DETECTION OF RIFT VALLEY FEVER VIRUS INTEREPIDEMIC ACTIVITY IN LOWER MOSHI AREA OF KILIMANJARO REGION, NORTHEASTERN TANZANIA: A COMMUNITY SURVEY

Medard Sostenes Kumalija

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master's in Life Sciences of the Nelson Mandela Institution of Science and Technology

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ABSTRACT

Rift Valley fever virus (RVFV) is a zoonotic viral disease of public health importance that affects livestock, wildlife and humans, mainly in sub-Saharan Africa. Despite its public health importance, the ways in which RVFVs circulate during the inter-epidemics (IEPs) stages and potentially spread to new areas remain unclear. The IEPs are the period during which transmissions pass without being detected, whereas surveillance is not performed. This study aimed to compare the rates of exposure to RVFV and RVFV infection among humans, goats and mosquito vectors in an agricultural and pastoral community in the lower Moshi area of the Moshi rural district. The area is located along the Great Rift Valley and is characterized by animal maintenance and heavy seasonal rains, making it swampy and favoring mosquito reproduction throughout. Although the spread of RVFV by mosquito vectors to animals and humans during interepidemic periods has been established, less is known about the roles that animals, humans, and vector mosquitoes play in maintaining the virus during IEPs. The reservoirs in the IEPs have become an area of interest for research. Therefore, a cross-sectional study survey was conducted in Lower Moshi in the Kilimanjaro region from June to December 2020. The RVFV exposure was determined via the detection of IgG/IgM to RVFV via a competitive enzyme-linked immunosorbent assay (cELISA), whereas infection was determined via real-time quantitative polymerase chain reaction (RT–qPCR). In humans, male sex was more related to RVFV seropositivity than female sex was ($\chi^2 = 5.351$; p=0.030). People aged 50 years and above were seropositive (χ^2 =14.430; p=0.006). The RVFV seropositivity in goats was related to increased seropositivity in humans (χ^2 =6.003; p=0.021, χ^2 =23.213; p=0.000 and 27.053; p=0.000). The RVFV antibody concentrations were only marginally higher in humans than in goats [t (112) =0.526; p=0.60)]. The RT–qPCR revealed that goats presented the highest RVFV infection rate (4.1%), followed by humans (2.6%), *Aedes aegypti* (2.3%), and *the Culex pipiens* complex (1.5%). Similarly, a greater proportion of goats (23.3%) were RVFV seropositive than were humans (13.2%). These findings suggest that northeastern Tanzania may serve as a persistent hotspot for RVF transmission and call for the implementation of integrated, health-based active surveillance strategies targeting livestock, humans and vectors. Enhanced diagnostics, targeted vaccination and climate-informed risk mapping are recommended to enable timely interventions and reduce the likelihood of future RVF outbreaks.

DECLARATION

I, Medard Sostenes Kumalija, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my original work and that it has neither been submitted nor concurrently submitted for degree awards at any other institution.

Medard Sostenes Kumalija Date

The above declaration is confirmed by:

Dr. John-Mary Vianney Date

Prof. Jaffu Othniel Chilongola Date

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance and approval by the Senate of the Nelson Mandela African Institution of Science and Technology a dissertation titled: "*Detection of Rift Valley Fever Virus Interepidemic Activity in Lower Moshi area of Kilimanjaro Region, Northeastern Tanzania*" submitted in partial fulfilment of the requirements for the Award of the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology, Arusha Tanzania.

Dr. John-Mary Vianney Date

Prof. Jaffu Othniel Chilongola Date

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DEDICATION

This work is dedicated to my family, staff mates and friends, who have always been there for support throughout my study.

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LIST OF ABBREVIATIONS AND SYMBOLS

cELISA	Competitive ELISA
IEPs	Inter epidemic Periods
IgG	Immunoglobulin G
IgM	Immunoglobulin M
RNA	Ribonucleic acid
RT–qPCR	Real Time quantitative Polymerase Chain Reaction
RVF	Rift Valley Fever
RVFV	Rift Valley Fever Virus

CHAPTER ONE

INTRODUCTION

1.1 Background of the Problem

The Rift Valley fever virus (RVFV) is a member of the genus Phlebovirus in the family Bunyaviridae. It is a zoonotic arbovirus disease affecting livestock and humans, mainly in Africa (Georges *et al*., 2018; Gudo *et al*., 2016). Reports indicate the presence of RVF in other parts of the world such as the Great Britain (Gale *et al*., 2015). According to the World Health Organization (WHO, 2020) and Tanzania, RVF is among the prioritized diseases due to its significant public health impression in areas where it occurs and the scarce intrusions to control it (Sanderson *et al*., 2020). The virus is also thought to be an important risk to livestock production in African countries, including Tanzania (Fawzy & Helmy, 2019). The RVFV spreads to faunas, including humans, mostly through bites by infested *Aedes* and *Culex* mosquitoes and direct interactions with the tissues of RVFV disease-ridden animals (Bashir *et al*., 2019).

