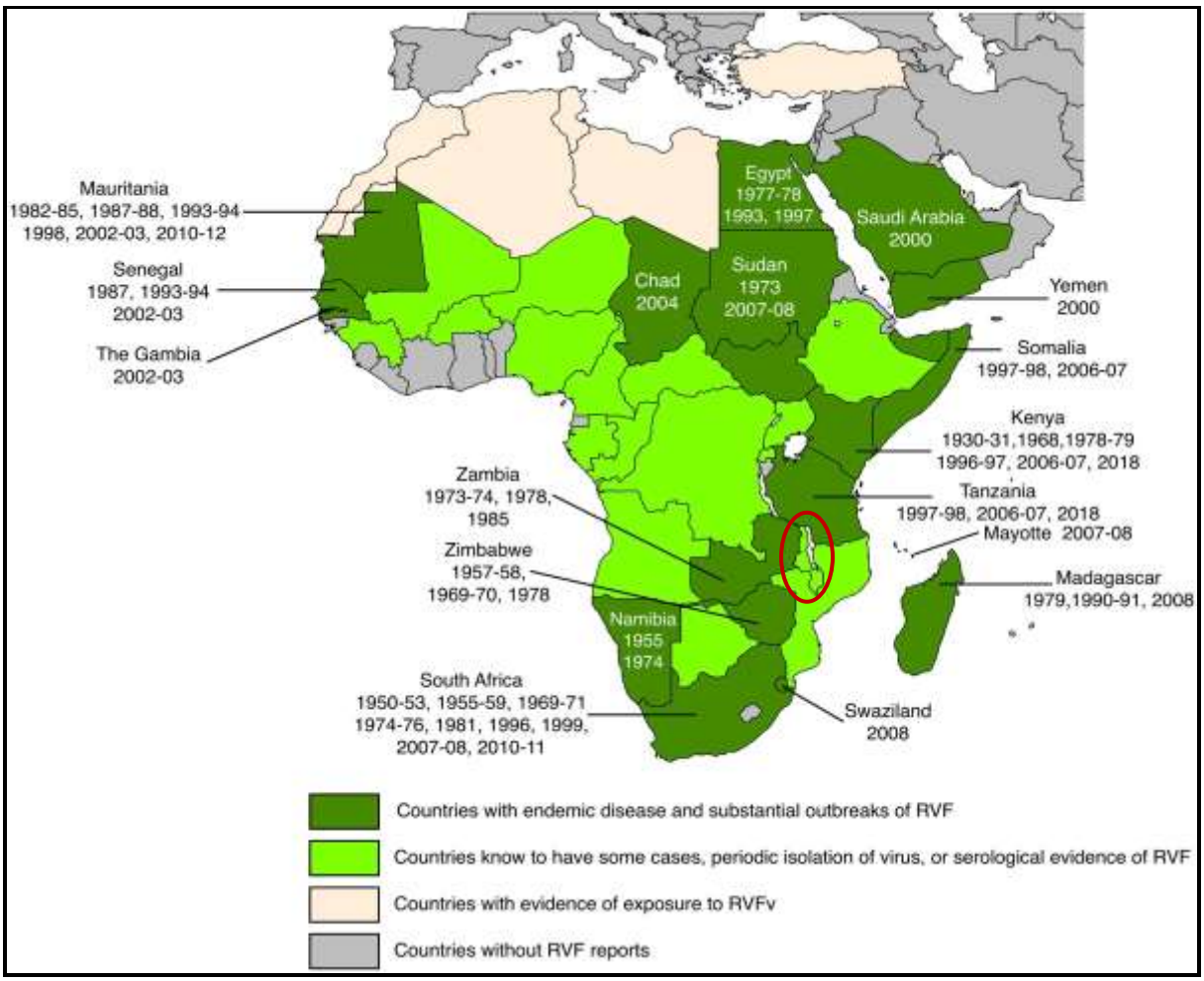

Figure 1- 1: Update on RVF outbreak report in Africa, 2014.
Source: Sumaye et al., 2019.

Since 1970s, periodic epidemics of RVF have been reported in many countries in Eastern and Southern Africa including Kenya, Somalia, Sudan, Tanzania, Zimbabwe, and South Africa (Figure 1) (Nderitu et al., 2010). The RVF epidemic of 1997–1998 that affected Kenya, Somalia, and Tanzania was characterized by outbreaks that originated from north eastern province of Kenya in November 1997 and spread to the north-central region of Tanzania in June 1998 (WHO, 2007; CDC, 2007). Another wave of RVF epidemic occurred in 2006–2007 in these three countries, with cases first reported in the north eastern province of Kenya and later in Tanzania, where the last livestock and human cases were reported in June 2007 (Mohamed et al., 2010, Munyua et al., 2010). Recent outbreaks in East Africa were reported in 2018 in Kenya, Rwanda Uganda and in South Sudan, with severe cases in livestock and humans (Anyamba et al., 2018; CDC, 2018).

RVF was first officially reported in Malawi in 1968-69; another outbreak was suspected in 1988-89, and two serological surveys were conducted in the years 1974-75 and 1990-92 using cattle serum demonstrated RVFV antibody in all regions of Malawi (Edelsten, 1990; Bryony, 1992). The disease was predominantly reported in Zomba district (Bryony, 1992). The district is located in the eastern region and shares a boundary with Chiradzulu and Machinga districts. There was a probable outbreak of RVF in 2006-2008 agricultural production seasons in Chiradzulu and Thyolo districts, which was not documented because of lack of diagnostic capacity. The lack of diagnostic capacity for RVF in Malawi resulted in misdiagnosis as most suspected cases were handled as ECF or Brucellosis which usually tested negative (Kothowa et al., 2021).

2.2 Overview of Rift Valley fever virus

Rift Valley fever virus is a prototype species of the genus *Phlebovirus* of the family *Phenuiviridae* which was first recognized and characterized during an epidemic among sheep in Rift Valley of Kenya in 1931 (Daubney et al., 1931, Adams et al., 2017). The RVFV virion particles are spherical in shape and approximately 100nm in size (Figure 2-1). The outer surface of the virion comprises capsomers of the structural glycoproteins Gn and Gc, which are embedded in a lipid bilayer (Knipe et al., 2001). The virion contains an RNA genome that is divided into 3 segments, each named after their respective size. The large (L) segment encodes the viral RNA-dependent RNA polymerase while the medium (M) segment encodes a glycoprotein precursor that is co-translationally cleaved into Gn and Gc and 2 accessory proteins. The first of these proteins is a 14-kDa nonstructural protein named NSm, which was shown to have an anti-apoptotic effect (Bird et al., 2009; Pepin et al., 2010). The function of the second protein, a 78-kDa protein, is presently unclear but is thought to suppress virus-induced apoptosis in the host cell. The small (S) segment encodes a nucleocapsid (N) protein and a nonstructural protein, named NSs, which counteracts host innate immune responses and is, therefore, considered the major virulence factor of the virus (Bird et al., 2007a). Of note, RVFV is divided into 15 major genetic lineages (A-O), based on the sequences of the S, M and L segments despite having over 33 strains in circulation (Bird et al., 2007a; Grobbelaar et al., 2011; Tshilenge et al., 2018). Juma et al., (2022) reported a total of 234 sequences of RVFV which their distribution among the 15 major genetic lineages is A (n=10), B (n=1), C (n=88), D (n=1), E (n=3), F (n=2), G (n=2), H (n=105), I (n=2), J (n=1), K (n=4), L (n=8), M (n=1), N (n=5) and O (n=1) and are correctly classified at phylogenetic level.

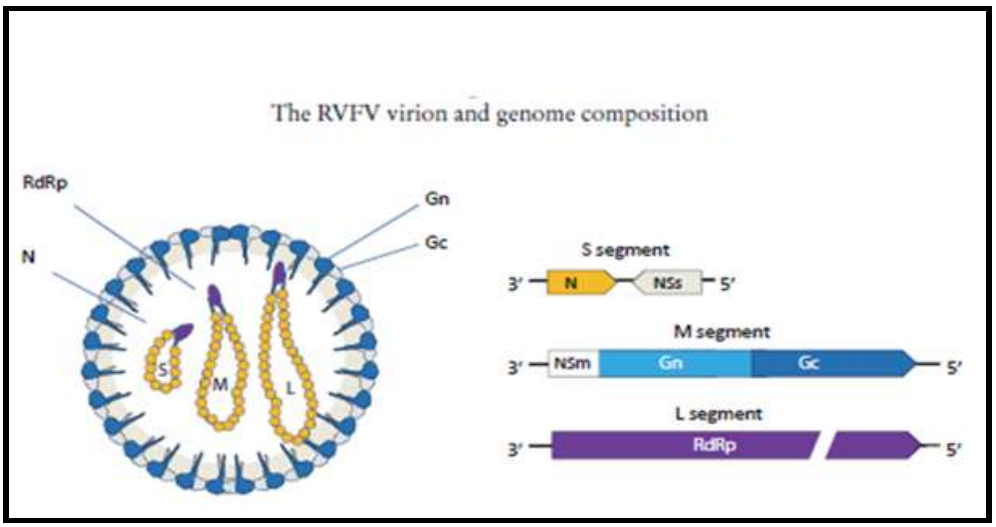

Figure 2- 1: Morphological organization of various structures of RVFV.
Source: FAO, 2014.

2.3 Transmission of Rift Valley **fever virus**

Rift Valley fever virus adopts both vertical and horizontal transmission to promote survival under a range of ecological conditions and pressures. The vertical transmission is possible with *Aedes* mosquito (Linthicum et al., 1985; Pepin et al., 2010; Mwaengo et al., 2012). When the RVFV is hatched from the *Aedes* mosquito eggs, it is amplified in ruminant hosts such as cattle, goats and sheep (Pepin et al., 2010; Dar et al., 2013; Diop, 2015). This phase of virus amplification is followed by massive virus transmission by some species of mosquito such as *Culex*, *Anopheles* and *Aedes* (Turell et al., 2007; Linthicum et al., 2016). RVFV can also be mechanically transmitted by the ticks *Amblyomma variegatum* and *Rhipicephalus appendiculatus* vectors (Omuse, 1994; Nchu and Rand, 2013). Other arthropods such as stable flies (*Stomoxys* spp.), tsetse flies (*Glossina morsitans*), sandflies (*Lutzomyi longipalpis*), biting midges (*Culicoides variipennis*) and blackflies (*Simulium* spp) are also involved in mechanical transmission (Pepin et al., 2010; Braack et al., 2018). The main mode of transmission to livestock is through mosquito bite (Pepin et al., 2010; Tantely et al., 2015). Humans can be infected via the bite of infected mosquitoes, although most cases are attributed to contact with bodily fluids when contact with viraemic animals or their products (Laughlin et al., 1979; Rakotoarivelo et al., 2011). No human-to-human transmission has been documented. Indeed, although not resolved, humans are considered dead end hosts for RVFV, in those viral titres in the blood, are not sufficient to infect mosquitoes (Lumley et al., 2017).

Malawi is reported to have several mosquito species belonging to at least four genera namely Aedes, Culex, Anopheres, and Mansonia (Mzilahowa, et al., 2016). In addition, mosquito vectors, mainly of the *Aedes* and *Culex* genera, increase the likelihood of RVFV dispersal and establishment in nonendemic regions (Javelle et al, 2020).

Rift Valley fever virus shedding from mammalian hosts is a subject of debate. Nevertheless, the infectivity of blood during acute infection is high with extremely high titers of virus (Boshra et al., 2011). Aborted materials constitute another route of virus transmission, through direct contact with fetal envelopes, placenta, and the fetus. Virus within these tissues may remain infectious over a few days due to its resistant to inactivation in a protein-rich environment (Pepin et al., 2010; Peyre et al., 2015). The shedding of RVFV into milk has potentially large consequences for public health as consumption of raw milk is reported as a potential risk factor for exposure to the virus (Pepin et al., 2010; Bird and Nichol, 2012).

2.4 Dynamics of Rift Valley fever virus in host and environment

Apart from the possible long-term persistence of RVFV in *Aedes* mosquito eggs, it is generally accepted that the virus can circulate with no apparent clinical signs in both domestic and sylvatic cycles (Matiko et al., 2018). After the mass hatching of mosquito eggs during periods of heavy rainfall, the virus can "spillover" from wild ruminants to herds of domesticated ruminants, resulting in large epizootics (Clements et al., 2007; Chevalier et al., 2010; Pepin et al., 2010). Ruminants are the major target species of RVFV, of which sheep are the most susceptible. Lambs under the age of 2 weeks generally do not survive the infection. In adult sheep, mortality can approach 30% (Peyre et al., 2015). A characteristic feature of RVF outbreaks is the so-called "abortion storms" where nearly all gestating animals in sheep herds abort. Goats and cattle are somewhat less susceptible to disease, but high mortality ratios and abortions also occur in these species. The susceptibility of the vertebrates is presented as very high in sheep and in the order of decreasing susceptibility goats, cattle, and camels (Pepin et al., 2010; Bird and Nichol, 2012; Dodd et al., 2012). The infection in humans is generally benign and manifests as a flu-like illness. However, an estimated 1% of infected humans develop severe complications, which can result in blindness or fatal encephalitis or hemorrhagic fever (Anyamba et al., 2010; Nicholas et al., 2014; Faburay et al., 2016) (Figure 2-2).

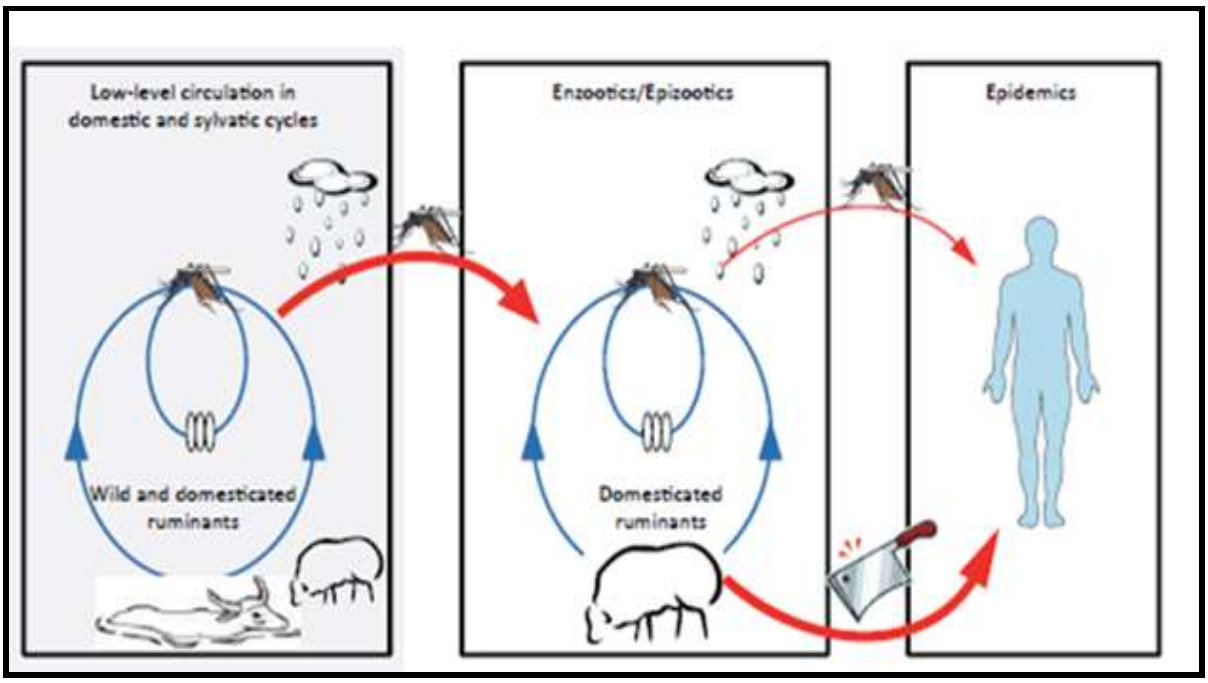

Figure 2- 2: Role of mosquitos in RVFV transmission.
Source: FAO, 2014.

2.5 Role of Climate Change on Rift Valley fever outbreaks

The results of the greenhouse effect include changes in average daily temperature, precipitation, flooding, droughts and other climate-related disasters (Métras et al., 2013; Oyas et al., 2018). Other major catastrophic livelihood challenges are reported in many geographical areas following disease outbreaks, spread and increase of disease vectors (Braack et al., 2018; Bett et al., 2019). The amount of rainfall is considered to be the main factor initiating RVF outbreaks as the outbreaks are preceded by heavy rains associated with flooding (Vizcaíno et al., 2013). The wide distribution of competent vectors in non-endemic areas coupled with global climate change poses a significant threat of the transboundary spread of RVF (Bett et al., 2019). The trends of unexpected high rainfall which causes increased flooded dambos and booming of dambo mosquitoes make prediction of disease occurrence, vector increase and disease spread difficult (Pepin et al., 2010). It is assumed that RVFV adopts both vertical and horizontal strategies to promote survival under a range of ecological conditions and pressures (Pepin et al., 2010; Mwaengo et al., 2012). With this climate change background, update on RVF epidemiological information in countries like Malawi that

frequently experience heavy rains and dambo flooding is a preliquisite for proper control and preventive strategies in sub-Saharan Africa.

2.6 Prevention and control of Rift Valley fever

The containment of active RVF outbreaks and long-term protection from RVF exposure to infected mosquitoes are important goals for RVF vaccination (Ikegame, 2017; Smith et al., 2018). In endemic and non-endemic areas, vaccinating livestock against RVF represents the most sustainable strategy to mitigate the impact of RVF on livestock. The earliest vaccines such as inactivated vaccines and the live-attenuated Smithburn vaccine were developed from virulent RVFV isolates using conventional technologies but their use has been associated with certain level of risks especially when used in non-endemic areas and they do lack the properties of Differentiation of Infected from Vaccinated Animals (DIVA) (Faburay et al., 2016; LaBeaud et al., 2017; Smith et al., 2018). The live-attenuated Smithburn vaccine, which was made in 1940s, causes considerable abortion risk in pregnant animals and is therefore only used for non-pregnant ruminants in endemic countries. On the other hand, a formalin-inactivated RVF vaccine derived from the Entebbe strain was not efficacious for protecting pregnant ewes with a single dose (Faburay et al., 2016; LaBeaud et al., 2017; Smith et al., 2018). Despite having endless list of the vaccines, currently, RVF is endemic in many parts of Africa (Dodd et al., 2012; Faburay et al., 2017).

The proper control measures of RVF include; (i) proper disposal of dead animals; (ii) active surveillance survey to detect cases of RVF among animals and humans and to identify target areas for animal vaccination; (iii) apply a vaccination campaign; (iv) restrict movement of animals outside the affected areas and a ban on animal imports from RVF-enzootic countries, (v) conducting awareness campaign and training of surveillance team members on case definition and how to manage suspected cases, (vi) performing epidemiological investigation to identify risk factors and lastly, (vii) conducting entomological study to search for the mosquito breeding ground and subsequent mosquito control program with spraying (Pepin et al., 2010; Himeidan, 2016; Oyas et al., 2018). These strategies are robust to limit the effect of the outbreak and contain the disease from spreading to other areas. However, in face of all good management and control strategies documented for RVF, the disease is still a challenge in sub-Saharan Africa where it is declared

endemic. Hence, the current study will provide epidemiological information of RVF that will guide proper diagnosis, prevention and control of the disease in Malawi.