Conservation of the virus in animals during interepidemic periods (IEPs) is still a challenge; thus, the spread of the virus to novel zones through animal movement may require special attention to control it. Disease pathology and endemic maintenance within mammalian hosts have been observed (Balkhy & Memish, 2003; Pépin, 2011). Although the spread of RVFV by mosquito vectors to animals and humans during IEPs has been established, less is known about the role that animals, humans and vector mosquitoes play in maintaining the virus during IEPs.

The reservoirs associated with IEPs and their characteristics have become potential areas of interest for research. Some clarifications have been made concerning the likely reservoirs by which the virus is preserved during IEPs. For example, RVFV reservoirs are suggested to include mosquitoes, livestock and humans (Lorenzo *et al*., 2015; Sissoko *et al*., 2009). This phenomenon has been revealed in previous studies, where as stated in previous studies, most people infected by RVFV show no symptoms (Sumaye, 2013).

Additionally, reports have theorized mechanisms for the subsistence of RVFV during long interepizootic stages, such as vertical transmission through mosquito progenies to mosquito descendants (Lorenzo *et al*., 2015; Terasaki & Makino, 2015). Although the spread of RVFV by mosquito vectors to animals and humans during outbreaks has been extensively explored (Sumaye *et al.*, 2013), less is known about whether humans, animals or vector mosquitoes

maintain the virus during IEPs. Similarly, RVFV transmission depends on various factors, such as excessive rains, high animal density and temperatures that sustain vector progression and pathogen replication (Breiman *et al*., 2008).

However, the exposure of RVFV in high-risk agricultural and pastoralist communities in northern Tanzania during the IEPs has been inadequately scrutinized despite their ability to support virus transmission. The current study aimed to examine exposure to RVFV and RVFV infection among humans, goats and mosquitoes in an agropastoral community in the lower Moshi area of the Moshi rural district during IEPs.

1.2 Statement of the Problem

Available serological evidence suggests that, across many countries and Africa in particular, RVFV continues to circulate in livestock and humans between large epidemics, that is, interepidemic periods. The RVFV and transmission depend on various factors, such as excessive strains, animal density and temperatures that sustain vector progress and pathogen replication (Anyamba *et al*., 2009); however, little is known about whether humans, animals, or vector mosquitoes maintain virus transmission during IEP transmission in potential areas, such as northern Tanzania, where repeated large outbreaks have occurred (De Glanville *et al*., 2022).

In Tanzania, limited RVF cases have been reported during the pandemic; however, it has been assumed that RVFV transmission may continue undetected (Georges *et al*., 2018). Lower Moshi, Kilimanjaro region in Tanzania, is the area that has all potential characteristics for RVF endemicity, such as its location along the Great East African Rift Valley, livestock keeping, seasonal heavy rains and irrigation agriculture (Mweya *et al.,* 2015; Nanyingi *et al*., 2015). Therefore, the present study was carried out to determine the exposure of RVFV in livestock (goats) and humans, the presence of RVFV in vector mosquitoes and the risk factors for infections among the agropastoral community in the Lower Moshi area during the interepidemic period.

1.3 Rationale of the Study

According to the World Health Organization (WHO) and Tanzania, RVF is among the prioritized zoonotic diseases because of its significant public health impression in areas where it occurs and scarce intrusions to control it. The virus is also thought to be an important risk to

agronomy in African countries, including Tanzania (Fawzy & Helmy, 2019). The RVFV spreads to faunas, including humans, mostly through bites by infested *Aedes* and *Culex* mosquitoes and direct interactions with the tissues of RVFV disease-ridden animals (Bashir *et al.*, 2019). In Tanzania, limited RVF cases have been reported during the pandemic; however, it has been assumed that RVFV transmission may continue undetected (Georges *et al*., 2018).