2.7 Rift Valley fever diagnosis and investigation

During the early phase of illness in the blood and in postmortem tissue, the virus may be detected using virus isolation, antigen-detection Enzyme-Linked Immunoassay (ELISA), and molecular techniques reverse transcription polymerase chain reaction (RT-PCR), antibody testing using ELISA can be used to confirm presence of IgM antibody, which appears as an early, transient response, and IgG antibodies, which persist for several years (Garcia et al., 2001; Ikegami and Makino, 2011). Hence, two serological tests using Enzyme-Linked Immunoassay (ELISA) are conducted to screen for the presence of RVF antibodies (IgG and IgM). The purpose for running IgG ELISA is to investigate presence of IgG antibodies that suggestes non-recent RVFV infection, while IgM ELISA is specific antibodies against RVFV which indicates recent infection (Paweska et al., 2003; Kortekaas et al., 2013). Recent study by Saasa et al., (2018) has demonstrated that the recombinant nucleocapsid protein (rNP) based indirect immunofluorescence antibody assay (IFA) is a safe and useful diagnostic tool for sero-survaillance of RVF infection among cattle. The rNP –based IFA has the capacity to detect circulation of RVFV in a herd without reports of active cases.

Diagnostic assays are available for RVF, but availability can be limited in resource limited areas such as Malawi, and there is a need for global harmonization. continued improvement of standard serological and viral genome amplification approaches. The genome amplification method uses Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR) method. Standard RT-PCR assays are available for both mammalian and mosquito samples (Wilson et al., 2014). Closed tube real-time RT-PCR methods are favored, because they are less labor intensive and less prone to contamination. Three realtime RT-PCR assays have been developed for RVF. In addition, there are two real-time reverse transcription-loop-mediated isothermal amplification (LAMP) assays available that provide alternative amplification detection tools for RVF viral RNA (Wilson et al., 2014).

2.8 Agro-ecological zones and livestock husbandry in Malawi

Most livestock are kept on a 6.1 million hectares of customary land within family operated smallholdings (Chintsanya et al., 2004). Majority of livestock activities are conducted under extensive management systems in all the three agro-ecological zones. Ecological zone 1 (EZ1) covers low lands of semi-arid areas found mainly on the shores of lake Malawi and in the Rift valley areas of the Shire valley like areas of Salima, Mangochi, Chikwawa and Nsanje districts. EZ1 lies between 500–1,000m above sea level and receives an annual rainfall of less than 1,000 mm. Ecological zone 2 (EZ2) covers the highland plains of the Shire highlands, Lilongwe, Kasungu and Mzimba lying at 1,000–1,500 m above sea level, Thyolo and Chiradzulu inclusive. This zone receives an annual rainfall of 1,000–1,500 mm. Ecological zone 3 (EZ3) covers high-altitude areas of the Vipya and Nyika plateaus and the Chitipa and Karonga districts lying over 1,500m above sea level. It has a total annual rainfall of over 1,500 mm. A high proportion of this zone encompasses forest reserves and national parks. There are approximately two million smallholder families and 30,000 estates in Malawi. Most family-operated smallholdings depend upon subsistence farming based on mixed crop and livestock farming (Chintsanya et al., 2004; Tebug et al., 2012).

In general, the low latitude area where Malawi is located provides the land with wet season (November to April) and dry season (May to October) (Chingala, 2018). During the wet season livestock graze in communal ground especially in higher lands while during the dry season, livestock graze in crop fields and communal grounds located in dambo lands. Most of dairy farming is concentrated in Shire Highlands and many parts of Lilongwe-Kasungu plains where cut and carry/stall feeding/zero-grazing management practice is dominant (Baur et al., 2017a). Tebug et al., (2012) reported that poor animal health as the second important constraint of livestock production. Mastitis, ECF, brucellosis, tuberculosis, abortion, peri-natal calf mortality and retained placenta were among the most common animal health concerns.

Further, the EZs varied in livestock cencus distribution during the Agricultural Production Estimates (APES) first round of January, 2019 (GOM, 2019). The EZ1 had about 1,536,864 cattle, 292,921 sheep and 7,837,127 goats while EZ2 had approximately 80,552 cattle, 3,872sheep and 9,523 goats. The EZ3 had approximately 146,288 cattle, 34,479 sheep and 1,301,187 goats.

Ecological Zone 1 possess several permanent water bodies such as Lake Malawi, Shire River and several other tributaries to the lake as well as Shire River (Musa, et al., 2018). In addition, EZ1 has annual reports of flooding and overflowing of both Lake Malawi and Shire River (GOM, 2019). In addition, EZ1 has several irrigation schemes and man-made dams for agricultural purposes. The vegetative cover is dense in the wet season unlike in dry season (Pangapanga et al., 2012). The EZ3 also shares part of Lake Malawi and Songwe river. The EZ3 has several natural running waters from mountaneous and hilly areas which facilitate presence of cool-wet weather throughout the year. On the contrary, EZ2 has few perennial rivers such as Namadzi, Msuwadzi, Luchenza and Nthuchira Rivers. The EZ2 has several Tea and Macadamia estates which has several perennial water bodies used as water reserviour for tea production (GOM, 2019).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Study area

Malawi is a landlocked and agriculture-economy based country covering 118,484 km^2 in southeast Africa, situated within latitudes 9^o and 18^{o}S, and longitudes 32^o to 36^o E. Malawi shares borders with Tanzania to the north, Mozambique to the southeast and southwest, and Zambia to the west. The study was conducted in eight districts selected from all three ecological zones of Malawi (Table 3-1, Fig 3- 1). The eight districts were purposively selected to include different annual rainfall patterns, vegetation and livestock density. The districts were Chitipa (CP), Karonga (KA), Salima (SA), Mangochi (MH), Chiradzulu (CZ), Thyolo (TO), Chikwawa (CK) and Nsanje (NE). CP and KA constitute the EZ3 and are located in the northern part of Malawi along Songwe river bordering Tanzania and Zambia. The EZ1 has SA in the central part of Malawi and MH in the southern part of Malawi which are located along the shores of lake Malawi with characteristic wide range of dambo areas and dense vegetation cover. The CZ1 also has CK and NE are on the southernmost part of Malawi along the Shire River valley bordering Mozambique. These two districts have high ruminant population and experience frequent flooding. The EZ2 includes CZ and TO in the southern part of Malawi and are located adjacent to Zomba district where RVF was previously reported (Edelsten, 1990; Bryony, 1992).

Table 3- 1: Geographical positioning system coordinates for study areas[a]

Ecological Zones	District name	District mapping code	Latitude	Longitude
EZ1	Salima	SA	-13° 44' 59.99" S	34° 29' 59.99" E
	Mangochi	MH	-14° 28' 41.34" S	35° 15' 52.13" E
	Chikwawa	CK	-16° 09' 60.00" S	34° 44' 59.99" E
	Nsanje	NE	-16° 44' 59.99" S	35° 09' 64.00" E
EZ2	Chiradzulu	CZ	-15° 41' 59.99" S	35° 09' 60.00" E
	Thyolo	TO	-16° 04' 3.90" S	35° 08' 25.66" E
EZ3	Chitipa	CP	-9° 44' 59.99" S	33° 14' 60.00" E
	Karonga	KA	-9° 55' 59.99" S	33° 55' 59.99" E

[a]Source: www//GPS Coordinates, Latitudes, and Longitudes, of geolocated articles in Malawi; accessed 2019-03-29.

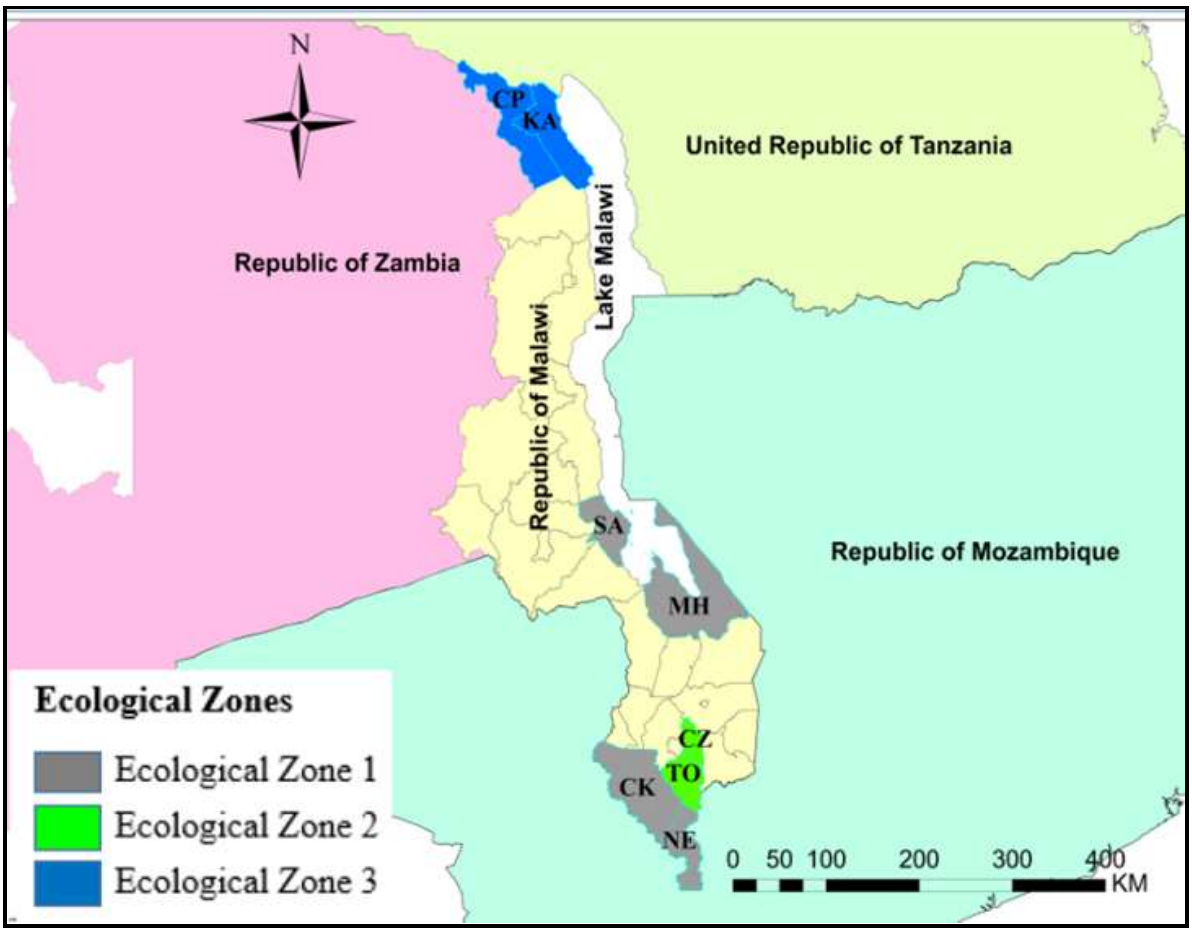

Figure 3- 1: Map of study area and the selected districts
CP = Chitipa, KA = Karonga, SA = Salima, MH = Mangochi, CZ = Chirazulu, TO = Thyolo, CK = Chikwawa and NE = Nsanje.
Map was developed using ArcGIS

3.2. Study design, sample size and sampling strategy

3.2.1. Study design

A cross sectional design was used to conduct this study. Livestock were sampled from ecological zones according to total district livestock census. At each District Agriculture Office (DAO), two veterinary stations were identified. An average of 5 livestock (cattle, goats and sheep) per farmer was suggested. Farmers were randomly selected from the updated livestock census books basing on sample size allocated to each district. At household level, all livestock were sampled when the herd size was < 7 and a maximum of 15 was sampled if the herd size was > 7 (Thrusfield, 2005; Sikasunge et al., 2008; Sumaye et al., 2013). Assistant veterinary officers and lead farmers were

consulted to identify livestock owner and herd size after selecting from the livestock census book. Since individual animal could not be identified within herds, arbitrary numbers were assigned to individual animals within a herd which was later used for simple random selection by a raffle draw. The number of livestock herds were obtained by counting the number of herds included in the study (Phonera et al., 2021).

3.2.2. Sample size estimate for human participants in knowledge, attitude and practices study

A cross-sectional survey was conducted among the livestock farmers in eight districts. An exploratory research method was used for sample size estimate because there was paucity of literature review on knowledge and determinants of RVF in Malawi. The Thumb's rule of 30% was used in determining the sample size using Cochran's formula (Cochran, 1977):

$$\mathbf{n_0} = \frac{Z^2pq}{e^2} = 384$$

Where; **n** =required sample size, **Z** =1.96 (confidence level at 95%), **p** =prevalence rate of the RVF virus (50% estimated because prevalence was unknown in the study area), **q** =1-p, **e** =level of precision at 5% (standard value of 0.05)

The investigators considered 10% attrition rate as previously described by (Adegoke et al., 2020):

$$\text{Attrition} = \frac{\text{Calculated samples size} \times \text{Attrition rate}}{\text{Attrition rate} - 1 \text{ attrition}} = \frac{384 \times 10}{10 - 1} = \frac{3840}{9} = 426$$

Attrition = 426 – 384 = 42. Therefore, new sample size = 384 + 42 = 426 sample size.

3.2.3 Sample size estimate for Livestock samples

Livestock population for the districts were obtained from Malawi National Agricultural Production Estimates, Livestock census for first round conducted in January 2019 (GOM, 2019). The larger sample size was required to assess whether the observed effects was true reflection of the study area that had greater variability (Homogeneity was low) such as livestock husbandry (grazing type, herd sizes, livestock night shelters), ecological condition, purpose of livestock production,

transboundary livestock trade activities and veterinary services. The sample size was estimated using Cochran's Formula (Cochran, 1977).

$$\mathbf{n_0} = \frac{Z^2pq}{e^2} \quad \text{..... [Eqn 1]}$$

Where; **n** =required sample size, **Z** =1.96 (confidence level at 95%), **p** =prevalence rate of the RVF virus (50% estimated because prevalence was unknown in the study area), **q** =1-p, **e** =level of precision at 5% (standard value of 0.05)

However, RVF is a less contagious disease among livestock as such in order to increase the precision of study estimates and obtain similar accuracy to that with simple random sampling for districts, and the sample size was recalculated considering the finite population. The study expected at least five (5) livestock to be available in each sampled farmer/household, as such the average rate of RVF homogeneity (*p*) was estimated to be 0.156, and then sample size was subsequently recalculated as:

$$N_{new} = n\,[1+p\,*(m-1)] \quad \text{..... [Eqn2]}$$

Where n= 384, *p*= 0.156 and *m* = 5, representing the average number of animals to be sampled from each veterinary station. The adjusted sample size was 2,220 distributed proportionately among the three ecological zone districts (Table 3-2). The distribution was conducted using a proportion-to-size sampling formula (X/N * 2,220) (Monje et al., 2020).

Where X= population size of the district in question

N= sum of the total population of the eight districts chosen for the study.

Table 3- 2: Sampling proportion of livestock to ecological zones and districts

		EZ 1			EZ 2			EZ 3	
		Sampling allocations							
		1101			409			710	
	Districts	SA	MH	CK	NE	TO	CZ	CP	KA
Livestock Species									
Cattle	938	109	119	124	131	98	78	141	138
Goat	790	103	85	108	81	52	40	171	150
Sheep	492	58	54	78	51	68	73	59	51
Total	2,220	270	258	310	263	218	191	371	339

EZ1= Ecological zone 1 (SA= Salima, MH=Mangochi, CK=Chikwawa, and NE=Nsanje), EZ2= Ecological zone 2 (TO= Thyolo, and CZ=Chiradzulu), EZ3= Ecological zone 3 (CP= Chitipa and KA= Karonga)

3.2.4 Inclusion and exclusion criteria

The study included sheep and goats that were above 6 months of age and cattle of 12 months of age since were not suckling. All livestock without previous vaccination history and animals which stayed were with the herd for over 6 months. However, the study excluded livestocks with the following characteristcs;

i. Sheep and goats aged less than 6 months and cattle of less than12 months of age. This was to avoid testing animals with maternal antibodies.
ii. Livestock with RVF vaccination history.
iii. Livestock introduced into the herd within the previous 6 months.

The study included livestock farmers with cattle, goat and sheep for not less than 6 months and were registered by the village livestock committees and district Agriculture Office. The study did not include participants without cattle, goat and sheep and that were not registered with the village livestock committee and district agriculture office.

3.2.5 Age determination

Information about the age of individual animals was obtained from livestock farmers during sample collection as well as from farm records when available. Usually, records were available from farmers keeping exotic dairy or beef cattle. Where records were missing, age estimation was achieved by examining the dentition (Greenfield and Arnold, 2008; Mushonga et al., 2020). The three livestock categories (cattle, sheep, goat) were split into age categories based on biological functionality (suckling, pregnant, non-pregnant, active bulls/bucks, growing heifers/does and

young bulls/bucks) which influences importance, activities and interaction of animals in the herd (Table 3-3).

Table 3- 3: Age categories for the three livestock species

Age (years)	Cattle	Goat	Sheep
Young	< 2	<2	<2
Sub adult	2-4	2-3	2-3
Adult	5-8	4-5	4-5
Old	>9	>6	>6

3.3. Determining knowledge, attitudes and practices (KAP) of livestock farmers towards reporting susceptible cases of RVF

The knowledge, attitude and practices (KAP) scores and the associated determinants were established using quantitative methods so that the results could be generalized to the wider population of livestock famers unlike qualitative methods, as described below.

3.3.1. Establishing knowledge, attitude and management practices

This was the first steps to establish the KAP scores of the study participants as described below.

3.3.2. Data collection instrument for the study

A structured questionnaire was adapted from Focus Group Discussion (FGD) guide which were conducted earlier and further developed through an in-depth literature search, that was translated and pretested in Chichewa language (Appendix 1)**.** The questionnaires were pre-validated for relevance, accuracy, clarity, simplicity and understandability and the Cronbach`s alpha coefficient of 0.72, 0.86 and 0.78 for questionnaire for knowledge, attitude and practices, respectively, indicating the internal consistency and reliability of the study instrument. A pilot study of the questionnaire was carried out on 16 participants that were excluded from the final analysis. Further, adequate training was provided to enumerators to address the observed discrepancies and improve the quality of the data. The training was conducted at the Department of Epidemiology and Public Health, Faculty of Veterinary Medicine, Lilongwe University of Agriculture and Natural Resources (LUANAR).

3.3.3. Participants identification and data collection

A structured questionnaire with mostly categorical questions was administered in Chichewa language. Only smallholder farmers of cattle, goat and sheep were included in the study. The study strictly considered participants above 18 years of age to include only those responsible for keeping animal health records. At each District Agriculture Office, 10% of the recorded livestock farmers were considered using simple random sampling method by a raffle draw. The questionnaire was administered face to face to 400 smallholder livestock owners. Herd size was categorized into small (< 24) and large (25 and above) (Namgyal et al., 2021). The distribution of participants per district were 39 CP, 45 KA, 60 SA, 54 MA, 36 TO, 48 CZ, 52 CK and 66 NE. Data was collected in the period of May – June 2020.

3.3.4. Establishing KAP score

Thematic questions regarding expected knowledge, management practices and attitudes on RVF, were answered on a "yes" or "no" basis. A correct answer was assigned 1 point, while an incorrect and "don't know" answer was assigned 0 point. The total knowledge score ranged from 0 to 100%, with a higher score denoting a better knowledge of RVF. To determine the KAP score level, the cut-off value was based on ability of the participant to provide correct answers on the cause, host range, clinical signs and mode of transmission of RVF. An average KAP score of 80% or more was classified as "good knowledge", indicating satisfactory knowledge to suspect and report any probable case of RVF. On the other hand, average KAP scale of less than or equal to 79% "poor knowledge" indicating unsatisfactory knowledge. A similar scoring approach was used to classify "positive attitude", and "negative attitude", "good practice", and "poor practice" adapted from (Roy et al., 2020; Habib et al., 2021). The high-level participants were obtained by the total sum of each participant`s scores and cut-off of 50% was used (Vijay et al., 2021), then comparisons were conducted by Student t-test, then chi-square (Memon, et al., 2015).

3.3.5. Establishing determinants of RVF knowledge for participants

Data was entered, cleaned and validated in Microsoft Office Excel®2019. The data was grouped according to intended information as socio-demographic, awareness, attitude and management practices. Data was entered in SPSS Ver. 21 (SPSS Inc., Chicago IL) statistical software and run

through three steps of analysis; univariate, a bivariate and multivariate analysis. At multivariate analysis, independent variables with *p* value less than 0.05 were considered significant determinants of RVF knowledge.