The lower Moshi, Kilimanjaro region in Tanzania, is an area that has all potential characteristics for RVF endemicity, such as its location along the great east African Rift Valley, livestock keeping, seasonal heavy rains and irrigation agriculture (Mweya *et al.*, 2015; Nanyingi *et al*., 2015). However, exposure to RVFV in high-risk agricultural and pastoralist communities in northern Tanzania during IEPs has not been adequately scrutinized despite having characteristics that support virus transmission. The present study aimed to examine exposure to RVFV and RVFV infection among humans, goats and mosquitoes in an agropastoral community in the lower Moshi area of the Moshi rural district during IEPs.

1.4 Research Objectives

1.4.1 General Objective

To determine the seroprevalence of RVF and the type of host that causes RVFV during interepidemic periods in Lower Moshi, Kilimanjaro Region, for appropriate management of the disease in humans and goats.

1.4.2 Specific Objectives

(i) To determine the prevalence of antibodies to RVFV in humans in Lower Moshi, Kilimanjaro region, Tanzania.

(ii) To determine the prevalence of antibodies to RVFV in goats in Lower Moshi, Kilimanjaro region, Tanzania.

(iii) To determine the RVFV infection rate in mosquito vectors in Lower Moshi, Kilimanjaro region, Tanzania.

(iv) To assess the risk factors for exposure to RVFV during interepidemic periods in Lower Moshi, Kilimanjaro region, Tanzania.

1.5 Research Questions

(i) What is the prevalence of antibodies against RVFV in humans in Lower Moshi, Kilimanjaro Region, Tanzania?

(ii) What is the prevalence of antibodies against RVFV in goats in Lower Moshi, Kilimanjaro region, Tanzania?

(iii) What is the infection rate of RVFV in mosquito vectors in Lower Moshi, Kilimanjaro region Tanzania?

(iv) What are the risk factors for RVFV among humans in Lower Moshi, Kilimanjaro region, Tanzania?

1.6 Significance of the Study

This study identified potential hot spots for RVFV transmission during interepidemic periods and revealed that there is a possibility that humans and livestock are exposed to RVFV during interepidemic periods in relation to vector mosquito infection rates. These findings suggest that the interepidemic period is the best timing for RFV control surveillance. These findings provide important information to the scientific community with interest in RVFV studies from a One Health perspective. The knowledge that RVFV is maintained in hosts during epidemics may help with intervention strategies to control and prevent future epidemics.

1.7 Delineation of the Study

This was a longitudinal study across seasons. To achieve the study's main aim of assessing the degree of exposure to Rift Valley Fever Virus (RVFV) among goats and humans, as well as isolating RVFV from humans, goats and mosquitoes, a combination of Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR) techniques were employed. The ELISA was used to detect RVFV-specific IgG and IgM antibodies in serum samples collected from both humans and goats, where the presence of IgG antibodies indicated past exposure and IgM antibodies signified recent infection. This serological approach facilitated the estimation of seroprevalence and helped determine the level of RVFV exposure during inter-epidemic periods (IEPs) in the agro-pastoral community of Lower Moshi, Moshi Rural District. In parallel, Reverse Transcription PCR (RT-PCR) was applied to detect RVFV RNA in blood samples from humans and goats, as well as in pooled mosquito samples collected

from the study area. This molecular technique enabled the identification of active infections and supported attempts to isolate the virus. Additionally, mosquito samples that tested positive by PCR were further analyzed to determine species identity, with a specific focus on Aedes spp. and Culex spp., to assess their potential role as key vectors in the transmission and maintenance of RVFV in the environment.

CHAPTER TWO

LITERATURE REVIEW

2.1 Disease Overview

Rift Valley fever (RVF) is an acute viral disease that can cause severe disease in domestic animals (such as buffalo, camels, cattle, goats and sheep) (Gerdes, 2004). The disease is characterized by fever, severe illness, abortions and high morbidity and mortality rates (Bird & Nichol, 2012). The virus that causes RVF belongs to the genus Phlebovirus in the family Bunyaviridae. Many related Bunyaviridae viruses can cause fever and encephalitis (Park *et al*., 2018). Another commonly known Bunyaviridae virus is Hantavirus (Anyamba *et al*., 2009), which is also zoonotic.

Although epizootic Rift Valley fever (RVF) has occurred in sub-Saharan Africa, it has occurred more than four times in East Africa in the last 30 years (Lumley *et al*., 2017). Widespread, frequent, and heavy rainfall has been a feature of these epizootic periods (Himeidan *et al.,* 2014). Heavy rainfall increases the level of the water table in certain areas, which results in flooding of the grassland, which is the habitat of the immature forms of breeding mosquitoes, including the genus Aedes (Mondet *et al*., 2005). The RVF virus is probably transmitted transovatically in Aedes species under these damp conditions (Chamchod *et al*., 2014).

2.2 Etiology

Bunyaviridae is a large family of viruses and contains five genera, four of which infect vertebrates, whereas the remaining genus, *Tospovirus*, contains a group of plant viruses (Ikegami & Makino, 2011). Three of the genera, *Bunyavirus, Phlebovirus* and *Nairovirus,* are associated with vertebrates, whereas the last genus, *Hantavirus*, has no known invertebrate association (Girma *et al.,* 2011). The RVF virus is a typical member of the *Bunyaviridae* family of the genus *Phlebovirus* (Coetzer & Barnard, 1977). The RVFV is an enveloped spherical virus approximately 120 nm in diameter, with short glycoprotein spikes projecting through a bilayer lipid envelope (Bird *et al.*, 2009). The single-stranded ribonucleic acid (RNA) genome is divided into three segments each in its own nucleocapsid; these segments are the large (L), medium (M) and small (S) segments, which express many copies of the N (nucleocapsid) and few copies of the L (transcriptase) structural proteins (Gerdes, 2004).

2.3 Epidemiology

The occurrence of RVF can be endemic and/or epidemic depending on the climatic and ecological characteristics of different geographic regions. In the high-rainfall forest zones of coastal, eastern and central Africa, RVF is reported to occur in endemic cycles, which are poorly understood (Sindato *et al.*, 2011). Virus activity is often detected either by serological studies in susceptible animals, the appearance of sporadic human disease cases, or fortuitous virus isolation (Wandinger *et al*., 2011). Currently, available evidence suggests that RVF epidemics may occur annually if there is heavy rainfall or may occur at least every two or three years in the absence of heavy rainfall (Kanouté *et al.,* 2017).

Interestingly, the disease is endemic in some semiarid zones, such as northern Senegal (Conley *et al*., 2014), indicating that not only heavy rainfall, which enhances its spread but also spread, can be enhanced under various conditions, such as in plateau grasslands with relatively high rainfall, such as East Africa (Himeidan *et al*., 2014), semiarid zones (Saudi Arabia, West Africa) (Nanyingi *et al*., 2015) and irrigated zones (Egypt, Yemen) (Kenawy *et al.,* 2018). In these areas, RVF epidemics appear up to 5-15-year cycles, and the generation of epidemics seems to be associated with the simultaneous intensification of virus activities over the vast livestock areas where it is already present as cryptic endemic foci (Budodo *et al.,* 2020). Historical information has shown that pronounced periods of RVF virus activity in East Africa occur during periods of heavy, widespread and persistent rainfall associated with El Niño events (Martin *et al.,* 2008).

Uncontrolled livestock movement has been suggested to be responsible for geographical expansion and cumulative effects, whereas the amount of rainfall is considered the main cause of outbreaks (Swanepoel, 1976). It has been suggested that the bimodal rainfall pattern experienced in this ecosystem provides an environment for mosquito species to emerge in large numbers at the onset of the rainy season, resulting in high biting rates and transmission of the virus in animals and humans (Kanouté *et al.,* 2017).

2.4 Clinical Signs in Humans

The RVF clinical signs following exposure in humans include fever, malaise, myalgia, chills, backache, eye pain, headache, rash, red eyes, photophobia, poor appetite, flushing, nausea, vomiting, meningism, poor vision, epistaxis, hematemesis, hematochezia, bruising, confusion, vertigo, stupor and coma (LaBeaud *et al.,* 2008).

2.5 Clinical Signs in Animals

Clinical signs in animals depend on the species of animal affected and conditions such as age and pregnancy. During epidemics, the occurrence of numerous abortions and mortalities among young animals has been reported (Breiman *et al.,* 2008). Pregnant sheep and cattle affected by this disease almost always abort approximately 80–100% (Girma *et al.,* 2011). Young lambs and calves that develop fever become weak and die very quickly (Nyarobi, 2020). The mortality rate in young animals is very high, whereas the mortality rate in adult sheep is approximately 20% and approximately 10% in adult cattle (Budodo *et al.,* 2020). Adult sheep and cattle may experience nasal discharge, excess salivation, and loss of appetite, weakness or diarrhea (Venkatesh, 2019).