3.4 Determine seroprevalence of RVFV in domestic ruminants

RVFV seroprevalence was determined by ELISA tests specific for the IgG and IgM antibodies in serum samples. The purpose for running IgG ELISA is to investigate presence of IgG antibodies that suggestes non-recent RVFV infection, while IgM ELISA is specific antibodies against RVFV which indicates recent infection (Paweska et al., 2003; Kortekaas et al., 2013).

3.4.1. Sample collection, storage and transportation

Whole blood samples were aseptically collected from cattle, goats, and sheep of all eligible age groups using jugular or coccygeal venipuncture approaches. One set of whole blood sample was collected in plain vacutainers for serum and the other in EDTA tubes (5mL in each). Serum was separated by centrifugation at 1000× *g* for 15 min as per World Organization for Animal Health protocol (OIE, 2018a). The samples were immediately stored at -80ºC, at African Union Center of excellence for Tick and Tickborne Diseases (AU-CTTBD). Samples were triple packed and transported by road, in a frozen state, to the Department of Disease Control, in the Samora Machel School of Veterinary Medicine, University of Zambia (UNZA), for processing and analysis. At UNZA, the samples were stored at -80ºC.

3.4.2. Serum samples laboratory analysis

Tests for the presence of IgG and IgM antibodies in serum samples were carried out in the Department of Disease Control. The two serological tests for screening for the presence of IgG and IgM antibodies were conducted in parallel using ID Screen® Rift Valley Fever Competition Multi-species ELISA a commercial, indirect competition ELISA (cELISA) kit and IgM Antibody Capture Enzyme-Linked Immunoassay (MAC-ELISA) kit, respectively, supplied by (ID. Vet Innovative Diagnostics, Grabels, France). The test kits are reported to have a validated high diagnostic sensitivity and specificity of 98% and 100%, respectively (Cêtre-Sossah et al., 2009; Comtet et al., 2010; Kortekaas et al., 2013).

3.4.3. IgG competition multi-species ELISA

ID Screen® Rift Valley Fever Competition Multi-species ELISA a commercial, indirect competition ELISA (cELISA) kit was used according to the manufacturer's instructions for detecting RVFV specific IgG antibodies.

3.4.3. 1 IgG competition multi-species ELISA protocol

Reagents provided in the kit included the following: i) microplates coated with recombinant RVF nucleoprotein, ii) Ant-RVF-NP-Po conjugate (10 X), iii) positive control, iv) negative control, v) dilution buffer 19, vi) wash concentrate (20X), vii) substrate solution and viii) stop solution (0.5M).

The preparation of reagents and working dilutions was done prior to the onset of running the assay. Wash Buffer (1X) was prepared by adding 50 mL of Wash Concentrate (20X) to 950 ml distilled water. Anti-nucleoprotein-horseradish peroxidase (Anti-RVF-NP-HRP Conjugate 1X) was prepared by adding 1 mL of Anti-RVF-NP-Po conjugate (10X) to 9 mL Dilution Buffer 19. Other reagents that were ready to use without dilution include Dilution Buffer 19, Substrate Solution, Stop Solution and Control sera (Positive and Negative Control sera).

Sera samples were diluted in plates, where 50 µL of dilution buffer 19 was added to each well of the dilution plates, followed by 50 µL of either the control sera or the test sera. Test sera was dispersed in duplicate. Next, 50 µL per well of the test samples were transferred to pre-coated test plates using a multichannel pipette. The plates were incubated for 1 hour at 37° C and then washed three times with 300 µL per well of washing buffer. The anti-RVF-NP-HRP conjugate was diluted in dilution buffer 19 at a ratio of 1:10 and 100 µL added per well. The plates were then incubated for 30 minutes at room temperature and washed as before, after which 100 µL of ready-to-use TMB substrate (ID. Vet Innovative Diagnostics) was added to each well and incubated in the dark for 15 min at room temperature. Then, 100 µL of stop solution was added per well and the absorbance by measure of Optical Density (OD) was read at 450 nm.

3.4.3.2 Validation of IgG competition multi-species ELISA protocol

All tests were run in duplicate and recorded on a plate map. The test was valid if the mean value of the positive control optical density (OD_{PC}) was less than 30% of the negative control (OD_{NC}) given as [OD_{PC}/ OD_{NC} < 0.3] and if the mean value of the negative control optical density (OD_{NC}) was greater than 0.7 given as [OD_{NC} >0.7] and all runs were valid on both criteria (Appendix 3). Interpretation: The results were calculated as percentage inhibition (competition), using the following formula: Suspect or negative (S/N) = (OD Sample/OD Negative control) x 100. A suspect or negative (S/N) value of ≤ 40% was considered to be positive, while (S/N) value of 40% < S/N % ≤ 50% was doubtful and (S/N) value of > 50% was negative.

3.4.4. IgM Antibody Capture (MAC) – ELISA

All samples were run for IgM investigation. ID Screen® RVF IgM Capture Multi-species ELISA kit was used for detection of anti-RVF virus nucleoprotein (NP) IgM specific antibodies against RVFV.

3.4.4.1 IgM Antibody Capture (MAC) – ELISA protocol

Reagents provided in the kit included the following: i) microplates coated with anti-bovine-ovine-caprine IgM antibodies, ii) freeze dried RVFV Nucleoprotein (10 X), iii) reconstitution buffer, iv) anti-RVFV NP conjugate (10 X), v) positive control, vi) negative control, vii) dilution buffer 14, viii) wash concentrate (20X), ix) substrate solution and x) stop solution (0.5M). Working reagents were prepared as follows: An amount of 1ml of reconstitution buffer was dispersed to the freeze-dried RVFV nucleoprotein vial. After 2 minutes the mixture was well mixed to homogenize the solution. Then reconstituted RVFV nucleoprotein was stored at 5ºC. The reconstituted RVFV nucleoprotein (10X) with 1mL of the reconstitution buffer was further diluted with dilution buffer 14 (ratio 1:10). The wash solution was prepared by diluting the wash concentrate (20X) down to 1X with distilled water prepared in our laboratory (ratio 1:20). The Conjugate solution (1X) was prepared by diluting the conjugate 10X to 1/10 in dilution buffer 14 in our laboratory. All the reagents and samples were equilibrated to room temperature before use.

Ninety-six well ELISA microplates were used and wells of each plate were partitioned as follows: A1 and A2, B1 and B2 wells for negative control, C1 and C2, D1 and D2 for positive control. To start with, 40 µL of dilution buffer was dispersed to all the wells using multi-channel pipette. Then, 10 µL of the negative control was added to wells C1, D1, C2 and D2 wells. Following was loading of the test serum samples in duplicates to the remaining wells. Positive controls were loaded after the samples to avoid contamination. The plates were covered, genteelly mixed and then incubated at 37ºC for 60 minutes. After incubating, all the wells were washed three times with 300 µL of wash solution per wash per well. Thereafter, 50 µL of RVFV nucleoprotein 1X to the even-numbered columns and 50 µL of dilution buffer 14 was dispersed to odd-numbered columns. The plate was then covered and incubated at 37ºC for 60 minutes. Then each well was washed three times with 300 µL wash solution per well per wash. After washing, 50 µL of conjugate 1X was dispersed to each well. The plate was then covered and incubated at 37ºC for 60 minutes. Then each well was washed three times with 300 µL wash solution per well per wash. Then 100 µL of substrate solution was added to each well. The plate was then covered and incubated in dark at 21ºC for 15 minutes. The substrate reaction was stopped by adding 100 µL of stop solution, in the order the substrate was added, to avoid variation in substrate reaction time. The optical density was read and recorded at 450nm as per the manufacturer's instruction.

3.4.4.2 Validation of IgM Antibody Capture (MAC) – ELISA protocol

All tests were run in duplicate and recorded on a plate map. The test was valid if the mean optical density of the positive control (OD_{PC}) was greater than 0.350, given as [net $OD_{PC} > 0.350$] and the ratio of the mean OD_{PC} to mean optical density for negative control (OD_{NC}) was greater than three, given as {[net OD_{PC}/|net OD_{NC}|] > 3}. All runs were valid on both criteria. Interpretations of the sample ODs were based on the ratio of the mean sample optical density to OD_{PC}, expressed as a percentage (S/p *100). Samples with S/P % ≤ 40% were considered negative, while S/P% value of 40% < S/P% < 50% was considered doubtful and S/P% value of ≥ 50% were considered positive.

3.5 Evaluation of risk factors associated with RVFV sero-positivity of livestock

Risk factors associated with RVFV sero-positivity of livestock were determined from the data collected through questionnaire administration.

3.5.1. Questionnaire administration

A structured questionnaire was developed and administered face to face with the livestock owners to capture information on potential risk factors for RVFV sero-positivity at individual and herd level. The questionnaire was administered at the household of the herd owner in either Chichewa or English language (Appendix 2). At each District Agriculture Office, a sampling frame of district livestock farmers was developed from an updated record books. Participating farmers were considered using simple random sampling method by a raffle draw. Selected herd owners were asked to complete a written consent form and herd owners who did not consent for themselves or their herds, were replaced by other herd owners and corresponding herds within the veterinary stations (Appendix 3; Appendix 4). The questionnaire had four parts: the first part gathered information about demographics of herd owners (age, sex, marital, status and education level); the second part obtained information about livestock species in stock, herd dynamics and livestock management [herd size (small <25, medium 26-50 and large > 51)] (Otte and Chilonda, 2003), source of livestock (within or outside the district), cross border livestock interaction (present or absent), grazing system (stall feeding or communal grazing), type of grazing land (private grazing grounds or mixed species grazing grounds) and presence of livestock market. The third part of the questionnaire collected information about herd owners' knowledge of RVF, causative agent, clinical signs, occurrence of abortion, neonatal death, mode of transmission, knowledge of *Aides spp* of mosquito, and knowledge of zoonotic nature of RVF. The fourth part gathered information about ecological factors such as occurrence of heavy rainfall, flooding, occurrence of mosquito, presence of permanent water points, and degree of vegetative cover.

3.6 Detection of RVFV RNA in livestock samples

RNA was extracted from serum samples. Detection of RVFV RNA was conducted using two molecular methods: real time and conventional RTPCR.

3.6.1. RNA Extraction from serum

Viral RNAs were extracted from the serum samples using a QIAamp Viral RNA kit (QIAGEN, 2012, Valencia, CA, USA) according to the manufacturer's instructions. Briefly, 141 µL of serum was added to 560 µL AVL buffer containing carrier RNA into a 1.5 mL micro-centrifuge tube and mixed by pulse-vortexing for 15 seconds followed by incubation at room temperature for 10 min. About 560 µL of absolute ethanol was added and mixed by pulse-vortexing for 15 sec. About 630 µL of the mixture was transferred to QIAamp spin column mounted on 2 mL collection tube and centrifuged at 6000x for 1 min. The column was then transferred to another collection tube and the remaining 630 µL of the mixture was passed through it. The column was then washed twice with 500 µL of washing buffers AW1 and AW2, followed by centrifuge at 6000 x g for 1 min and at full speed (20,000 x g) for 3 min, respectively while placing the QIAamp Mini column in a clean 2 mL collection tube in between. The QIAamp Mini column was placed in a new 2 mL collection tube and centrifuge at full speed for 1 minute. Finally, RNAs were carefully eluted by 60 µL of buffer AVE equilibrated to room temperature. Then the cap was closed, and incubated at room temperature for 1 min. This was then centrifuged at 6000 x g for 1 min to elute RNA. The eluted RNA was stored at -80 ºC until required for use.

3.6.2. One-Step quantitative Reverse-Transcription Real-Time PCR (RT-qPCR)

For screening samples for RVFV RNA, RVF Screen_Luna® UniversalProbe One-Step RT-qPCR kit (New England Biolabs®, Massachusetts, USA) targeting L- segment of RVFV genome was used. The protocol was followed as per the manufacturer`s instructions. The reaction mixture of 20 µL contained, 0.8 µL of RVF-primer mix, 0.2 µL probe, 10 µL Luna Universal Probe One- Step Reaction Mix (2X), 1µL Luna Warmstart RT enzyme mix (20X), 3µL nuclease free water and 5 µL RNA template. The fluorescence dyes selected for the LightCycler® 96 Real-Time PCR system were fluorophores (FAM) and black hole quencher (BHQ) and the primers used were as given in (Table 3-4). The primers and probe were reported to have high throughput (Bird et al., 2007b).

Table 3- 4: Description of primers and probe for the One- Step RT-qPCR

Primer/probe	Nucleotide sequence	Details
Probe	FAM-CAATGTAAGGGGCCTG TGTGGACTTGTG-BHQ-1	RVFL-probe-2950
Forward primer	TGAAAATTCCTGAGACACATGG	RVFL-2912fwdGG
Reverse primer	ACTTCCTT GCATCATCTGATG	RVFL-2981revAC

The cycling condition for the LightCycler® 96 Real-Time PCR were as provided in (Table 3-5).

Table 3- 5: Cycling conditions for RVF One-Step RT-qPCR

Condition	Temperature	Time	Cycles
Reverse transcription	55 ° C	10 min	1
Initial denaturation	95 ° C	1 min	1
Denaturation	95 ° C	10 sec	45
Extension	60 ° C	60 sec	45

3.6.3. Conventional Reverse-Transcription PCR (RT-PCR)

3.6.3.1 cDNA Synthesis

For cDNA synthesis, Primescript one step RT-PCR kit version 2.0 Dye plus (Takara Bio, Shinga, Japan) was used targeting the NSs coding region of the smallest segment (S). The protocol was followed as per manufacturer`s instruction. The primers used for RT-PCR are listed in (Table 3-6) (Salli et al., 2002; Drosten et al., 2002; Wilson et al., 2013). The reaction mixture of 15 µL contained 0.6 µL of 10 µM enzyme, 7.5µL of 10X buffer, 0.6 µL of 10 µM RVF-forward primer, 0.6 µL of 10 µM RVF-reverse primer, 2.7 µL of 10 µM RVF-forward primer, 2.7 µL nuclease free water. The master mix was prepared by mixing (after thawing) all the reagents, with exception of the template RNA. The master mix was thoroughly mixed by tapping and spinning before dispersing 12 µL of the master mix into the PCR tubes. Thereafter, 3 µL of template RNA was added to a PCR tube containing master mix. All steps of the protocol up to this stage were performed on ice (4ºC). The temperature profile for thermal cycler was set as follows: Reverse transcriptase reaction at 50 ºC for 30 minutes and denaturation at 94ºC for 2 minutes; followed by amplification reactions of 45 cycles with: denaturation at 94ºC for 30 sec; annealing at 44ºC for 30 sec and extension at 72ºC one minute. Final reaction was extension at 72 ºC for 10 minutes.

Table 3- 6: Primers used to amplify the NSs coding region of S- segment

RT-PCR round	Primers*	Nucleotide sequence	Map position
First	F1:NSca	5′-CCTTAACCTTAACCTCTAATCAAC-3′	841- 824
	R1: NSng	5′-TATCATGGATTACTTTCC-3′	31 - 48
Second	F2:NS3a	5′- ATGCTGGGAAGTGATGAGCG-3′	729 - 710
	R2:NS2g	5′- GATTTGCAGAGTGGTCGTC-3′	62 - 80

*Primers sets adapted from OIE terrestrial manual, 2018b

3.6.3.2 Nested PCR detection of RVFV RNA gene of S- segment

To screen for RVFV, KOD one PCR Master Mix Blue (Toyobo Co. Osaka, Japan) and RVFV F2/R2 set of primers targeting 668bp long region of the S segment were used (Table 3-6) as described by (OIE, 2018b). The following reagents and volumes were used for each nested reaction: 8 µL of nuclease free water, 12.5 µL of KOD one PCR Master Mix Blue, 0.75 µL of RVFV F2/R2 (Table 3-6), and 2 µL of cDNA template (the Primescript one-step RT-PCR product). Total volume was 15 µL. The programing of thermal cycler was as follows: Initial denaturation at 92ºC for two minutes. This was followed by 40 cycles of amplification as follows: denaturation at 98°C for 10 sec, annealing at 55°C for 5 sec and extension at 68°C for 1 sec. The final extension was set at 68°C for 30 sec. The order of preparing the master mix and adding the samples was the same as described for Primescript one-step RT-PCR above and in each run, we used nuclease-free water as negative control. PCR products were stored at 4 ºC in waiting for gel electrophoresis.

3.6.3.3 Agarose Gel Electrophoresis

Amplicons were visualized by 1.5% ethidium bromide agarose gel prepared by dissolving agarose powder (Cleaver Scientific Ltd, Rugby, UK) in TAE buffer (Tris-base 2M, Acetic acid 2M, 0.005M EDTA, with pH 8.0 and distilled water) in a ratio of 1:5:1 (mass (g)/volume (mL)) and colored with a drop of ethidium bromide. Five microliters of each sample PCR product were mixed by pipetting with a drop of loading dye, and loaded onto the electrophoresis gel and later in the electrophoresis chamber and run for 30 minutes. A 1000bp ladder/ marker was used in a Benchtop 3UV transilluminator (BioDoc-it Imaging system UVP, CA, U.S.A)

3.7. Data analysis

3.7.1. Validation of data (KAP) collection instruments

The data collection tools for the study (Semi-structured questionnaires), were developed through an in-depth literature search and were translated and pretested in Chichewa. The questionnaires were pre-validated for relevance, accuracy, clarity, simplicity and understandability and the Cronbach`s alpha coefficient of 0.86 and 0.78 for questionnaire for KAP and RVFV sero-positivity risk factors, respectively, indicating the internal consistency and reliability of the study instrument. A pilot study of the questionnaire was done on 16 and 30 participants for questionnaire of objective 1 and objective 3, respectively, that were excluded from the final analysis.

3.7.2. Data acquisition, curation and organization

Quantitative data for analysis were obtained through recording herd attributes, observing study environmental factors (presence of permanent water points and degree of vegetative cover) methods, administering questionnaire on knowledge, attitude and practices towards RVF and on livestock management practices for the RVFV sero-positivity and laboratory analysis of whole blood and serum samples. All datasets were entered, cleaned and validated in Microsoft™ excel spread sheets (Microsoft Office™ Excel ®2019). Finally, data was organized according to the purpose and objective of interest. Statistical packages IBM SPSS ver. 21 (IBM Corp, Armonk, NY, USA) were used for univariate, bivariate and multivariate analysis.

3.7.3. Analysis of determinants for the knowledge of RVF

Data analysis of determinants for the knowledge of RVF study was conducted in three stages; first, univariate analysis for descriptive statistics such as percentages and frequencies of each independent variable. In the second stage, a bivariate analysis was conducted to assess the association between each independent variable and the dependent variable “knowledge of RVF”, and potential determinants with *p* value less than 0.05 were considered significant. Only potential determinants with *p* value less than 0.250 were considered for the next stage of multivariate analysis. Prior to multivariate analysis, three univariable linear regression models with the observed level of knowledge, were generated for the categories of knowledge, attitude and practice, respectively. The univariable linear regression model was fitted with all significant

independent variables for each category to check for Multicollinearity. Thereafter, a multivariable linear regression model was fitted, which included variables that retained significance (p<0.05) at univariable analysis (Abdi et al., 2015; Ngoshe et al., 2020). For the multivariate analysis, Stepwise regression method and Forward algorithms were used, then independent variables with p value less than 0.05 were considered significant predictors of RVF knowledge. Prior to assessing multivariate analysis results, the generated model was tested for goodness of fit using Hosmer-Lemeshow test and Omnibus test.

3.7.4. Analysis of prevalence and risk factors for the RVFV sero-positivity

Further, the second data set was on livestock management practices for the RVFV sero-positivity which was analyzed in three stages. The univariate analysis involved largely calculating counts, means, frequencies, relative frequencies and providing survey summaries. Results obtained after running RVFV ELISA test (positive or negative) were dependent variable in bivariate analysis for association. The test for association between RVFV sero-positivity and the independent variables was done using Person Chi-Square test of association (and Fisher`s exact test, where appropriate). The following were independent variables (potential risk factors) species, age, sex, livestock management, district, herd composition, herd size, rainfall amount, knowledge of RVF, education level of farmers, district, vegetation cover, night shelter and ecological zones.