2.6 Diagnosis and Control

2.6.1 Laboratory Diagnosis

Diagnostic tests for RVF are mainly based on serology (Chevalier *et al.,* 2010). The most common detection technique for detecting IgG for previous infections and IgM for recent infections is the enzyme-linked immunosorbent assay (ELISA). Real-time polymerase chain reaction is the only technique that detects RVFV (Sall *et al.,* 2002).

2.6.2 The RVF Control

(i) Animal Control Measures

The spread of RVF among animals can be controlled primarily through vaccination and movement restrictions. In South Africa, killed vaccines for sheep and cattle have been used effectively for several years and similar vaccines have also been produced and employed in Egypt (Martin *et al.,* 2008). Additionally, a modified live vaccine is available for veterinary use (Ikegami & Makino, 2009). However, this live vaccine presents limitations; it can cause abortion in a small percentage of sheep and has not been adequately studied for the risk of reverting to virulence (Ikegami & Makino, 2009), making it generally not recommended (Bird & Nichol, 2012).

Quarantine measures have also been implemented to control the movement of imported livestock (Martin *et al.,* 2008). Nonetheless, in many developing countries like Tanzania, these measures are often impractical due to economic and logistical constraints. As a result,

restricting the movement of animals from areas experiencing an RVF outbreak is considered a more feasible approach to prevent disease spread (Shope *et al.,* 1982).

(ii) Human Control Measures

For humans, person-to-person transmission of RVF is rare (Anyamba *et al.,* 2010). However, humans can become infected through direct contact with the blood, tissues or organs of infected animals, especially during slaughtering or veterinary procedures. Therefore, vaccination of high-risk individuals, such as veterinarians and laboratory personnel, is recommended to reduce the risk of occupational exposure (Morrill *et al.,* 1997). Given that vaccines are often expensive and not readily available in many affected regions, emphasis should also be placed on educating at-risk populations about safe handling of animals and using protective measures to avoid contact with infectious materials.

2.7 Rift Valley Fever Outbreak in Tanzania

Investigations have shown that spatial and temporal patterns of RVF outbreaks in Tanzania have occurred over the past 80 years (Mohamed *et al.,* 2010). All RVF outbreaks reported from 1930--2007 occurred between December and June (Mohamed *et al.,* 2010). During the period between 1930 and 1957, less than 1% of the districts affected were affected, whereas the 1977/1978 outbreak wave was reported to involve 3.33% of the districts (Woods *et al.,* 2002). A relatively larger outbreak wave in 1997/1998 involved 7.70% of districts, and the widespread outbreak in 2006/2007 involved 39.17% of districts in the country (Jost *et al.,* 2010).

However, despite this expansion of infection in districts that were not previously involved, RVF outbreaks still show significant spatial and temporal clustering in the eastern Rift Valley (Sumaye *et al.,* 2013). Space-time clustering of domesticated animal and human cases has shown a tendency to spread from the northern to the central eastern and western parts of the country (Jost *et al.,* 2010). While uncontrolled livestock movement has been suggested to be responsible for geographical expansion and cumulative effects, the amount of rainfall is considered the main factor that leads to outbreaks (Sindato *et al.,* 2014).

It has been suggested that the bimodal rainfall pattern experienced in this ecosystem provides a conducive environment for Aedes mosquito species to emerge in large numbers at the onset of the rainy season, resulting in extensive biting rates and transmission of the

virus in animals and humans (Nanyingi *et al.,* 2015). Arbovirus disease transmission is often heterogeneous because of its vectors, host distribution and underlying social and ecological determinants (Budodo *et al.,* 2020a; Kajeguka *et al.,* 2017).

The determination of locations where diseases are clustered and associated risk factors is useful for early estimation of case distributions, which provides an evaluation of risk at a small geographical scale for targeted surveillance, prevention, control and future research (Budodo *et al.,* 2020a; Kajeguka *et al.,* 2017). The lower Moshi, Kilimanjaro region is an area that has all potential characteristics for RVF endemicity, such as its locations along the Northeast African Rift Valley, livestock keeping, seasonal heavy rains and irrigation agriculture, which makes mosquito vectors abundant and persistent.