At bivariate analysis, independent variables with moderate significance ($p \leq 0.250$), were further assessed for strength of association using univariate linear regression. Univariable linear regression models with the expected outcome RVFV ELISA test results, were generated to check Multicollinearity. Multicollinearity was checked by Variance Inflation Factors (VIF), acceptable when (VIF value < 1.0) and Tolerance (value > 0.2). Thereafter, a multivariable linear regression model was fitted, which included variables that retained significance (p<0.05) at univariable linear regression analysis (Abdi et al., 2015; Ngoshe et al., 2019; 2020). Stepwise regression and enter algorithms with a liberal p-value for exclusion (p<0.05) to fit the multivariable model. Prior to assessing results for multivariate analysis, the generated model was tested for goodness of fit and predictability using Hosmer-Lemeshow test and Omnibus test, respectively.

3. 8 Ethical considerations

Animal Health Committee of the Department of Animal Health and Livestock Development (DAHLD-AHC: Ref. AHC/10/2019), Malawi (Appendix 5) and University of Zambia Biomedical Research Ethical Committee (UNZABREC: Ref. 617-2019), Zambia; independently reviewed and approved the research protocol (Appendix 6). The ethical clearance clearly described the protocols that were used and requested farmers to provide verbal or written consent to participate in the study. Further, participants were assured that the information and the samples collected were confidential and would be used in academic manner only and not necessarily linked to particular individuals. The farmers that declined to give consent were replaced.

3.9 Dissemination of study results

Study results were made available to all stakeholders namely, MoAIWD, DAHLD, Ministry of Health, Community Health Sciences Unit (CHSU) and the livestock famers through sharing of reports and organized formal meetings. Meeting with farmers was facilitated by District Animal Health and Livestock Development Officers (DAHLDOs). Meetings with other stakeholders was facilitated by the Director of Department of Animal Health and Livestock Development at CHSU Research Dissemination Halls. Thereafter, the results were accepted to be published for easy access of interested parties.

CHAPTER FOUR

4.0 RESULTS

4.1 Knowledge, attitude and management practices (KAP) of livestock farmers towards RVF

4.1.1 Socio-demographic characteristics

Of a total of 400 participants enrolled in the study (Table 4- 1), 67.25% were males, while 32.75% were females. The age group of 46 years and above were in the majority 39.75% followed by the age group of 36 – 45, 35.50%. The majority of the participants 67.50% had primary education whilst few 0.75 attained tertiary education.

Table 4- 1: Background information of the participating members

Variable	Category	Frequency *n*=400	Proportion (%)	95% CI
Gender				
	Male	269	67.25	62.38-71.78
	Female	131	32.75	28.21-37.62
Age (years)				
	18-25	44	11.00	8.19-14.58
	26 – 35	55	13.75	10.38-17.33
	36 – 45	142	35.50	30.85-40.43
	≥ 46	159	39.75	34.95-44.75
Education				
	None	42	10.50	7.75-14.03
	Primary	270	67.50	62.63-72.02
	Secondary	85	21.25	17.41-25.65
	Tertiary	3	0.75	0.19-2.36
Marital Status				
	Married	352	88.00	84.31-90.93
	Single	18	4.50	2.77-7.15
	Divorced	24	6.00	3.96-8.92
	Widowed	6	1.50	0.16-3.40
Herd size				
	Small herds < 25	379	94.75	91.96-96.64
	Large herds ≥ 25	21	5.25	3.36-8.04

n= Number of participants; CI= 95% Confidence Interval

Table 4-1 continues

Variable	Category	Frequency	Proportion (%) *n*=400	95% CI
District				
	CP	39	9.75	7.11-13.19
	KA	45	11.25	8.40-14.86
	SA	60	15.00	11.72-18.97
	MH	54	13.50	10.39-17.33
	CZ	36	9.00	6.47-12.35
	TO	48	12.00	9.06-15.69
	CK	52	13.00	9.94-16.79
	NE	66	16.50	13.07-20.57
Species on the farm				
	Cattle	35	8.75	6.25-12.07
	Cattle, goat	187	46.75	41.79-51.77
	Cattle, goat, sheep	106	26.50	22.30-31.16
	Goat	48	12.00	9.06-15.69
	Goat, sheep	21	5.25	3.36-8.04
	Sheep	3	0.75	0.19-2.36

n= Number of participants; CI= 95% Confidence Interval

4.1.2 Knowledge of participants on Rift Valley fever

Of the participants, 10.25% knew RVF, despite only 8.25% knew its causative agent and 8.75% it`s clinical signs (Table 4- 2). The host species of RVF were mentioned by 9.50% of the participants. Further, only 9.50% of the participants knew its transmission pattern and only 9.50% believed that the mosquitoes could transmit RVFV. Majority of the participants, 92.50% witnessed abortion in livestock. The study also found that 9.50%, of the participants knew that RVF is zoonotic. The average knowledge score of the participants was found to be 17.94% which indicated unsatisfactory with regard to the ability to suspecting and reporting probable cases of RVF.

Table 4- 2: Participants knowledge towards Rift Valley fever

Factors under knowledge	Category	Frequency (n = 400)	Proportion (%)	95% CI	Knowledge score (%)
Did your livestock abort					
	Yes	370	92.50	89.35-94.80	
	No	30	7.50	5.19-10.64	
Which months					
	Jan, Feb, Mar	349	87.25	83.86-90.27	
	Apr, May, Jun	51	12.75	9.72-16.51	
What causes abortion					
	Diseases	313	78.25*	73.81-82.12	78.25
	Poor feeding	48	12.00	9.06-15.69	
	Misfortune	39	9.75	7.10-13.19	
Do you know RVF					
	Yes	41	10.25*	7.54-13.75	10.25
	No	359	89.75	86.25-92.46	
Do you know clinical signs of RVF?					
	Yes	35	8.75*	6.25-12.07	8.75
	No	365	91.25	87.93-93.75	
Do you know what causes RVF					
	Yes	33	8.25*	5.83-11.50	8.25
	No	367	91.75	88.49-94.17	
Do you know how it is transmitted					
	Yes	38	9.50*	6.89-12.91	9.50
	No	362	90.50	87.09-93.11	
Do you know RVF host species					
	Yes	38	9.50*	6.89-12.91	9.50
	No	362	90.50	87.09-93.11	
Can mosquito transmit RVF					
	Yes	38	9.50*	6.89-12.91	9.50
	No	362	90.50	87.09-93.11	
Do you know that it is zoonotic					
	Yes	38	9.50*	6.89-12.91	9.50
	No	362	90.50	87.09-93.11	
Average knowledge score on RVF knowledge of participants (143.5/8)					17.90%

n = number of participants; *= Proportion considered as knowledge score; CI= Confidence interval

4.1.3 Management practices of livestock towards Rift Valley fever

Of the participants, 84.75% witnessed neonatal death in livestock. About 44.75% of the participants handle the aborted materials with unprotected hands and 26.75% of the participants handled neonatal death materials with unprotected hands. Over half of the participants, 55.50% did not bury the aborted materials. Nevertheless, 9.50% of the participants reported to be capable to suspect RVF in livestock. On the other hand, only 9.50% indicated to be capable of preventing the spread of RVF among livestock (Table 4-3). The average practices score of participants was found to be 41.23% which indicated unsatisfactory with regard to the ability to suspecting and reporting probable cases of RVF.

Table 4- 3: Participants practice and management of livestock towards Rift Valley fever

Factors under management practices	Category	Frequency (*n* = 400)	Proportion (%)	95% CI	Practice score (%)
Did you experience neonatal death					
	Yes	339	84.75	80.76-88.05	
	No	61	15.25	11.95-19.24	
Did you experience retain placenta					
	Yes	228	57.00	51.98-61.88	
	No	172	43.00	38.11-48.02	
Are young and old livestock raised together					
	Yes	233	58.25*	53.23-63.10	58.25
	No	167	41.75	36.89-46.76	
How did you handle aborted materials					
	Protected hands	221	55.25*	50.22-60.17	55.25
	Unprotected hands	179	44.75	39.83-49.77	
How did you handle neonatal death materials					
	Protected hands	293	73.25*	68.58-77.47	73.25
	Unprotected hands	107	26.75	22.53-31.42	
Can you suspect RVF in livestock					
	Yes	38	9.50*	6.89-12.91	9.50
	No	362	90.50	87.09-93.11	

n= number of participants; *= Proportion considered as practice score; CI= Confidence interval

Table 4- 3 continues

Factors under management practices	Category	Frequency (n = 400)	Proportion (%)	95% CI	Practice score (%)
Can you prevent RVF in livestock					
	Yes	38	9.50*	6.89-12.91	9.50
	No	362	90.50	87.09-93.11	
How did you dispose aborted materials					
	Buried	178	44.50*	39.58-49.52	44.50
	Unburied	222	55.50	50.47-60.42	
Mode of night shelter					
	Communal	177	44.25	39.34-49.27	54.00
	Private	216	54.00*	48.97-58.94	
	None	7	1.75	0.77-3.73	
Type of grazing grounds					
	Communal	224	56.00	50.97-60.90	44.00
	Private	176	44.00*	39.09-49.02	
Type of herd composition					
	Mixed species	293	73.25	68.57-77.46	26.75
	Single species	107	26.75*	22.53-31.42	
Average practices score on RVF practices of participants (375.1/9)					41.23%

n= number of participants; *= Proportion considered as practice score; CI= Confidence interval

4.1.4 Attitude of participants towards Rift Valley fever

Of the participants, 84.25% associated heavy rainfall and flooding with the destruction of crops and homes while 15.75% of the participants associated heavy rainfall and flooding with the spread of RVF. Further, 8.50% of the participants associated increased mosquito population with the spread of RVFV. Furthermore, 91.50% of the participants did not associate abortion and neonatal death with possible presence of RVF. Only a few participants 8.50% could associate production losses with the possibility of RVF infection. In addition, 8.50% of participants did not fear suffering RVF (Table 4- 4). The average attitude score of participants was found to be 9.40% which indicated unsatisfactory with regard to the ability to suspect and report probable RVF cases.

Table 4- 4: Attitude of participants towards Rift Valley fever

Factors under attitude	Category	Frequency (*n* = 400)	Proportion (%)	95% CI	Attitude score (%)
How do you feel on heavy rainfall and flooding towards occurrence of RVF?					
	Destroy crops	337	84.25	80.22-87.60	
	Promote spread of RVF	63	15.75*	12.39-19.78	15.75
How do you feel on increased mosquito population, can it spread RVF?					
	Yes	34	8.50*	6.04-11.78	8.50
	No	366	91.50	88.21-93.95	
Do you think RVF cause abortion?					
	Yes	34	8.50*	6.04-11.78	8.50
	No	366	91.50	88.21-93.95	
Do you think RVF cause neonatal death?					
	Yes	34	8.50*	6.04-11.78	8.50
	No	366	91.50	88.21-93.95	
Do you think vendors bring RVF infected livestock?					
	Yes	34	8.50*	6.04-11.78	8.50
	No	366	91.50	88.21-93.95	
Do you think there is production losses on your farm due to RVF?					
	Yes	34	8.50*	6.04-11.78	8.50
	No	366	91.5	88.21-93.95	
Do you fear suffering RVF?					
	Yes	34	8.50*	6.04-11.78	8.50
	No	366	91.50	88.21-93.95	
Are you capable to prevent RVF in livestock?					
	Yes	34	8.50*	6.04-11.78	8.50
	No	366	91.50	88.21-93.95	
Average attitude score on RVF attitude of participants (75.2/8)					9.40%

n= number of participants; *= Proportion considered as attitude score; CI= Confidence interval

4.1.5 Mean knowledge, attitude and practices across socio-demographic characteristics

The study found that men had higher mean score 63.50 ±17.90, 24.88 ± 8.13 and 147.44 ± 72.52 than female for knowledge, attitude and practices, at *p* - 0.019, *p* - 0.014, and *p* - 0.003, respectively. Age group of greater than 46 had higher mean score 37.62 ± 15.63, followed by 36 – 45 age group 21.87± 15.04. Also, mean scores statistically differed among marital status groups and varied significantly with herd size categories (Table 4- 5). Mean knowledge scores were higher 25.13 ± 3.52 and 16 ± 11.51 for Thyolo and Chiradzulu districts, respectively, than other districts.

Table 4- 5: Mean knowledge, attitude and practice scores socio-demographic characteristics

Variable	**Mean knowledge score**		**Mean attitude score**		**Mean practice score**	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Gender						
Male	63.50	17.90	24.88	8.13	147.44	72.52
Female	8.25	4.82	12.75	2.12	45.22	36.86
p-value	**0.019**		**0.048**		**0.003**	
Age (years)						
18-25	3.50	4.24	1.38	1.06	23.89	15.35
26 – 35	6.25	5.57	8.38	2.20	33.44	17.72
36 – 45	21.87	15.04	14.63	5.60	66.44	39.94
≥ 46	37.62	13.81	13.25	3.15	58.11	37.33
p-value	0.071		0.092		0.051	
Education						
None	5.50	6.74	1.13	0.35	24.56	12.78
Primary	30.62	72.10	8.25	1.83	106.00	60.28
Secondary	32.62	18.78	25.25	8.46	59.11	24.04
Tertiary	3.00	0.00	2.88	0.35	3.00	0.00
p-value	0.991		**0.048**		0.063	
Marital Status						
Married	54.87	16.97	25.00	8.29	155.56	86.86
Single	7.88	4.15	5.38	1.40	13.44	3.35
Divorced	3.88	6.17	4.13	2.80	18.22	5.33
Widowed	5.13	0.64	3.13	2.03	5.44	0.882
p-value	**0.031**		**0.044**		**0.026**	

Std. Deviation =Standard Deviation, boldface indicates statistical significance at p<0.05

Table 4- 5 continues

Variable	Mean knowledge score		Mean attitude score		Mean practice score	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Herd size						
Small herds <25	62.13	16.63	174.22	96.10	23.50	8.142
Large herds ≥25	9.00	4.92	18.44	2.12	14.63	3.739
p-value	**0.011**		**0.001**		**0.047**	
District						
CP	3.88	10.96	0.00	0.00	16.89	10.99
KA	5.00	11.31	1.00	0.00	21.33	12.63
SA	4.50	12.72	0.00	0.00	28.44	19.61
MH	4.75	13.43	0.00	0.00	23.44	14.03
CZ	25.13	3.52	16.87	4.91	23.67	4.58
TO	16.13	11.51	16.12	4.91	26.11	8.16
CK	6.00	16.97	0.00	0.00	21.11	12.53
NE	6.48	18.03	0.00	0.00	31.67	20.35
p-value	0.721		0.994		0.898	
Overall	17.90	12.78	9.40	2.55	41.23	22.11
Range	3.00-80.50		0.00-270.32		3.00-242.42	

Std. Deviation =Standard Deviation, boldface indicates statistical significance at p<0.05

4.1.6 Distribution of participants` KAP score across the categories

A higher proportion of participants obtained poor grades for the three categories, for practice (p=0.016) and for knowledge (p=0.031), than good practice and knowledge. A similar observation was made for negative and positive attitude (p=0.023) (Table 4- 6).

Table 4- 6*:* Summary of grades for the participants` KAP levels towards RVF

Grades for knowledge	**Scale**	**Proportion** (*n* =400).	**Grades for practice**	**Scale**	**Proportion** (*n* =400).	**Grades for attitude**	**Scale**	**Proportion** (*n* =400).
Poor	< 80	367 (91.75)	Poor	< 80	362 (90.50)	Negative	< 80	366 (91.50)
Good	> 80	33 (8.25)	Good	> 80	38 (9.50)	Positive	> 80	34 (8.50)
p-value		0.031			0.016			0.023

n= Number of participants

4.1.7 Analysis for association between knowledge of RVF and potential determinants of knowledge

The study found only 8.25% participants were knowledgeable on RVF at cut of point of 80% and above. Pearson chi-square was run to assess association between the potential determinants and the knowledgeable. There was association between knowledgeable and the observed level of knowledge on what causes RVF (X^2= 7.989, p=0.018); the observed level of knowledge on clinical signs (X^2= 4.007, p=0.045); the observed level of knowledge on mode of transmission (X^2= 13.214, p=0.001) under the knowledge category. Also there was association between the knowledgeable and lack of ability to prevent spread of RVF (X^2= 18.105, p=0.001); the practice of mixing young and old livestock (X^2= 14.192, p=0.001) under the management practices. There was no association between the knowledgeable and potential predictors under negative attitude category. Thereafter, the variables were screened for multicollinearity using univariate linear regression (Table 4- 7) and (Table 4- 8).

Table 4- 7: Summary of univariate regression analysis of potential determinants under knowledge category and the observed level of knowledge for RVF

Factors under knowledge	Number of participants (*n*=400)	Knowledgeable (*n*=33)	Proportion (%)	OR	95% CI	*p* -Value
Months for occurrence of abortions (*n*= 400) ***						
Jan, Feb, Mar	349	26	7.45	Ref		
Apr, May, Jun	51	7	13.73	3.531	1.40-8.90	0.008
What causes abortion (*n*= 400)*						
Diseases	313	21	6.71	Ref		
Poor feeding	48	9	18.75	1.159	0.32-4.07	0.818
Misfortune	39	3	7.69	3.209	1.37-7.50	0.007
Do you know what causes RVF (*n*= 400)***						
No	367	29	7.90	Ref		
Yes	33	4	12.12	3.531	1.40-8.90	0.008
Do you know RVF clinical signs (*n*= 400)*						
No	365	27	7.40	Ref		
Yes	35	6	17.14	2.590	1.98-6.78	0.053
Do you know the affected species (*n*= 400)***						
No	362	31	8.56	Ref		
Yes	38	2	5.26	0.216	0.07-0.62	0.005
Do you know how it is transmitted (*n*= 400)***						
No	362	24	6.63	Ref		
Yes	38	9	23.68	4.371	1.85-10.27	0.001

n = Number of participants; CI = Confidence interval, Significant level < 0.05; OR=Odds ratio; *** = Significant at 0.05, considered for multivariate analysis; * = considered for multivariate analysis (cut-off p = <0.250); Ref = reference category.

Table 4- 7 continues

Factors under knowledge	Number of participants (*n*=400)	Knowledgeable (*n*=33)	Proportion (%)	OR	95% CI	*p* -Value
Can you suspect RVF cases in livestock (*n*= 400)***						
No	362	23	6.35	Ref		
Yes	38	10	26.32	5.264	2.28-12.15	< 0.001
Can mosquito transmit RVF (*n*= 400)***						
No	362	23	6.35	Ref		
Yes	38	10	26.32	5.264	2.28-12.15	< 0.001
Do you know that it`s zoonoses (*n*= 400)***						
No	362	23	6.35	Ref		
Yes	38	10	26.32	5.264	2.28-12.15	< 0.001

n = Number of participants; CI = Confidence interval, Significant level < 0.05; OR=Odds ratio; *** = Significant at 0.05, considered for multivariate analysis; * = considered for multivariate analysis (cut-off p = <0.250); Ref = reference category.