Furthermore, RVFVs have been detected in a diverse range of wild species, such as African buffalo, giraffe, black rhino, Impala and African elephant species, among many others; domesticated ruminants include cattle, sheep and goats (Wright *et al.,* 2019).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Design and Site

The study was a community- and laboratory-based cross-sectional survey conducted in the lower Moshi in Moshi district, Kilimanjaro region of Tanzania. Information (data) was collected from March to June 2020 in three villages: Mikocheni, Chemchem and Arusha Chini. The lower Moshi is situated on the southern hills of Mount Kilimanjaro (Fig. 1). West China, the Lower Moshi bordered the Kikuletwa River, Hai District and Manyara Region. To the east are the borders of the Mwanga district. The lower Moshi elevation arrays are between 700 and 800 m above sea level. The area is characterized by RVF vectors, which are mosquitoes of a variety of species, such as *Culex* spp., *Mansonia* spp., *Anopheles* spp., and *Aedes* spp. (Lumley *et al.,* 2017). The area is abundant in watercourses for irrigation networks for puddy and sugarcane farming. Paddy irrigation schemes involve organized and formless canal channels that cover an area of approximately 1100 hectares (Tarimo *et al.,* 1998). The area has two rainy seasons: Extended rains, which run from March-May and short rainy periods, which last from November-December. The average yearly rainfall is approximately 900 mm per year (Chamchod *et al.,* 2014).

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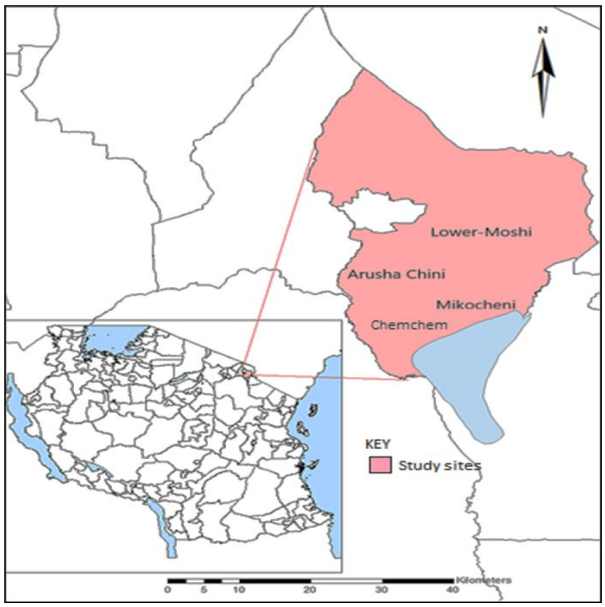

Figure 1: Map of Tanzania developed from Google Earth showing the study site

3.2 Sample Size Estimation

According to previous publications, the RVF seroprevalence rates in Tanzania, humans and animals (goats) are 23.3% and 08.2%, respectively (Wachtmeister, 2015; Wensman *et al.,* 2015). To estimate the sample size for humans, the following formula adopted from Arya *et al.* (2012) was used:

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

n= number of samples

Z= statistic for the level of confidence

p= Expected prevalence

d=allowed error margin

Q=Number of goats

W=Number of humans

$$W = \frac{(1.96^2)0.233\ (1-0.233)}{0.05^2} = 238$$

A total of 266 blood samples were examined for presence of RVF antibodies, with 10% included as a replacement for dropout participants. To estimate the sample size for goats, the following formula adopted from Arya *et al*. (2012) was used.

$$\mathrm{Q}= \frac{(1.96^2)0.\,082(1-0.\,082)}{0.05^2} = 115$$

With 10% included as a replacement for dropout participants, a total of 125 blood samples from goats were examined.

3.3 Participants and Sample Collection

The members who participated in this study were both males and females aged 10 to 70 years. The participants aged 18 years and above were either smallholder crop agriculturalists or cattle keepers. Only participants who were willing to take part were included in the study. For participants aged younger than 18 years, their parents or legal guardians consented on behalf of their children to participate in the study. Animal sampling was supported by the help of animal health experts from the Moshi Local veterinary office. Up to 5 goats were carefully chosen from each flock via a systematic sampling method in which each 3rd and/or 5th animal

was selected depending on the size of the flock. A total of 125 goats were sampled from 25 herds.