Table 4- 8: Summary of univariate regression analysis of potential determinants under management practices and knowledge of RVF

Factors under practices	Number of participants (*n*=400)	Knowledgeable (*n*=33)	Proportion (%)	OR	95% CI	*p* -Value
Age (*n*= 400)*						
≥ 46	159	12	7.55	Ref		
36-45	142	15	10.56	2.228	1.60-8.14	0.022
26-35	54	4	7.41	0.484	0.06-3.71	0.486
18-25	44	2	4.55	0.000	0.00-0.00	1.000
Gender (*n*= 400)***						
Female	269	18	6.69	Ref		
Male	131	15	11.45	1.808	1.00-3.70	0.008
Education (*n*= 400)*						
None	42	7	16.67	Ref		
Primary	270	23	8.52	0.393	0.01-0.74	0.826
Secondary	85	3	3.53	0.100	0.00-0.42	0.038
Tertiary	3	0	0.00	5.370	0.00-0.00	0.177
Did you experience neonatal death (*n*= 400) ***						
No	61	33	54.10	Ref		
Yes	339	0	0.00	0.350	0.17-0.72	0.004
Mode of night shelter (*n*= 400)*						
Private	177	7	3.95	Ref		
Communal	216	26	12.04	3.323	1.41-7.85	0.006
None	7	0	0.00	0.000	0.00-0.00	0.999
Type of grazing grounds (*n*= 400)***						
Communal	224	7	3.13	Ref		
Stall feeding	176	26	14.77	2.583	1.25-5.36	0.011

n = Number of participants; CI = Confidence interval, Significant level < 0.05; OR=Odds ratio; *** = Significant at 0.05, considered for multivariate analysis; * = considered for multivariate analysis (cut-off p = <0.250); Ref = reference category.

Table 4-8 continues

Factors under practices	Number of participants (*n*=400)	Knowledgeable (*n*=33)	Proportion (%)	OR	95% CI	*p* -Value
Herd composition (*n*= 400)***						
Mixed species	293	13	4.44	Ref		
Single species	107	20	18.69	2.855	1.39-5.88	0.004
Abortion management (*n*= 400)***						
Not buried	222	29	13.06	Ref		
Buried	178	4	2.25	0.190	0.08-0.44	0.001
How did you handle aborted materials (*n*= 400)***						
Protected	221	29	13.12	Ref		
Unprotected	179	4	2.23	0.321	0.15-0.70	0.004
Can you prevent spread of RVF? (*n*= 400)***						
Yes	38	3	7.89	Ref		
No	362	30	8.29	5.264	2.28-12.15	0.001
Management of neonatal materials (*n*= 400)***						
Unprotected	107	12	11.21	Ref		
Protected	293	21	7.17	0.350	0.17-0.72	0.004

n = Number of participants; CI = Confidence interval, Significant level < 0.05; OR=Odds ratio; *** = Significant at 0.05, considered for multivariate analysis; * = considered for multivariate analysis (cut-off p = <0.250); Ref = reference category.

4.1.8 Determinants of RVF knowledge for the participants

After adjustment for other variables in the stepwise binary logistic regression model, significant determinants of RVF knowledge for participants were (*p*-value <0.05). Variables with (*p*-value < 0.250) in the bivariate analysis were included in the model. The test had insignificant Hosmer-Lemeshow goodness-of-fit statistic (*p*=0.828) and Omnibus Test of Model Coefficients values of

(p<0.000) were obtained, indicating goodness of fit of the generated model. The significant determinants were knowledge on the cause of abortion and knowledge of how RVFV transmitted and the respective adjusted Odds ratio (aOR) are presented in (Table 4- 9). Farmers with the knowledge that misfortune caused abortion were (aOR: 3.861, 95% CI= 1.14-13.05) times more likely to be less knowledgeable on RVF than farmers with knowledge that diseases cause abortion (p=0.001). Farmers without knowledge of how RVFV spread/transmitted were (aOR: 5.65, 95% CI= 1.76-18.12) times more likely to be less knowledgeable about RVF than farmers with knowledge on how RVFV spread/transmitted (p=0.004). Farmers with knowledge on the affected species were (aOR: 0.140, 95% CI= 0.03-0.62) times more likely to be knowledgeable about RVF than farmers without knowledge on the affected species (p=0.009).

Table 4- 9: Summary of maximum likelihood estimates for determinants associated with RVF knowledge

Variable	**Level**	**aOR**	**95% CI**	***p*-Value**
What causes abortion (n=400)	Diseases	Ref		
	Poor feeding	1.879	0.47-7.52	0.372
	Misfortune	3.861	1.14-13.05	0.001***
How RVF is transmitted (n=400)	Yes	Ref		
	No	5.652	1.76-18.12	0.004***
Do you know the affected species (n=400)	No	Ref		
	Yes	0.140	0.03-0.62	0.009***

*** = Significant at 0.05; aOR = adjusted Odds ratio; CI = Confidence interval; Significant level < 0.05; Ref = Reference category

4.2 Rift Valley fever virus seroprevalence

4.2.1 Descriptive statistics of the study population

4.2.2 Descriptive statistics for livestock farmers and herds

The study recruited 361 livestock farmers who gave consent for questionnaire administration and whole blood sample collection from their livestock. Of the 361 participants, 58.17% were males and 41.83% were female. Farmers had a median age of 39 years and mean 41 years (minimum 21 years, maximum 68 years), unfortunately 37 farmers did not disclose their age. Of the 361 participants, 98.06% depend on subsistence farming for their livelihoods while 1.94% had other income generating activities. The species composition at herd level was 62.05% (95% CI= 56.94–66.90), 34.07%, (95% CI: 29.39-39.11) and 3.88%, (95% CI= 2.32–6.40) for cattle, goat and sheep, respectively. In this study, herd size distribution varied with 84.49% small herds (≤ 25 animals), 4.98% medium herds (26-50 animals), and 10.53% large herds (> 50 animals). The age of cattle was divided into four categories, young < 2 years old were 24.85%, sub-adults 2-4 years old were 29.87% adults 5-8 years were 40.61% and old age group for those above 9 years were 4.66%. Similarly, the age of goats was divided into four categories, < 2 years old 30.50%, 2-3 years 40.54%, 4-5 years 26.26% and those above 6 years 2.70%. The age for sheep were also in four age categories < 2 years old 31.08%, 2-3 years were 45.95%, the age categories 4-5 years were 21.62% and those above 6 years were 1.35%. There were mostly small herds, with the majority keeping at least two animal species

4.2.3 Descriptive statistics at individual livestock level

The study collected 1,523 out of the target of 2,220 samples indicating 68.60% sample collection success (Table 4-10). Some of the challenges were that sampling of sheep was not possible because farmers were not interested to bleed sheep in Thyolo and Chiradzulu, whilst in Chitipa flocks of sheep were located along impassable roads and then time and fuel resources constrained the sampling team to revisit the sites. Sampling of cattle in all districts had challenge of low man power because crushes were not in good condition for restraining cattle.

Table 4- 10: Distribution of blood sample collection across the ecological zones and districts

			EZ 1			EZ 2		EZ 3	
			Sampling allocations						
			1101			409		710	
	Districts	**SA**	**MH**	**CK**	**NE**	**TO**	**CZ**	**CP**	**KA**
Livestock Species									
Cattle	857	120	144	53	108	47	61	162	162
Goat	518	59	60	59	84	104	102	23	27
Sheep	148	23	18	67	36	0	0	0	4
Total	1,523	202	222	228	179	163	151	185	193
Total EZ samples			831			314		378	

EZ1= Ecological zone 1 (SA= Salima, MH=Mangochi, CK=Chikwawa, and NE=Nsanje), EZ2= Ecological zone 2 (TO= Thyolo, and CZ=Chiradzulu), EZ3= Ecological zone 3 (CP= Chitipa and KA= Karonga)

With this background, the whole blood samples were collected from 1,523 livestock, cattle 56.27%, goat 34.01% and 9.72% sheep. Female livestock were in majority, 90.54%, (95% CI= 89.01-92.01) than male livestock 9.46%, (95% CI= 7.99–10.99). There were 90.90%, female livestock for cattle, 90.93% female livestock for goats and 87.84% females for sheep.

4.2.4. Rift Valley fever virus IgG and IgM sero-positivity results for livestock

The study screened 1,523 livestock (857 cattle, 518 goats and 148 sheep) for antibodies against RVFV using an ID Screen® Rift Valley fever competition multi-species ELISA, for IgG antibodies and ID Screen® RVF IgM Capture Multi-species for IgM antibodies (Table 4-11).

Table 4- 11: Distribution of serum samples across the ecological zones and antibody sero-positive results at individual livestock level

		EZ 1								EZ 2				EZ 3				
		Number of animals sampled																Total sampled
		831								314				378				1,523
	District	**SA**		**MH**		**CK**		**NE**		**TO**		**CZ**		**CP**		**KA**		
Livestock species	**Sampled**	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	Total reactors
Cattle	857	32	1	37	8	5	1	21	2	12	2	14	1	33	7	6	1	183
Goat	518	7	0	9	0	2	2	7	1	4	2	1	1	0	3	2	0	40
Sheep	148	8	0	3	0	2	13	8	0	0	0	0	0	0	0	3	0	38
Total	1,523	47	1	49	8	9	16	36	3	16	4	15	2	33	10	11	1	261
EZ total reactors		169								37				55				

IgG= Immunoglobin G; IgM= Immunoglobin M; EZ1= Ecological zone 1 (SA= Salima, MH=Mangochi, CK=Chikwawa, and NE=Nsanje), EZ2= Ecological zone 2 (TO= Thyolo, and CZ=Chiradzulu), EZ3= Ecological zone 3 (CP= Chitipa and KA= Karonga)

4.2.5. Rift Valley fever virus seroprevalence by livestock species at individual animal level

Sheep had higher seroprevalence 25.68%, (95% CI= 19.31-33.26) than cattle 21.35%, (95% CI= 18.74-24.22) and goat 7.72%, (95% CI= 5.72-10.34), (p=0.047). The crude seroprevalence was 17.14% (95% CI= 15.33-19.11) at individual livestock level. The IgG overall seroprevalence was 14.18% (95% CI= 12.49-16.06), while IgM overall seroprevalence was 2.95% (95% CI= 2.19-3.97). Cattle had highest IgG seroprevalence 18.67% (95% CI= 16.20-21.41) than sheep 16.22% (95% CI= 11.14-22.99) and goat 6.18% (95% CI= 4.41-8.59). The results indicated higher IgM seroprevalence in sheep 9.46% (95% CI= 5.71-15.25) than cattle 2.68% (95% CI= 1.79-3.99) and goat 1.54% (95% CI= 0.78-3.01) at species level. Goats had the lowest seroprevalence for both IgG and IgM (Table 4-12).

Table 4- 12: RVFV seroprevalence for the livestock species and prevalence for the antibodies

Livestock spp.	Antibody test	*n*	Reactors	Seroprevalence (%)	95% C I
Cattle	IgG	857	160	18.67	16.20-21.41
	IgM	857	23	2.68	1.79-3.99
	Overall	857	183	21.35	18.68-24.28
Goat	IgG	518	32	6.18	4.41-8.59
	IgM	518	8	1.54	0.78-3.01
	Overall	518	40	7.72	5.64-10.45
Sheep	IgG	148	24	16.22	11.14-22.99
	IgM	148	14	9.46	5.71–15.25
	Overall	148	38	25.68	19.02-33.62

n = number of livestock; IgG= Immunoglobin G; IgM= Immunoglobin M; CI= Confidence interval; Significant level < 0.05

4.2.6 Seroprevalence in ecological zones and districts

The seroprevalence varied across ecological zones and districts. EZ1 had the highest seroprevalence followed by EZ3 and then EZ2 (Table 4-13). The seroprevalence for districts had ranged from 6.22%-25.68%. The following districts had seroprevalence above the overall prevalence, Salima 23.76% (95% CI= 18.19-30.35), Mangochi 25.68% (95% CI= 20.17-32.04), Nsanje 21.79% (95% CI= 16.12-28.69) and Chitipa 23.24% (95% CI= 17.50-30.12) (Table 4-13).

Table 4- 13: RVFV seroprevalence for the ecological zones and districts

Ecological zones	Districts	*n*	Reactors	Seroprevalence	95% CI
EZ 1	Chikwawa	228	25	10.96	7.54–15.68
	Nsanje	179	39	21.79	16.37-28.39
	Salima	202	48	23.76	18.42–30.09
	Mangochi	222	57	25.68	20.38–31.81
	Overall	831	169	20.34	17.68–23.27
EZ 2	Chiradzulu	151	17	11.26	7.15-17.29
	Thyolo	163	20	12.27	8.09-18.19
	Overall	314	37	11.78	8.53–15.99
EZ 3	Chitipa	185	43	23.24	17.74–29.84
	Karonga	193	12	6.22	3.59–10.55
	Overall	378	55	14.55	11.23– 18.61

n = Number of herds; 95% CI= Confidence interval; % = Percent; EZ1= Ecological zone 1, EZ2= Ecological zone 2, EZ3= Ecological zone 3

4.2.7 Seroprevalence according to sex and age

The overall seroprevalence according to the sex of livestock had similar trend across species, despite not being significantly different in goats. The overall seroprevalence appeared to be higher in male livestock than female counterparts (Table 4-14). Similarly, males had higher IgG seroprevalence than female livestock (p=0.031). Further, the overall seroprevalence across the age groups was higher in sub adults (2-4 and 2-3 age groups) for cattle and goats (p=0.023 and p=0.046, respectively), while old age (≥ 6) had higher seroprevalence in sheep (p=0.029). Age effect was noted in higher overall seroprevalence of adults and old age groups, compared to young animals (< 2) in cattle. Similarly, age effect was observed in sheep and goats where young age had lowest seroprevalence compared to sub-adults, adults, and old age (Table 4-15).

Table 4- 14: Distribution in seroprevalence of RVFV by the sex categories

Species	Sex category	Antibody	*n*	Reactors	Seroprevalence (%)	95% CI
Cattle	Male	Overall	77	22	28.57	19.13-40.17
	Female	Overall	780	161	20.64	17.88-23.69
	Male	IgG	77	19	24.67	15.86-36.05
	Female	IgG	780	141	18.08	15.47-20.99
	Male	IgM	77	3	3.89	1.01-11.73
	Female	IgM	780	20	2.56	1.61-4.01
Goat	Male	Overall	47	4	8.51	2.76-21.27
	Female	Overall	471	36	7.64	5.48-10.52
	Male	IgG	47	4	8.51	2.76-21.27
	Female	IgG	471	28	5.94	4.05-8.58
	Male	IgM	47	0	0.00	0.00-9.41
	Female	IgM	471	8	1.69	0.79-3.45
Sheep	Male	Overall	18	7	38.89	18.26-63.86
	Female	Overall	130	31	23.85	17.00-32.27
	Male	IgG	18	6	33.33	14.35-58.84
	Female	IgG	130	18	13.84	8.63-21.26
	Male	IgM	18	1	5.55	0.29-29.37
	Female	IgM	130	13	10	5.65-16.81

n = Number of livestock; CI= Confidence interval; IgG= Immunoglobin G; IgM= Immunoglobin M

Table 4- 15: Distribution in seroprevalence of RVFV across age categories

Species	Age groups (Years)	Antibody	Reactors	*n*	Seroprevalence (%)	95% CI
	< 2	Overall	11	213	5.16	2.74-9.30
	2-4	Overall	97	256	37.89	31.98-44.17
	5-8	Overall	64	348	18.39	14.54-22.95
	≥9	Overall	11	40	27.50	15.14-44.13
	<2	IgG	8	213	3.85	1.75-7.53
Cattle	2-4	IgG	89	256	34.76	29.01-40.98
	5-8	IgG	54	348	15.52	11.96-19.85
	≥9	IgG	9	40	22.50	11.40-38.85
	<2	IgM	3	213	1.41	0.36-4.39
	2-4	IgM	8	256	3.12	1.46-6.29
	5-8	IgM	10	348	2.87	1.47-5.39
	≥9	IgM	2	40	5.00	0.87-18.21
	< 2	Overall	6	158	3.79	1.55-8.45
	2-3	Overall	23	210	10.95	7.21-16.17
	4-5	Overall	10	136	7.35	3.78-13.45
	≥6	Overall	1	14	7.14	0.37-35.83
	< 2	IgG	4	158	2.53	0.81-6.76
Goat	2-3	IgG	18	210	8.57	5.30-13.41
	4-5	IgG	9	136	6.62	3.26-12.55
	≥6	IgG	1	14	7.14	0.37-35.83
	< 2	IgM	2	158	1.26	0.22-4.97
	2-3	IgM	5	210	2.38	0.87-5.77
	4-5	IgM	1	136	0.74	0.04-4.64
	≥6	IgM	0	14	0.00	0.00-26.76

n = Number of livestock; CI= Confidence interval; IgG= Immunoglobin G; IgM= Immunoglobin M

Table 4- 15 continues

Species	Age groups (Years)	Antibody	Reactors	*n*	Seroprevalence (%)	95% CI
	<2	Overall	3	46	6.52	1.69-18.92
	2-3	Overall	24	68	35.29	24.36-47.90
	4-5	Overall	10	32	31.25	16.74-50.14
	≥6	Overall	1	2	50.00	9.45-90.55
	<2	IgG	2	46	4.34	0.75-16.03
	2-3	IgG	11	68	16.17	8.75-27.52
Sheep	4-5	IgG	9	32	28.13	14.39-46.97
	≥6	IgG	1	2	50.00	9.45-90.55
	<2	IgM	1	46	2.17	0.11-12.96
	2-3	IgM	13	68	19.11	10.95-30.82
	4-5	IgM	1	32	3.12	0.16-17.99
	≥6	IgM	0	2	0.00	0.00-80.21

n = Number of livestock; CI= Confidence interval; IgG= Immunoglobin G; IgM= Immunoglobin M

4.2.8. Sampling and antibody investigation results for livestock herds

The study recruited 361 livestock herds of which 62.05%, (95% CI= 56.80-67.04), 34.07%, (95% CI= 29.24-39.25) and 3.88%, (CI= 2.21-6.57) were cattle, goat and sheep, respectively, (Table 4-16). The sampling for sheep flocks was not as expected due to fear of unknown by the herd owners in Chitipa, Thyolo and Chiradzulu districts. The IgG antibody investigation results for the herds were observed in all sampled districts unlike the IgM results which were observed in five out of eight districts (Table 4-16).

Table 4- 16: Distribution of serum samples across the ecological zones and districts and antibody sero-positive results for herds

		EZ 1						**EZ 2**						**EZ 3**				
		EZ Sampled																Total sampled
		85						143						133				361
Districts		**SA**		**MH**		**CK**		**NE**		**TO**		**CZ**		**CP**		**KA**		
District sampled		29		19		20		17		63		80		70		63		
Antibody tests		IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG	IgM	
Livestock species	Sampled																	Total reactors
Cattle	224	6	1	6	4	2	1	5	2	8	2	10	1	21	6	5	1	81
Goat	123	3	0	4	0	1	2	3	0	3	2	1	1	0	3	2	0	25
Sheep	14	3	0	2	0	1	4	2	1	0	0	0	0	0	0	1	0	14
District total		13		16		11		13		15		13		30		9		120
EZ total reactors		29						52						39				

IgG=Immunoglobin G; IgM= Immunoglobin M; EZ= Ecological zone; EZ1= Ecological zone 1 (SA= Salima, MH=Mangochi, CK=Chikwawa, and NE=Nsanje), EZ2= Ecological zone 2 (TO= Thyolo, and CZ=Chiradzulu), EZ3= Ecological zone 3 (CP= Chitipa and KA= Karonga)

4.2.9 Rift Valley fever virus seroprevalence for livestock herds

The overall herd seroprevalence was 33.24%, (95% CI= 28.18-38.11). The study found sheep herd seroprevalence higher than other two species 100%, (95% CI= 73.23–1.00) (p=0.019) and it consisted of 64.29% for IgG seroprevalence and 35.71% for IgM seroprevalence. The herd seroprevalence for cattle were 36.16%, (95% CI= 29.51–42.41) of which 77.78% were IgG seroprevalence and 22.22% were IgM seroprevalence. The herd seroprevalence for goats were 20.33%, (95% CI= 13.82–28.73) of which 68.00%, were IgG seroprevalence and 32.00% were IgM seroprevalence.

Further, the herd seroprevalence for EZ2 was comparatively higher at 36.36%, (95% CI= 28.61-44.55) followed by EZ1, then EZ3. The seroprevalence for EZ1 was 34.12%, (95% CI= 24.40-45.88) and 29.32%, (95% CI= 21.92–37.95) was the herd seroprevalence for EZ3 indicating slight variations.