Human blood collection was performed by professional phlebotomists from Kilimanjaro Christian Medical Center (KCMC). A total of 3 ml of blood was collected from the median cubital vein via venipuncture using standard procedures. The sampled animals were manually restrained, and 3 ml of blood was collected through the jugular vein via a vacutainer needle with anticoagulant. Each sample from both animals and humans was divided into two aliquots of 1.5 mil each and put into plain and EDTA vacutainer tubes. To each sample in an EDTA tube, 4.5 mil of Tri Reagent (Zymo Research, Irvine, CA, U.S.A.) was added. The blend was gently mixed by shaking for 1 min and stored in a cool box at 4°C for until when transported to the KCRI biotechnology laboratory for analysis. Plain tubes were also stored in a cool box at 4°C for until when transported to the KCRI biotechnology laboratory for analysis. Demographic data from participants were collected via electronic forms designed with the Open Data Kit (ODK) tools (https://opendatakit.org/) deployed in Android tablets.

3.4 Mosquito Trapping

A Biogents Sentinel (BGS) trap (Biogents AG, Regensburg, Germany) was used to target outdoor host-seeking adult mosquitoes, predominantly *Aedes* spp*., Ochlerotatus* spp*., Culex* spp*., Mansonia* spp. and *Anopheles* spp. (Pedro *et al.,* 2016). The BGS traps were used in combination with the BGS-Lure, a slot machine that releases discharges such as those found on human skin (lactic acid, ammonia and caproic acid) (Breiman *et al.,* 2008). The BGS-Trap, developed by Biogents GmbH (Regensburg, Germany), is easy to transport and is a portable white bucket with white gauze that covers its opening.

In the middle of the gauze cover, there is a black tube through which a downflow is formed by a 12 V DC supporter, which causes any mosquito in the vicinity of the opening tube to lap into a catch bag (Breiman *et al.,* 2008). Mosquito vectors were instantly morphologically recognized in the field and subsequently sorted according to genus through their wings and color, including fed and unfed; a stereo microscope was used to identify their sex. Among the mosquitoes trapped, two groups were obtained, *Culex* spp. and *Aedes spp*., which were subjected to qPCR analyses for RVFV RNA in pools of 50 s. The *Aedes* spp. and *Culex* spp. 347 and 130 mosquito pools, respectively, were collected and analyzed.

3.5 Laboratory Procedures

3.5.1 The RVFV Competitive ELISA (cELISA)

Plasma and serum were obtained by centrifugation of samples from EDTA and plain tubes, respectively, at 3000 rpm for 5 min. Plasma and serum samples were then transferred into 2 ml sterile cryovials via a sterile Pasteur pipette. All the serum samples were tested for the presence of antibodies against RVFV via a competitive enzyme-linked immunosorbent assay (cELISA) via the ID Screen RVF Competition Multi-Species Kit (ID-vet, Grables, France), which detects both immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies directed against RVFV nucleoprotein (NP). Plasma samples were used for testing for presence of the RVFV. Validation tests for the test kit revealed a sensitivity between 91% and 100% and a specificity of 100%.

Competitive ELISA is based on the competitive binding of the 1' antibody between the target antigen in a sample and the same antigen that is coated into the multiwell plate. The 1' antibody is first added to the sample to form antigen–antibody complexes. The cELISA was performed according to the instructions and protocol of the manufacturer, which involves the following steps: binding samples to the support, adding substrate, adding primary antibody and washing, adding secondary antibody-enzyme conjugates and washing (Lowassa *et al.,* 2012; Nyarobi, 2020). To control the validity of each plate, the mean value of the two negative controls (ODNCs) was computed, whereby a plate was considered valid if the ODNC was >0.7. For a valid plate, the mean value of the two positive controls divided by the ODNC had to be <0.3. For each sample, the competition percentage was calculated by dividing ODsample/ODNC) × 100. If the value was ≤0.4, the sample was considered positive, whereas a value > 0.5 was considered negative. Only samples that tested positive by cELISA were subjected to RT–qPCR for RVFV detection.