Furthermore, the overall herd seroprevalence (for both IgG and IgM) at district level had a range of (14.37%-84.25%, n = 8) and the median was 43.93%. Mangochi and Nsanje districts had herd seroprevalence over 75% while Chiradzulu and Karonga districts had 16.13% and 14.33%, respectively. The herd seroprevalence varied among the three species such that sheep herds had high seroprevalence of (100%, n = 5) for both median and mean compared to cattle and goats (Table 4-17).

Table 4- 17: RVFV herd seroprevalence across districts and species.

Factor	District	*n*	Seroprevalence (%)	95% CI
Districts	Salima	29	44.83	26.95-64.02
	Mangochi	19	84.24	59.51-95.83
	Chikwawa	20	55.00	32.04-76.17
	Nsanje	17	76.50	49.80-92.18
	Chiradzulu	80	16.13	9.36-26.55
	Thyolo	63	23.78	14.35-36.49
	Chitipa	70	42.89	31.28-55.22
	Karonga	63	14.33	7.14-25.97
	Total	361	33.24	28.18-38.11
Cattle	Salima	10	7.01	35.37-91.91
	Mangochi	12	83.32	50.88-97.06
	Chikwawa	10	30.00	8.09-64.63
	Nsanje	8	87.51	46.68-99.34
	Chiradzulu	39	28.24	15.55-45.10
	Thyolo	32	31.34	16.75-50.14
	Karonga	53	11.30	4.69-23.72
	Chitipa	60	45.00	32.33-58.31
Goat	Salima	16	18.76	4.97-46.31
	Mangochi	5	80.00	29.88-98.95
	Chikwawa	5	60.00	17.04–92.74
	Nsanje	6	50.00	18.76–81.23
	Chiradzulu	41	4.92	0.81–17.05
	Thyolo	31	16.12	6.09–34.47
	Chitipa	10	30.00	8.09–64.63
	Karonga	9	22.22	3.95–59.81
Sheep	Salima	3	100	19.79–100.0
	Mangochi	2	100	19.79–100.0
	Chikwawa	5	100	56.55–100.0
	Nsanje	3	100	39.58–100.0
	Karonga	1	100	5.46–100.0

n = Number of herds; CI= Confidence interval; % = Percent

4.3. Determining potential risk factors for RVFV sero-positivity at herd and individual livestock

4.3.1 The frequency and proportions of epidemiological factors at individual livestock level

Frequency and proportions of 15 epidemiological factors at individual livestock level were determined by using univariate analysis of variables coded from the questionnaire (Table 4-18).

Table 4- 18: Frequency and proportions of epidemiological factors at individual livestock level

Factor	Level	Frequency	Percent (*n* = 1,523)
Gender of farmers	Male	1226	80.50
	Female	297	19.50
Species	Cattle	857	56.27
	Goat	518	30.01
	Sheep	148	9.72
Sex of livestock	Male	143	9.39
	Female	1380	90.61
Ecological zones	EZ1	831	54.56
	EZ2	314	20.62
	EZ3	378	24.82
Districts	SA	202	13.26
	MH	222	14.58
	CK	228	14.97
	NE	179	11.75
	TO	163	10.70
	CZ	151	9.91
	KA	185	12.15
	CP	193	12.67
	None	19	1.25
Night shelter	Communal	862	56.60
	Private	642	42.15
Herd composition	Single species	109	7.16
	Mixed species	1414	92.84
Grazing site	Communal	1214	79.71
	Stall feeding	309	20.29
Vegetative cover	Trees	632	41.50
	Trees & green grass	513	33.68
	Forest	378	24.82
Permanent water	Swaps	634	41.53
	Swaps and rivers	889	58.47

n = Number of livestock; EZ1= Ecological zone 1, EZ2= Ecological zone 2, EZ3= Ecological zone 3; District names: SA= Salima, MH=Mangochi, CK=Chikwawa, NE=Nsanje, TO= Thyolo, CZ=Chiradzulu, CP= Chitipa, and KA= Karonga; IgG=Immunoglobin G; IgM= Immunoglobin M

Table 4- 18 continues

Factor	Level	Frequency	Percent (*n* = 1,523)
Education level	None	69	4.53
	Primary	1010	66.32
	Secondary	412	27.05
	Tertiary	32	2.10
Routine management	No	385	25.28
	Yes	1138	74.72
Average rainfall	<1,000mm	393	25.80
	1,001-1,500mm	660	43.34
	>1,600mm	470	30.86
Herd size	< 25	864	56.72
	26-50	196	12.87
	≥ 51	463	30.40
RVF awareness	Yes	54	3.55
	No	1469	96.45

n = Number of livestock; EZ1= Ecological zone 1, EZ2= Ecological zone 2, EZ3= Ecological zone 3; District names: SA= Salima, MH=Mangochi, CK=Chikwawa, NE=Nsanje, TO= Thyolo, CZ=Chiradzulu, CP= Chitipa, and KA= Karonga; IgG=Immunoglobin G; IgM= Immunoglobin M

4.3.2 Analysis of association between potential risk factors and RVFV sero-positivity at individual livestock level.

The bivariate analysis included 15 epidemiological factors for analysis of association of potential risk factors and RVFV sero-positivity. It was found that 46.67%, variables were significant at p=0.05, for individual livestock. Thereafter, the variables were screened for multicollinearity using univariate linear regression (Table 4-19).

Table 4- 19: Summary of univariate regression analysis of potential risk factors and RVFV sero-positivity at individual livestock level

Potential risk factors	**Number tested (*n*=1,523)**	**Reactors (*n*=261)**	**Sero-positivity (%)**	**OR**	**95% CI**	***p* =Value**
Species (*n*= 1,523)***						
Cattle	857	183	21.35	Ref		
Goat	518	40	7.72	0.30	0.21-0.44	0.001
Sheep	148	38	25.68	1.27	0.85-1.90	0.242
Sex (*n*= 1,523)***						
Male	142	33	23.24	Ref		
Female	1381	228	16.51	0.65	0.43-0.98	0.044
Education level (*n*= 1,523)***						
None	69	69	100.00	Ref		
Primary	1010	111	10.99	4.55	1.41-14.64	0.011
Secondary	412	72	17.47	4.96	1.51-16.20	0.008
Tertiary	32	9	28.12	8.60	2.14-34.56	0.002
Rainfall (*n*= 1,523)***						
< 1,000mm	393	75	19.08	Ref		
1,001-1,500mm	660	78	11.82	0.587	0.41-0.83	0.003
>1,600 mm	470	110	23.40	1.33	2.96-9.81	0.044
RVF awareness (*n*= 1,523)***						
No	1469	245	16.68	Ref		
Yes	54	16	29.63	2.10	1.15-3.83	0.015
Herd composition (*n*= 1,523)***						
Single spp.	109	29	26.61	Ref		
Mixed spp.	1414	232	16.41	0.513	0.37-70	0.001
Ecological zones (*n*=1,523)***						
EZ3	378	55	14.86	Ref		
EZ1	831	169	20.34	1.499	1.07-2.09	0.017
EZ2	314	37	11.78	0.784	0.50-1.22	0.287

n = Number of participants; CI = Confidence interval, Significant level < 0.05; OR=Odds ratio; *** = Significant at 0.05, considered for multivariate analysis; * = considered for multivariate analysis (cut-off p = <0.250); Ref = reference category.

4.3.3 Maximum likelihood estimates of risk factors for sero-positivity of individual livestock

A stepwise binary logistic regression model was used to determine variables that could be predisposing factors for RVFV sero-positivity of an individual livestock. Variables with a *p*-value less than 0.250 in the bivariate analysis were included in the model. The test had significant Hosmer-Lemeshow goodness-of-fit statistic (p=0.987) and Omnibus Test of Model Coefficients values of (p<0.000) were obtained, indicating goodness of fit of the generated model. Further, the models classified 82.8% of the expected outcome. The risk factors were significant when <0.05, *p*-value and when confidence interval did not include 1. After adjustment for other variables in the model, (20.00%, 1/5) of the variables was significant risk factors of RVFV sero-positivity for livestock at individual level and this was sex and the adjusted Odds ratio (aOR) was presented in (Table 4-20). Female livestock were (aOR: 1.74, 95% CI= 1.08–12.82) times more likely to be seropositive to RVFV than male livestock (p=0.016).

Table 4- 20: Summary of maximum likelihood estimates for risk factors associated with RVFV sero-positivity for individual livestock

Variable	Level	aOR	95% CI	*p*-value
Sex (n=1,523)	Male	Ref		
	Female	1.74	1.08-12.82	0.016***

***Statistically significant at p<0.05; Ref = Reference category; aOR = adjusted Odds Ratio, 95% CI = Confidence interval

4.3.4 The frequency and proportions of epidemiological factors at herd level

Frequency and proportions of epidemiological factors in the study population were summarized at herd level (Table 4-21).

Table 4- 21: Frequency and proportions of epidemiological factors at herd level

Factor	Level	Frequency	Percent (*n* = 361)
Gender of farmer	Male	210	58.17
	Female	151	41.83
	Goat	123	34.07
Species	Cattle	224	62.05
	Sheep	14	3.88
District	SA	29	8.03
	MH	19	5.26
	CK	20	5.54
	NE	17	4.71
	TO	63	17.45
	CZ	80	22.16
	KA	70	19.39
	CP	63	17.45
Ecological zone	EZ1	85	23.55
	EZ2	143	39.61
	EZ3	133	36.84
Night shelter	None	7	1.94
	Communal	179	49.58
	Private	175	48.48
Herd composition	Single species	69	19.11
	Mixed species	292	80.89
Grazing site	Communal	219	60.66
	Stall feeding	142	39.34
Permanent water	Swaps	56	15.51
	Swaps and rivers	305	84.49
Vegetative cover	Trees	199	55.12
	Trees & green grass	29	8.03
	Forest	133	36.84
	None	15	4.16
Education level	Primary	248	68.70
	Secondary	81	22.44
	Tertiary	17	4.71
Routine management	No	90	24.93
	Yes	271	75.07
Average rainfall	<1,000mm	28	7.76
	1,001-1,500mm	258	71.47
	>1,600mm	75	20.78

n= Number of herds; EZ1= Ecological zone 1, EZ2= Ecological zone 2, EZ3= Ecological zone 3; District names: SA= Salima, MH=Mangochi, CK=Chikwawa, NE=Nsanje, TO= Thyolo, CZ=Chiradzulu, CP= Chitipa, and KA= Karonga; IgG=Immunoglobin G; IgM= Immunoglobin M

Table 4- 21 continues

Factor	Level	Frequency	Percent (*n* = 361)
Average rainfall	<1,000mm	28	7.76
	1,001-1,500mm	258	71.47
	>1,600mm	75	20.78
Herd size	less 25	305	84.49
	26-50	18	4.99
	over 51	38	10.53
	< 4	236	65.37
Years in farming	5-8	114	31.58
	≥ 9	11	3.05
RVF awareness	No	349	96.68
	Yes	12	3.32

n= Number of herds; EZ1= Ecological zone 1, EZ2= Ecological zone 2, EZ3= Ecological zone 3; District names: SA= Salima, MH=Mangochi, CK=Chikwawa, NE=Nsanje, TO= Thyolo, CZ=Chiradzulu, CP= Chitipa, and KA= Karonga; IgG=Immunoglobin G; IgM= Immunoglobin M

4.3.5 Analysis of association between potential risk factors and RVFV sero-positivity at herd level.

The bivariate analysis included 15 epidemiological factors for analysis of association of potential risk factors and RVFV sero-positivity. It was found that 26.67%, variables were significant (p=0.05). Further, the variables were screened for multicollinearity using univariate linear regression (Table 4-22).

Table 4- 22: Summary of univariate regression analysis of potential risk factors and RVFV seropositivity at herd level

Potential risk factors	**Number tested (*n*=361)**	**Reactors (*n*=120)**	**Sero-positivity (%)**	**OR**	**95% CI**	***p* =Value**
Species (*n*= 361)***						
Cattle	224	81	36.16	Ref		
Goat	123	25	20.32	2.22	1.32-3.72	0.002
Sheep	14	14	100.00	0.00	0.00-0.00	0.998
Education level (*n*= 361)***						
None	15	3	20.00	Ref		
Primary	248	82	33.06	0.232	0.06-0.79	0.019
Secondary	81	34	42.0	0.163	0.04-0.58	0.005
Tertiary	17	1	5.88	0.23	0.01-0.37	0.014***
Rainfall (*n*= 361)***						
< 1,000mm	28	8	28.57	Ref		
1,001-1,500mm	238	73	30.37	4.925	1.44-23.35	0.024***
>1,600 mm	75	39	52.00	9.023	1.37-17.97	0.037***

n = Number of participants; CI = Confidence interval, Significant level < 0.05; OR=Odds ratio; *** = Significant at 0.05, considered for multivariate analysis; * = considered for multivariate analysis (cut-off p = <0.250); Ref = reference category.

Table 4- 22 continues

Potential risk factors	Number tested (*n*=361)	Reactors (*n*=120)	Sero-positivity (%)	OR	95% CI	*p* =Value
RVF awareness (*n*= 361)*						
No	349	118	33.81	Ref		
Yes	12	2	16.67	2.587	0.55-11.99	0.225
Herd composition (*n*= 361)						
Mixed *spp*.	292	99	33.90	Ref		
Single *spp*.	69	21	30.43	0.353	0.02-0.80	0.022*
Ecological zones (*n*= 361)***						
EZ3	133	39	29.32	Ref		
EZ1	85	53	62.35	6.802	3.72-12.42	0.001
EZ2	143	28	19.58	1.704	0.97-2.97	0.061

n = Number of participants; CI = Confidence interval, Significant level < 0.05; OR=Odds ratio; *** = Significant at 0.05, considered for multivariate analysis; * = considered for multivariate analysis (cut-off p = <0.250); Ref = reference category.

4.3.6 Maximum likelihood estimates of risk factors for seropositivity of herds

A stepwise binary logistic regression model was used to determine variables that could be predisposing factors for RVFV sero-positivity of herds. Variables with a *p* value less than 0.250 in the bivariate analysis were included in the model. The test had Hosmer-Lemeshow goodness-of-fit statistic (*p*=0.952) and Omnibus Test of Model Coefficients value of (*p* <0.000) were obtained, indicating goodness of fit of the generated model. The risk factors were significant when <0.05, *p*-value. After adjustment for other variables in the model, 33.33%, of the variables were significant risk factors of RVFV sero-positivity for livestock at herd level. These were areas receiving about 1,001-1,500mm of rainfall, and herd composition and the adjusted Odds ratio (aOR) was presented in (Table 4-23). Livestock herds found in areas of rainfall amount of

>1,600mm were (aOR: 2.239, 95% CI= 1.07-8.82) times more likely to be seropositive to RVFV than those in areas of rainfall amount of <1,000mm at (p=0.023). Further, livestock herds in areas of rainfall amount of 1,001-1,500mm, were (aOR: 2.470, 95% CI= 1.14-5.37) times more likely to be seropositive to RVFV than those in areas of rainfall amount of <1,000mm at (p=0.022). Livestock herds that belonged to mixed species were (aOR: 10.410, 95% CI= 3.04-35.59) times more likely to be seropositive to RVFV than those managed under single species (p=0.001).

Table 4- 23: Summary of maximum likelihood estimates for risk factors associated with RVFV sero-positivity at livestock herds level

Variable	Level	aOR	95% CI	p-value
Rainfall (n=361)	<1,000mm	Ref		
	1,001-1,500mm	2.475	1.14-5.37	0.022***
	>1,600 mm	2.239	1.07-8.83	0.023***
Herd composition (n=361)	Single spp	Ref		
	Mixed spp	10.410	3.04-35.59	0.001***

*** = Significant at 0.05; aOR = adjusted Odds ratio; CI = Confidence interval; Significant level < 0.05; Ref = Reference category

4.4 Demonstration of the circulating RVFV

RNA investigation was conducted on 45 samples that reacted to IgM ELISA test. The samples were split according to districts and were screened for RVFV RNA.

4.4.1. Detection of RNA gene on L- segment using RT-qPCR

The RT-qPCR detected one (1) sample at cycle 36 out of the set 45 cycles. The sample was collected from male cattle in Chitipa district which belong to EZ3.

4.4.2. Detection of RNA gene by NSs coding region on S- segment using nested PCR

The nested PCR showed amplified band of the expected band size of 668 bp corresponding to RVFV sequence in the NSs coding region of the S segment of the genome. The test detected three (3) samples (one detected using RT-qPCR test and two more samples). The sample detected using RT-qPCR was run in duplicate on the electrophoresis gel; as positive control (P) and sample one (1), as presented by electrophoresis gel below (Figure 4- 2). The two more samples (sample 2 and sample 3) were collected from Chitipa (EZ3) and Chikwawa districts (EZ1). The sample 2 was

collected from a cow in Chitipa district while sample 3 was collected from a ewe in Chikwawa district.

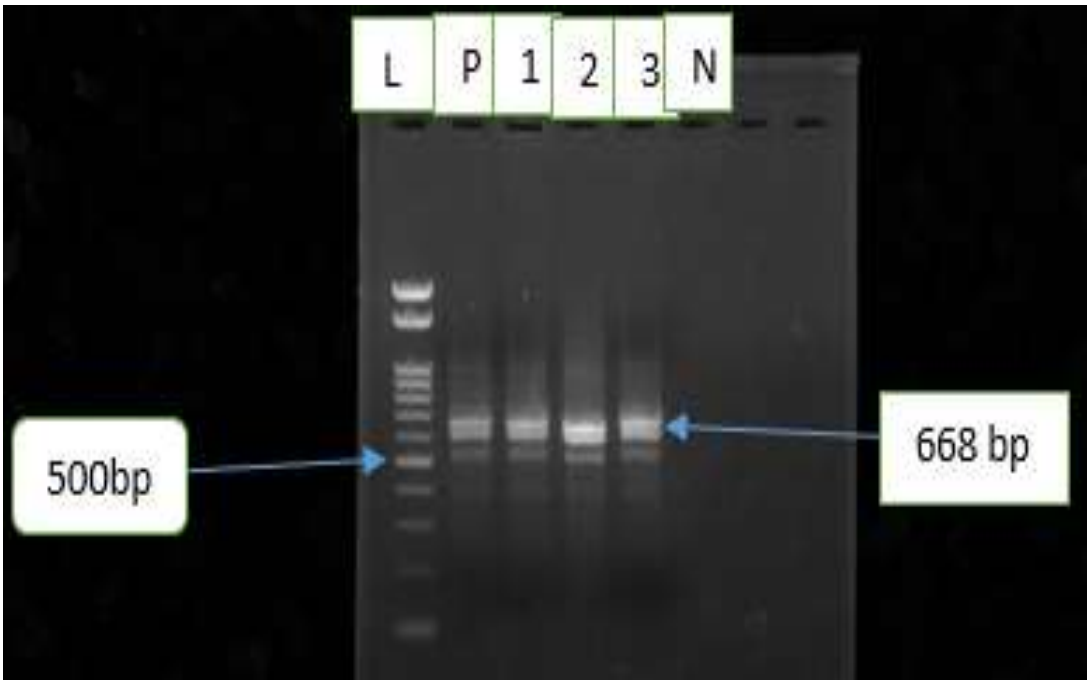

Figure 4- 1: NSs coding region of S-segment amplified from RVFV RNA extracted from serum samples.
L= ladder; P=positive control; 1= sample one, 2= sample two; 3= sample three; N= Nuclease Free water as negative control

CHAPTER FIVE

5.0 DISCUSSION

Good knowledge, attitudes and practices towards RVF amongst livestock farmers form a great foundation for infection control and prevention in areas at risk for RVF. The study aimed to assess livestock farmers' knowledge, attitude, and practices because of the critical role that knowledge plays in curbing the spread of viral hemorrhagic infectious diseases, including RVF (CDC, 2020). In addition, the understanding of the influence of management practices and attitude towards infectious diseases is crucial to improve prevention and control efforts.

Rift Valley fever is a re-emerging mosquito-borne viral hemorrhagic fever in Africa and the Arabian Peninsula, affecting humans and livestock. The current study revealed poor knowledge, negative attitude and poor management practices that were not satisfactory to suspect and report probable RVF cases. Consequently, it compromised the prevention and control efforts as previously reported by (Chengula et al., 2014).

The demographic information of the respondents included marital status, education, gender and age group. Gender and age are among the important demographic factors that could contribute to the knowledge, attitude and practices of farmers (Sarker et al., 2010; Seyed Abolhasan et al., 2010). By integrating gender and age in a sampling of the respondents, the response bias could be reduced and the findings could be easily generalized. Education opens for awareness and fosters a better understanding of conditions and topics in the production community. The positive influence of education was noted by the mean score of secondary and tertiary edication, as was observed in other studies (Tebug, et al., 2012; Chingala et al., 2017). Mean score for knowledge, attitude and practice for married farmers were higher compared to other categories (Table 4- 5), because livestock management was provided by family members. Family members endeavor to share information in order to get increased benefits from their farming in agreement with reports (Banda et al., 2012; Namgyal et al., 2021; Alemayehu et al.,2021).

The study established that few farmers knew about RVF, despite having unspecified sources of information (Table 4- 2; Table 4- 5). This level of awareness is believed to be resulted from a

previously suspected RVF outbreak (2006-2007), observed among smallholder dairy herds of Thyolo and Chiradzulu districts. Smallholder dairy farmers operate in Milk Bulking Groups (MBG), where they receive expert training on various livestock topics, including diseases, and share information and experiences among themselves. Certainly, MBG gatherings promoted awareness of RVF among livestock farmers. Nevertheless, the farmers had limited knowledge on RVF, causative agent, transmission, clinical signs, and host species, which are central pillars for describing the disease as previously reported by (Abdi et al., 2015; Monje et al., 2020). The observed basic and clinical knowledge of RVF was insufficient to enable farmers to suspect and report probable cases of RVF in livestock as previously reported (Chengula et al., 2014; Shabani et al., 2015). The current study found that farmers did not know that RVF was zoonotic infectious disease transmitted between animals and humans. Ng'ang'a et al. (2016), reported a lack of knowledge of zoonotic for RVF among pastoral communities in northern Kenya, a finding that is in agreement with the present study. The unsatisfactory knowledge of RVF as a zoonotic disease could be attributable to lack of knowledge of zoonotic among livestock farmers. This observation corroborated the results on management practices whereby participants handle aborted materials and remains of neonatal death without protection. These materials are potential risk factors for RVFV sero-positivity in humans and livestock, as reported by (Lernout et al., 2013).

The poor knowledge of farmers on the role of mosquitoes in the transmission of the disease draws particular concern as only a few of the participants knew that mosquito is the primary vector for transmission of RVF in livestock (Table 4- 2). Further, farmers did not know that human can contract RVF infection through mosquito bites and coming into contact with infectious aborted fetuses and other remains of neonatal death materials. Furthermore, participants were not aware that RVF is zoonotic which is a threat to human health which could be due to poor awareness. Many emerging diseases are zoonotic infectious diseases transmitted between animals and humans; examples include RVF (Owange et al., 2014). These observations showed that farmers would not practice preventive measures against these risk factors as previously reported (Abdi et al., 2015).

Poor management of potentially infectious materials was common as almost half of participants did not bury aborted materials and handled the aborted materials with unprotected hands, which

were practices of particular concern regarding RVF disease control and prevention. The possible risk of RVFV transmission in the study population could be assumed to be high considering deficiencies in management practices of aborted materials, dystocia and remains of neonatal death that were handled without protective equipment and sometimes incorrect disposal as alluded by (Tiongco et al., 2012; Alemayehu et al., 2021). This malpractice is common in areas where farmers have low knowledge of RVF as it was in Ijala district, Kenya (Owange et al., 2014).

Although not satisfactory, the participants had a reasonable management practice score (Table 4-3). The average practice score showed that the participants had potential to improve with enhanced awareness. Most of the management practices for RVF are similar for production practices and prevention to other diseases. It has been observed that farmers in participating districts allowed livestock to graze in communal grazing grounds with mixed- livestock species. Communal grazing and mixed-species grazing type accounted for intensive interaction of livestock herds of different villages. These interactions could potentially influence the spread of RVFV between livestock and possible spill-over to humans (Métras et al., 2020).

The observed attitudes of participants would be considered as a mediator between knowledge and practices and had a significant role in directing the choice of management practice. Attitudes support the process of changing individuals' behavior (Kabir, 2013). This was evident by the findings whereby cases of dystocia, abortion and neonatal death in livestock were perceived as a result of other diseases, misfortune or poor feeding (starvation and poisonous plants) similar to findings reported by (Baur et al., 2017a; 2017b). In addition, few farmers admitted losses caused by suspected cases of RVF on their farms. Failure to associate the cause of losses in livestock indicated ignorance that suggested weakness in RVF survaillane and could not facilitate capacity to suspect and report possible cases of RVF. Most farmers could not suspect RVF because they did not know the disease by name or provide a vernacular name and could not differentiate from other diseases with similar clinical signs such as brucellosis and bovine viral diarrhea (BVD) (McGowan et al., 1993; Azher, 2016). This suggested many diseases with similar clinical signs like RVF existed but were not confirmed and described. The foregoing information suggested that the occurrence of abortions could be attributed to other causes, RVFV inclusive. The poor attitude of farmers towards RVF was observed in failure to associate increased mosquito population, heavy

rainfall and flooding and increased abortions during the period of January, February and March as opposed to the frequency of abortions in April, May and June. Nevertheless, the attitude and the level of knowledge of livestock farmers towards RVF were not effective to suspect and report probable cases of RVF (Affognon et al., 2017) and were evident in the attitude and management practices of the majority of the farmers in the study area (Chengula et al., 2014).

Table 4-5 showed that adequate knowledge was observed in EZ2 districts, Thyolo and Chiradzulu, which would suggest that there were sporadic cases similar to the previous experience of suspected RVF cases (2006-2007) that sparked memory and sharing of information as previously reported (Abdi et al., 2015). Secondary and tertiary education coupled with age of greater than 45 years had better knowledge and attitude, subsequently, better practices of farmers. These observations may be perfect because education supports informed decisions while experience accounts for degree of familiarity with the presentations of the disease. Farmers with livestock less than 25 had better knowledge, attitude and practices, than farmers with livestock more than 25, as their main source of labor was family members. However, both groups of farmers (small herds < 25 livestock and large herds ≥ 25 livestock) indicated that they cannot prevent the disease may be because prevention of mosquito is likely to be cumbersome compared to the control of other external parasites like ticks. Further, having knowledge does not directly translate to capacity to prevent RVF, as previously reported (Affognon et al., 2017).

All viral hemorrhagic infectious diseases, RVF inclusive, require special attention in prevention and control (WHO, 2011). Conversely, this study reported associations between knowledge and observations under management practices and attitude categories that synergistically influenced negative attitude of farmers towards conducting good practices to prevent RVF. For instance, the association between the observed low knowledge with knowledge of clinical signs and causative agent of RVF was strong indicating that poor knowledge corroborated failure of farmers to find out the cause and clinical signs of RVF (Table 4- 6) (Monje et al., 2020). Another strong association was observed between low knowledge and ability to prevent RVF under practices is of particular concern (Table 4- 7). This association indicated the dependence between the low knowledge of RVF and inability to prevent it (Affognon et al., 2017). Additionally, there was association between low knowledge and the three observations under attitudes namely; RVF is not

a cause of neonatal death, RVF is not a cause of production losses and lack of fear of suffering from RVF. This association could indicate low awareness of the disease, consequently, limited efforts made towards detection of the disease.

Nevertheless, the study found two factors that influenced low knowledge of farmers on RVF (Table 4- 9). These were lack of knowledge on cause of abortion (misfortune) and lack of knowledge on transmission of RVFV. Misfortune in its context, blatantly obscure the inquisitive minds on the causes of abortion. On the other hand, poor feeding was not a best option to consider as the study was conducted in wet season when green grasses were in abundance. In addition, lack of knowledge on how RVFV is transmitted and the role of mosquitoes in the transmission of RVFV predisposes the farmers to the spread of RVFV among the livestock and/or spill-over to humans (Owange et al., 2014). These conducts could be influenced by lack of knowledge among the farmers and could not prompt the communities to suspect and report probable cases of RVF.

This sero-epidemiological investigation reports the detection of RVFV antibodies (IgG and IgM) in apparently healthy domestic ruminants and also evaluated the risk of exposure to the virus in livestock in three ecological zones of Malawi. Several RVF outbreaks in Africa and beyond have been documented in humans and livestock (Anyamba et al., 2018; Sumaye et al., 2019). The current study reports RVFV seroprevalence of 21.4% in cattle, 25.7% in sheep and 7.7% in goats with the overall seroprevalence of 17.1%, which is comparable to the previously reported 18.1% RVFV seroprevalence in cattle from Zomba district in Malawi in 1992 (Bryony, 1992). The detection of IgG and IgM in this study in livestock that had no clinical signs support the notion that RVF is endemic in sub-Saharan Africa countries in general, Malawi inclusive. The higher seroprevalence in sheep compared to cattle and goats was not a surprising since sheep has been reported to be more susceptible than the other two studied livestock species. These findings are in tandem with reports from the Democratic Republic of Congo, Tanzania and Chad where seroprevalence in sheep was higher than in other livestock (Ringot et al., 2004; Sindato et al., 2015; Tshilenge et al., 2019). The seroprevalence in sheep herds could indicate possible circulation of RVFV that caused the death of sheep without clinical signs which corroborated with prevailing fear among sheep farmers as previously reported (Kainga et al., 2022). Further, the odds of livestock being RVFV infected were lower with increased level of education and awareness.

Hence, the observed risk on livestock production could be minimized as demonstrated by binary regression findings that livestock of farmers with primary, secondary and tertiary education would be less likely to get the RVFV infection than livestock of farmers without formal education.

In addition, RVFV sero-positivity was detected across all age groups of ruminant species tested, with higher prevalence in sub-adults and old age groups than young age group (<2year). The high seroprevalence in sub-adults would be due to increased activity of the group members and their high proportions in the population. Higher seroprevalence in old age could be attributed to lower numbers of these age group in the population because most adult livestock were selected for slaughter except the breeding stock as previously reported (Tshilenge et al., 2018; Ngoshe et al., 2020). Similar influence of age could apply for seroprevalence observed in cattle compared to goats, given the fact that cattle live longer than goats (Ngoshe et al., 2020). RVF is usually less severe in cattle compared to goats and occurs as a subclinical infection in most adult susceptible and indigenous cattle, making them resistant to RVF as previously reported by Labeaud et al., (2008). Nevertheless, differences in seroprevalence between ruminant species were previously reported in other parts of the region, such as the Republic of South Africa (Ngoshe et al., 2020), Mozambique (Blomström et al., 2016), Tanzania (Sumaye et al., 2013; Sindato et al., 2015), Kenya (Lichoti et al., 2014) and Uganda (Nyakarahuka et al., 2018).

The overall difference in prevalence based on sex was reported in Chad (Ringot et al., 2004) and Madagascar (Jeanmaire et al., 2011) where the higher seroprevalence in males was attributed to their roles as draft and breeding animals. Similarly, the current study observed higher seroprevalence in male than female cattle, due to low participation of male compared to female livestock. It was learned that the overall prevailing breeding management strategy preserved cows for reproduction purposes than bulls, in a breeding ratio of about 1 bull to 15 cows or more. In areas dominated by indigenous cattle, they cull unwanted bulls while in dairy production districts, artificial insemination is the dominant means of breeding (DAHLD, 2004). Surprisingly, there was similar infection rate between male and female goats which could be a result of similar immunity capacity.

Higher seroprevalence was observed in EZ1 which had more permanent water bodies such as Lake Malawi and Shire River plus many tributaries to these two water bodies, with frequent reports of flooding thus providing some conducive environments for mosquito breeding. A similar observation was made along Lake Malawi in the Mbeya region, Tanzania (Heinrich et al., 2012). Variations in seroprevalence between districts could be due to multiple factors, among others are, climatic and environmental, as reported by (Morvan et al., 1991; La Beaud et al., 2008). Of note, the two districts of Salima and Mangochi that register high seroprevalence are along the permanent water bodies of Lake Malawi and Shire river, respectively.

Further, the detection of IgM antibodies suggested recent virus infections in the study area, as IgM antibodies to RVFV are usually detected up to two months after infection (Morvan et al., 1991, Paweska et al., 2003). The circulation of RVFV during the inter-epidemic period leaves open the possibility that clinical RVF cases may have occurred undetected, or may have been mistaken for other diseases as such were not reported due to lack of public awareness. The detection of IgG in other districts could be explained by increased increased water bodies for mosquito population growth as previously reported by (Heinrich et al., 2012). Further, cross-border movement could also contribute since some of the districts share boundaries with Tanzania, Mozambique and Zambia where RVF was reported by Tshilenge et al., (2018). Furthermore, herd size and mixed species herd composition could account for the high seroprevalence of IgG because the majority of livestock were under mixed species grazing grounds. Mixed species management in communal grounds accounted for intensive interaction of livestock herds of different villages due to scarcity of grazing grounds, for security reasons and to access breeding bulls with desirable traits, as seen in KAP study. Presumably, mixed species interaction were epicenters for increased frequency for the exposure to disposing factors such as contact with remains of neonatal death, after-birth materials, aborted/stillbirth materials (including fluids), and mosquito bites as observed in KAP study.

Most of the potential risk factors were components of the ecology. Hence, the three ecological zones presented potential risk factor that differently influenced the distribution of exposure both to individual livestock and herds levels. The significant risk factor for individual livestock was sex. Female livestock were at risk of RVFV infection than male livestock possibly due to higher

proportions in the community as previously reported by Nyakarahuka et al., (2018). At herd level, risk factors suggested that livestock herds living in areas with higher rainfall amounts (>1,000mm) were at risk of RVFV infection than livestock herds living in areas with low rainfall amount (<1,000mm), possibly due to increased favorable mosquito breeding habitats such as wetlands/dambos as previously observed in Nigeria, Kenya and Southern Africa (Owange et al., 2014; Glancey et al., 2015; Alhaji et al., 2020). In addition, livestock herds that belonged to mixed species were at risk of RVFV infection compared to livestock herds that belonged to single species. This could be due to increased exposure to RVFV predisposing factors such as contact with remains of neonatal death, after-birth materials, aborted/stillbirth materials (including fluids), and mosquito bites in communal grounds, as observed in the current study.

The study has presented seroprevalence of RVF in three ecological zones of Malawi, especially the selected districts that had high livestock population and diverse livestock management practices. The seroprevalence data generated from this study can be used in designing effective prevention and control strategies for a sustainable livestock industry at the national and regional level. However, it's imperative to investigate in mosquito vectors and humans to further improve our knowledge of the epidemiology of the disease.

RVFV genome was detected from three (3) out of forty- five (45) IgM ELISA positive samples, which was possible considering that the sampling was done at randomly conducted without targeting RVFV vireamic livestock. RVFV Vireamia in ruminants is short-lived of about 5 days (Faburay et al., 2016), making virus detection a challenge in cross-sectional sero-survey studies. Further, the quality of RNA would have been compromised during the process of running ELISA tests which involved multiple freezing and thawing, possibly causing degradation of RNA in the process. The poor quality of the RNA suggested use of high potent primer sets with very reliable high efficiency as previously described by (Sall et al., 2002) and are recommended for RVFV detection by OIE, (2018b).

Despite three samples demonstrated the RVFV genome, its evidence enough that RVF is present in the study area and should be considered as one of the livestock challenges. Interestingly, the observation of genome from two districts that are very far apart, suggested wide distribution of RVFV in Malawi. The demonstration of RVFV genome in livestock without clinical signs was also

reported in the Democratic Republic of Congo (Tshilenge et al., 2019). This suggested that there were more possible RVF cases that were not suspected, reported and subsequently, detected in the study area and in other parts of Malawi. These observations confirmed the common knowledge that RVF is endemic in Africa (WHO, 2012; 2021; Mariner et al., 2022) and is a potential global threat to livestock and public health, economy, and food security, thus, requires a "One Health" approach to the mitigation strategies in Malawi (Sumaye et al., 2013).

The findings of poor knowledge, poor management practices and negative attitude seen in KAP study were in tandem with the finding of high seroprevalence and the identified RVFV genome. This observation confirmed that the participants had no capacity to suspect and report probable cases of RVF as previously reported in Kenya (Owange et al., 2014). Ng'ang'a et al. (2016), reported a lack of knowledge of zoonotic for RVF among pastoral communities in northern Kenya, a finding that is in agreement with the present study. This suggested improving knowledge levels of farmers would subsequently improve attitude and practice, hence fostering the capacity to suspect and report probable cases of RVF. This suggested that currently there are socioeconomic challenges due to effects of RVF in livestock and most likely in humans as well, which are not quantified. However, for a good development progress in livestock industry requires the current policies and strategies on disease prevention and control to include measures to contain RVF. Livestock industry has a potential to transform economy of Malawi by contributing largely towards the national gross domestic products (GDP). This could produce enormous positive outcome to the economic growth of Malawi which has agro-based economy (Matchaya, et al., 2014).

The study encountered several challenges especially on random sampling. Bias could have occurred in areas where farmers declined to participate in the survey or refused to breed their animals. The next farm could have been selected based on convenience not far from originally selected position. The study tested IgG and IgM and not with virus neutralization test (VNT) which is a "gold standard" to account for false positive. The ideal was to test for IgG, IGM and VNT but due to budgetary constraint and time, it was not possible. Nevertheless, the PCR results showed presumptive evidence for the presence of the RVFV. The study experienced challenges of sequencing machines and associated supply of reagents as such the study failed to sequence the isolated genomes of the virus.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This study has revealed that participants of the study had low knowledge, negative attitude and poor practices that were not satisfactory to suspect and report probable cases of RVF. This study has demonstrated circulation of RVFV in livestock and has provides valuable evidence in relation to RVFV infection in livestock which has important livestock production implications. The study has established that sex was a risk factor at individual livestock level while mixed species and areas with rainfall amounts of > 1000mm were risk factors at herd level. The reported risk factors at individual and herd level are informative to the observed RVF seroprevalence, hence potential areas to improve prevention and control of the disease. The species specific sereprevalence suggested special attention to be made towards sheep production to improve its livestock census. The detection of RVFV genome across the study area confirmed the presence of the disease in Malawi.

6.2 Recommendations

To further understand the inter-epidemic RVFV circulation in livestock, mosquito vector and possibly in human populations, we should consider the following:

i. Raising community awareness on the risk of RVF is highly recommended among the members of livestock farmers and other communities at risk.
ii. Further entomological surveillance is needed to detect and/or isolate the virus in mosquitoes in the inter-epidemic period and typing of RVFV strains circulating in the area.
iii. Enhanced surveillance of livestock abortion events to detect the RVFV in each district and describe the circulating strain.
iv. Establishing the seroprevalence and typing the circulating strain in humans and wildlife to thoroughly describe the epidemiology of RVF in Malawi.

CHAPTER SEVEN

7.0 REFERENCE

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CHAPTER NINE

9.0 APPENDICES

Appendex 1: **KAP questionnaire for livestock farmers**

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

DIRECTORATE OF RESEARCH AND POSTGRADUATE STUDIES

Dear participants

I am a postgraduate student at the University of Zambia collecting information on " Epidemiological Investigation of Rift Valley fever in Cattle, Sheep and Goats in Malawi". This questionnaire wishes to collect knowledge, attitudes, and practices of livestock owners and its implication on the transmission of Rift Valley fever among livestock herds in Malawi. This study will be important in developing the outbreak response to cases of Rift Valley fever. Please be as honest as you can when answering the questionnaire. Your response will be kept confidential and will only be used for academic purposes.

Date:…… District ID:……Village name:…….....

A. Socio-demographic characteristics

i. Name of Enumerator.....

ii. What is name of the EPA.....

iii. What is the name of village.....

iv. What is the starting time of interview…….....

v. What is the name of the farmer.....

vi. What is gender of the farmer.....

vii. What is age of the farmer.....

viii. What is the education level of the farmer.....

Knowledge

1. Do you know what causes abortion in wet season yes ……or No……?
2. What are the associated factors for abortion? Yes….. or No……

1. Diseases…….
2. Misfortune……..
3. Poor feeding……

3. Do you know the disease RVF? Yes….. or No……
4. Do you know the host range? Yes….. or No……
5. Do you know the clinical signs? Yes….. or No……
6. Do you know what causes RVF? Yes….. or No……
7. Do you know how it is transmitted in livestock? Yes….. or No……
8. Do you think mosquito can transmit RVF?
9. Do you know that RVF is a zoonoses? Yes….. or No……
10. Do you know that you are at risk of getting RVF? Yes….. or No……

Management practices

11. Mode of night shelter
    i. Communal shelter……..
    ii. Private shelter…….
    iii. Houses……..
    iv. Along the road…….
    v. In crop field……
12. Type of night shelter
    i. Kraal…….
    ii. Houses/private……
    iii. Open places……..
13. Grazing type
    i. Communal grounds……
    ii. Zero grazing…….
14. Grazing site composition
    i. Mixed species……
    ii. Single species…….
15. Dystocia management
    i. Bare hands…….

ii. Use plastic……..

iii. Use gloves……..

16. Handling of aborted material

i. Bare hands……..

ii. Use plastics…….

17. Aborted material management

i. Not buried…….

ii. Buried……

18. Do you experience neonatal death? Yes….. or No……

19. Do you separate young and old livestock? Yes….. or No……

20. Do experience dystocia? Yes….. or No……

21. Can you suspect RVF in livestock ? Yes….. or No……

22. Can you prevent RVF in livestock ? Yes….. or No……

23. Can you report RVF probable cases to responsible authorities? Yes….. or No……

24.

Attitudes of farmers on RVF

25. What do you think of heavy rains and flooding towards occurrence of RVF

i. It promotes spread of RVF……

ii. It destroys crops and homes…….

26. How do you feel when mosquito population increases, do you fear spread of RVF? Yes or no

27. Is the presence of *Aedes* Spp draws any of your attention yes or no

28. What do you think when animals abort, is it RVF? Yes….. or No……

29. Do you think RVF causes abortion Yes….. or No……

30. Do you associate livestock interaction with infection transmission? Yes….. or No……

31. How do you feel when you experience neonatal death, could it be RVF? Yes… or No…

32. Do you feel like presence of the disease when dystocia occurs? Yes….. or No……

33. Do you think vendors can bring infected livestock into our village herd? Yes….. or No…

34. Do you think RVF can be prevented Yes….. or No……

35. Are you capable to prevent RVF in livestock? Yes….. or No……

36. Do you think RVF causes serious economic losses to your farm Yes….. or No……

37. Do you fear suffering from the disease? Yes….. or No……

THANKS VERY MUCH FOR YOUR PARTICIPATION

Appendex 2: Questionnaire for the risk factors of the RVFV positivity in three EZs in Malawi

THE UNIVERSITY OF ZAMBIA

SCHOOL OF VETERINARY MEDICINE

DIRECTORATE OF RESEARCH AND POSTGRADUATE STUDIES

Dear respondent

I am a postgraduate student at the University of Zambia collecting information on " Epidemiological Investigation of Rift Valley fever in Malawi". This questionnaire wishes to collect knowledge, attitudes, and practices of livestock owners and its implication on the transmission of Rift Valley fever disease among livestock herds in Malawi. This study will be important in informing the outbreak response to recent cases of Rift Valley fever. Please be as honest as you can when answering the questionnaire. Your response will be kept confidential and will only be used for academic purposes. You are not required to write your name on this questionnaire.

Date: District ID: Village name:

GPS ID:……..Flock ID:_____

1. Name of Enumerator.....
2. What is the Ecological Zone number.....
3. What is name of the EPA.....
4. What is the name of village.....
5. What is the starting time of questionnaire administration.....
6. What is the name of the farmer.....
7. What is gender of the farmer.....
8. What is the education level of the farmer.....
9. What is age of the famers……..
10. What is the primary occupation of the farmer.....

    Livestock Crops Fisheries

11. What is the status of vegetative cover…….
12. Do you or your household own domestic livestock Yes or No
13. What species of livestock do you have Goat Sheep Cattle Chicken

    Other, specify.....

14. How many livestock do you have Cattle..... Sheep..... Goat.....

15. Do you have a night house/shelter for livestock Yes or No

16. If yes, what is the type and condition of khola Concrete or Muddy

17. How do you feed/ graze your livestock

Within shelter Isolated dambo land Communal grounds

18. Do you mix your multiple species during grazing or sheltering

Yes or No

19. How many villages mix their livestock with your herd.....

20. What is the management system of your livestock within a year

Fixed-no travelling for grazing Mobile (free grazing) / Transhumans

21. If mobile type of flock, which places Maize fields only Dambo lands Higher lands (ku mapiri) Others .....

22. Do you do routine management to your livestock..... Yes or No

If yes, Which months

Do you have heavy rains and flooding this season Yes or No

23. In the past year, have you witnessed livestock (cattle, sheep, and goats) having difficulties to give birth Yes or No

24. In the past year have you witnessed abortion or still-births in the area for livestock (cattle, sheep, and goats) Yes or No

25. What are common diseases in your area? List six

26. Have you heard about Rift Valley Fever disease Yes or No

27. If yes, from whom

i. Health worker
ii. Radio
iii. Community leader
iv. Animal Health Workers
v. Others .....

28. What are the species of livestock affected Goat Sheep Cattle Chicken

Other, specify.....

29. Do you know the clinical signs and symptoms of RVF disease in livestock Yes or No

30. If yes, what are some signs and symptoms of RVF in livestock Bleeding Fever Nasal discharge Diarrhea Abortion Reduced milk production Prostration

31. Do you think your livestock can suffer RVF Yes or No

32. Do you know how RVF disease is transmitted among livestock Yes or No

33. If yes, which ones

    i. Mosquito bite

    ii. Contact with infected livestock tissue

    iii. Contact with infected fluids and fomites

    iv. Blowing wind

    v. Running water

34. Do you think your livestock are at risk of contracting RVF disease Yes or No

35. If no, why.....

.....

36. If yes , why.....

.....

37. Do you know how RVF disease can be prevented in Livestock Yes or No

38. If yes, how.....

.....

39. Do you know that people also suffer RVF disease Yes or No

40. Do you know how people can contract RVF disease Yes or No

41. If yes, how.....

.....

42. Do you know the signs and symptoms of RVF virus disease in humans Yes No

43. How do you think you can protect yourself from acquiring RVF disease?

    i. Vaccination

    ii. Avoiding contact with sick livestock

    iii. Traditional medicine

    iv. Avoiding sick people

    v. Sleeping in a mosquito net

44. How do you think RVF disease can best be treated

i. Spiritual healing

ii. Traditional medicine

iii. Modern medicine

iv. Herbal medicine

45. Do you think you are at risk of contracting RVF virus disease Yes or No

46. Do you know how RVF disease can be prevented Yes or No

47. If yes, how.....

.....

48. How many cases of retained placenta death in your herd from last rainy season…….

49. How many cases of neonatal death in your herd from last rainy season.....

50. How many cases of abortion in your herd during the rainy season.....

51. What happened to the aborted materials

i. Collected by Vet

ii. Buried

iii. Eaten

iv. Fed to other animals

52. How many cases of dystocia in your herd during the rainy season

53. How do you access veterinary extension service

i. From within the village

ii. From outside the village

54. Do you have transfer in of livestock to your herd Yes or No

55. If yes, where do they come from.....

56. Do you have transfer in of livestock into village Yes or No

57. If yes, where do they come from.....

58. Where is your nearest livestock market (distance in KM.....

59. How frequent in a month Once Twice Thrice Several

60. Who is responsible for certifying livestock before selling in your village

.....

61. What happens to a sick livestock at the market

.....

62. Do you have livestock health service providers in the area Yes or No

63. If yes how many .....

64. Do you keep records of diseases of your livestock. Yes or No

THANK YOU VERY MUCH FOR YOUR ACCEPTANCE AND PARTICIPATION

Appendex 3: Farmer participants` information sheet and consent form

Title of Study: Epidemiological Investigation of Rift Valley fever in Cattle, Sheep and Goats in Malawi

Principal Investigator: Dr. Henson Kainga
Co-Principal Investigators: Dr Ngonda Saasa and Dr Edgar Simulundu

The study shall sample at random livestock from village livestock committees register book. Then, follow up with the owner who shall by default requested to participate in the study following the below outline.

We are conducting a study on Rift Valley fever (RVF) in Cattle, Sheep and Goats in Malawi. The study involves selecting livestock to screen for the virus of RVF and the owner of the selected livestock also requested to share at will information and knowledge of the disease in this area. Therefore, you have been chosen as one of the study participants and I would like to ask you for some of your time to explain the work that we are doing and to request for you to be one of the study subjects. As we discuss the information below, please feel free to ask any questions.

Brief description of the Study

RVF is a disease of mainly ruminant animals and humans. It is caused by a virus which is transmitted by mosquitoes. Mostly, the disease outbreaks are associated with heavy rainfall and floods. These outbreaks are characterized by storm abortions in livestock and death of young livestock. The disease is prevented by vaccination in livestock but there is no treatment in both humans and livestock.

For this study we are interested in RVF virus from Cattle, Sheep and Goats and Mosquitoes managed in ecological zones that slightly differ in characteristics which favor the spread of the disease. The information gained from this study will be used to know if there is circulation of the virus, its molecular information, risk factors that contribute to transmission, spatial distribution and ecological niche model of RVF.

If you agree to take part in this study, we will ask a number of questions concerning you and your livestock herd. You may choose not to answer any question.

In addition, you will be asked if you will allow us to collect blood samples from your animals through jugular vein as part of the study. The blood will be used to screen and isolate the virus only.

If you do not join the study, you will not be victimised in any way. You may choose to leave the study at any time you like.
This exercise will take about 20-30 minutes. The questions that will be asked are general and not personal. There should be no risks to you if you agree to take part in the study.

You will not directly benefit from this study. The information we collect will be used to try to stop the virus from being passed from mosquito to animals and then protect people from the disease. ***THERE IS NO MONEY TO BE PAID TO YOU BY PARTICIPATION***. In an event that, your livestock get injured in the process of sample collection, free proper treatment shall be provided.

The information that will be collected in this study will be confidential and will be used in academic manner only and not necessarily linked to particular individuals.
Once I/ we have completed the testing for this study, we shall provide feedback and we may store blood sample for testing in the future. These tests would be done if new strains of virus are identified or if new tests become available. The sample will not have a name on it so that it cannot be linked to you.

If you have questions, complaints, or problems as a result of participating in this study, you may call Henson Kainga (+265 882 675 428 / 0996 618 212 or email: kaingahenson@gmail.com. You can also consult the Deputy Director of Veterinary Research and Investigation (Dr. Julius Chulu) on +265 991277492, Department of Animal Health and Livestock Development in Ministry of Agriculture and Water Development, Republic of Malawi.

What does your signature (or thumbprint/mark) on this consent form mean?

Your signature (**or thumbprint/mark)** on this form means:

- You have been informed about this study's purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to participate in this study.

Please indicate Yes or No

- I agree to allow my livestock to participate in this study ☐Yes ☐No

- I agree to be interviewed and allow data from my questionnaire to be used in the study ☐Yes ☐No

Name of participant: _____

_____ _____

Signature of participant or Thumb print Date

In signing here, I agree that I have read and understood the agreement/consent form and agree to participate in the study.

Name of Witness: _____

_____ _____

Signature of Witness Date

_____ _____

Signature of Enumerator Date

Appendex 4: Chichewa version of consent form

Gwirizano Wotenga mbari mu Kafukufuku wa Matenda a Rift Valley Fever

Mutu wa Kafukufuku: Kafukufuku wa Matenda a Rift Valley fever mu Ng`ombe, Nkhosa ndi Mbuzi ku Malawi

Ofufunza wa nkulu: Dr. Henson Kainga
Othandizira kafukufuku: Dr Ngonda Saasa ndi Dr Edgar Simulundu

Tikuchita kafukufuku wa matenda a Rift Valley fever mu Ng`ombe, Nkhosa ndi Mbuzi ku Malawi. Kafukufuku ameneyu ndiwopima ziweto ngati zili ndi kachirombo koyambitsa matenda amenewa. Ndipo pamene chiweto chasakhidwa kuti chitenge nawo gawo mukafukufuku ameneyi zikutathawuza kuti eni chiwetocho nawo asakhidwa kutenga nawo gawo. Kotero, inu mwasakhidwa kutenga nawo gawo mu kafukufuku ameneyi ndipo mokupephani, chonde timpatseni mpata okuti ticheze nanu za kafukufuku ameneyi. Pamene tikhala tikukambirana zina ndi zina, mukupephedwa kukhala omasuka.

Kafukufuyi mwachidule

Rift Valley fever ndi Matenda a Ng`ombe, Nkhosa ndi Mbuzi komanso anthu. Amayamba ndi kachirombo kosawoneka ndi maso ka mtundu wa virus kamene kamafalitsidwa ndi udzudzu. Matenda amenewa amakonda kugwa pamene kwagwa mvula yambiri ndi kusefukira kwa madzi. Zizindikiro zazikulu za matenda amenewa ndi kupoloza ndi kufa kwa ana aziweto. Matenda amenewa ali ndi katemera koma alibe mankhwala.

Kafukufuku amaneyi ali ndi cholinga chodziwa za kachirombo kamene kamayambita matenda a Rift Valley fever muziweto ndipo kamapezekanso mu udzudzu maka maka zimene zikupezeka mu madera amene kachirombo kameneka kamakonda kupezeka. Zotsatira za kafukufuku ameneyi zithandiza kudziwa za kupezeka ndi mitundu ya kachirombo kameneka kuno ku Malawi, komanso

tidziwa zithu zopititsa pa tsogolo kufala kwa kachiromboka, ndiponso tipeza njira yodziwira mmene kachiromboka kamafalira mu madera osiyana siyana.

Mukavomera kutenga nawo gawo mukafukufuku ameneyi, ticheza nanu pokufunsani mafunso okhudza inuyo ndi ziweto zanu.

Komanso, tikupemphani ngati tikatenge nawo magazi muchiweto chanu ngati mbari imodzi ya kafukufuku ameneyi chifukwa kachiromboka kamapezeka mumagazi aziweto.

Dziwani kuti, ngati muti mukane palibe vuto lililonse limene litakugwereni ngati chotsatira cha chisankho chanu. Komanso, ngati simukusangalatsidwa ndi kafukufukuyi mutha kusiya.

Ticheza kwa nthawi yosachepera theka ya ola ndipo mafunso ake siapatali kapena aupandu. Ndipo zimene tikambirane tizisunga mwa chinsisi komanso tikadzigwiritsa tchito pa za maphunziro ndi chitukuko cha ziweto mdziko muno basi.

Palibe mphindu lililonse kwa inu ngati munthu kuchokera ku macheza amenewa. Koma kafukufuku athandiza kuchepetsa kufala kwa kachirombo kameneka kuchoka ku udzudzu kupita kuziweto zimene nthawi zina zimatha kufalitsa matenda kwa anthu.

Tikadzamaliza kafukufuku ameneyi, zotsatira mudzawuzidwa komanso magazi a ziweto atha ku kusungidwa kuti tidzapitilize kafukufuku patsogolo pomwe mtundu wa kachirombo tadzapezeke wina kapena tikadzapeza zipangizo zamakono zogwilira tchitoyi.

Ngati muli ndi funso kapena ndemanga mwina vuto chifukwa chotenga nawo gawo mu kafukufuku ameneyi adziwitseni a Henson Kainga (+265 882 675 428 / 0996 618 212 or email: kaingahenson@gmail.com). Komanso ufulu wanu mu kafukufuku ameneyi ukulimbikitsidwa ndi Wachiwiri oyanganira kafukufuku wokhudza ziweto mu unduna wa zamalimidwe ndi chitukuko cha madzi pa on +265 991277492.

Kodi kusayinira kwanu kapena kudinda chala zikuyimira chani?

Chala chanu kapena sayini yanu ikutathawuza:

- Mwadziwitsidwa bwino za kafukufuku ameneyi
- Munapatsidwa mwayi ofunsa mafunso
- Mwapanga chisankho nokha opanda kukakamizidwa kutenga nawo gawo mu kafukufukuyi

Onetsani kuvomera kepena kukana

- Ndavomera kuti chiweto changa chitenge nawo gawo mu kafukufukuyi ☐Eya ☐Ayi

- Ndavomera kuti ndiyakha mafunso anu ☐Eya ☐Ayi

Dzina la Mlimi: _____

_____ _____

Sayini ya Mlimi kapena ku dinda chala Tsiku

Posayinira apa, ndichizindikiro chogwirizana ndi chimvano chotenga mbari mu kafukufuku wa matenda a Rift Valley fever mu Ng`ombe, Nkhosa and Mbuzi.

Dzina la Mboni: _____

_____ _____

Sayini ya Mboni Date

_____ _____

Sayini ya ofunsa Date

Appendex 5: Study ethical clearance and study site authorization by DAHLD

Government of Malawi

Tel: 01 766341
01 766348
Fax: 01 751349

Ministry of Agriculture & Food Security,
Department of Animal Health and
Livestock Development,
P. O. Box 2096,
LILONGWE - MALAWI

Ref: DAHLD/AHC/10/2019

23rd October, 2019.

Dr. Henson Kainga

Department of Disease Control

School of Veterinary Medicine,

University of Zambia

Dear, Henson Kainga

RE: ETHICAL CLEARANCE

Following the meeting held on 10th October, 2019 to evaluate your documents for ethical clearance, the Veterinary Board has no objection to your study. The Department of Animal Health and Livestock Development (DAHLD) was instructed to issue Ethical Clearance for your study titled "Epidemiological Investigations of Rift Valley fever in Cattle, Sheep and Goat in Malawi".

Veterinary Board and DAHLD wish you all the best as you will be conducting the study.

Yours sincerely

Dr. Patrick Chikungwa
Director, Department of Animal Health and Livestock Development

Cc: Deputy Director field Services
Deputy Director Research and Investigation
Training Coordinator

Appendex 6: Ethical clearance by UNZABREC

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

6th February 2020.

Your REF. No. 617-2019.

Dr. Henson Kainga,
University of Zambia,
School of Veterinary Medicine,
P. O Box 32379,
Lusaka.

Dear Dr. Kainga,

RE: "EPIDEMIOLOGICAL INVESTIGATIONS OF RIFT VALLEY FEVER IN CATTLE, SHEEP AND GOATS IN MALAWI" (REF. NO. 617-2019)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee Meeting on 4th February, 2020. The proposal is **approved**. The approval is based on the following documents that were submitted for review:

a) **Study proposal**
b) **Questionnaires**
c) **Participant Consent Form**

APPROVAL NUMBER : REF. 617-2019

This number should be used on all correspondence, consent forms and documents as appropriate.

- **APPROVAL DATE : 4th February 2020**
- **TYPE OF APPROVAL : Standard**
- **EXPIRATION DATE OF APPROVAL : 3rd February 2021**
  After this date, this project may only continue upon renewal. For purposes of renewal, a progress report on a standard form obtainable from the UNZABREC Offices should be submitted one month before the expiration date for continuing review.
- **SERIOUS ADVERSE EVENT REPORTING**: All SAEs and any other serious challenges/problems having to do with participant welfare, participant safety and study integrity must be reported to UNZABREC within 3 working days using standard forms obtainable from UNZABREC.
- **MODIFICATIONS**: Prior UNZABREC approval using standard forms obtainable from the UNZABREC Offices is required before implementing any changes in the Protocol (including changes in the consent documents).
- **TERMINATION OF STUDY**: On termination of a study, a report must be submitted to the UNZABREC using standard forms obtainable from the UNZABREC Offices.