3.5.2 Ribonucleic Acid (RNA) Isolation, Purification and Real-Time PCR Amplification

For the detection of RVFV RNA in humans and goats, RNA was extracted from TRIzol archived blood in EDTA tubes via a DirectZol miniprep kit (Zymo Research, Irvine, CA, U.S.A.) via the Boom method (Boom nucleic acid extraction method), which is a solid-phase extraction method for isolating nucleic acid from a biological sample (Boom *et al.,* 1990). To isolate RVFV RNA from mosquitoes, pools of 40–50 unfed monospecific mosquitoes were placed in cryovials and transferred into Lysing Matrix, impact-resistant tubes containing 1.4

mm ceramic beads (MP Biomedicals, CA, USA). The samples were disrupted by bead beating at 10,000 × g for 1 min and then spun at 1000 × g for 10 min at 4°C. The supernatant was transferred into labeled RNase-free tubes. Purification procedures were performed via a Direct-zol™ RNA miniprep kit (Irvine, CA, U.S.A.) following the manufacturer's instructions.

For both the human/goat and mosquito samples, RNA concentration and quality checks were performed via a NanoDrop™ 2000 Spectrophotometer (Thermo Scientific, NY, USA) before storage at -80°C. The RVFV RNA was detected via TaqMan probe-based one-step RT–qPCR targeting the RVFV Gn gene as described by Gudo and colleagues (Gudo *et al.,* 2016) via the Applied Biosystems ViiA7 PCR platform (Thermo Scientific, NY, USA). All laboratory procedures were carried out at KCRI Lab, Moshi, Kilimanjaro, Tanzania.

3.6 Data Analysis

Data analysis was performed via IBM SPSS v.26 (IBM® Corp., Armonk, NY, USA). Descriptive data are presented as frequencies and percentages, means and medians. Categorical data were reported as tabularizations of amounts and associations between humans and goats. The chi-square statistic (χ^2) was used to examine associations between seropositivity for RVFV and RVFV infection in both humans and goats. The mean IgM and IgG concentrations were compared between humans and goats via paired t tests. The percentages of positive RVFV infection in goats, humans, and mosquitoes are reported as histograms.

3.7 Ethical Considerations

This study obtained approval from the Kilimanjaro Christian Medical University College (KCMUCo) Research and Ethics Committee (CRERC) with approval certificate #2419. Permission to conduct this study was also obtained from the Kilimanjaro Regional and District Administrative Secretaries, District Medical and Veterinary Officers and local village and ward executive officers of respective villages. Before commencement of sample collection, written informed consent was obtained from all study participants aged 18 years and above by signing ‘informed consent’ forms, whereas parents and/or legal guardians of participants under 18 years and participants who could not read or write signed the ‘informed consent’ on their behalf. All authors hereby confirm that all procedures in this study were approved by CRERC and were performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. The authors also confirm that all procedures that involved animals in this study were conducted in compliance with the ARRIVE guidelines.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Demographic Characteristics of the Human Participants

The study included a total of 266 human participants, of whom more than half (56.4%) were females. The median age (interquartile range) of the participants was 45 (30-55). The majority of participants (74.4%) came from households with more than 4 persons in the same house. With respect to the participants' education, 63.2% had attained primary school education. Most participants (72.9%) kept livestock (cattle, sheep, goats and/or chicken). Three quarters (75.2%) of the participants reported having used an insecticide treated bed net the night before the interview.

Table 1: Demographic characteristics of the study participants

Characteristics	n	%
Age group (years)		
≤20	28	10.5
21–50	140	52.7
>50	98	36.8
(Median, IQR) years		45 (30–55)
Sex		
Male	116	43.6
Female	150	56.4
Individuals living in a household		
<4	68	25.6
≥4	198	74.4
Highest education		
No formal education	51	19.2
School education (primary/secondary)	168	63.2
Tertiary education (college)	47	17.7
Type of animals kept by the participant		
None	72	27.1
Chicken only	82	30.8
Goats and sheep only	54	20.3
Cattle only	55	20.7
Goats, sheep, cattle, and chicken	3	1.1
ITN use*		
Yes	66	24.8
No	200	75.2

IQR, Interquartile range; *72 missing entries.

4.1.2 Goats Involved in the Study

The Table 2 presents the distribution of study animals by age category, sex and flock size. For age, 66 animals (53.25%) were less than 12 months old and 59 animals (46.75%) were more than 12 months old. In terms of sex, 38 animals (30.1%) were male and 87 animals (69.9%) were female. Regarding flock size, 15 animals (61.7%) were from flocks with fewer than 20 animals, 5 animals (20.0%) were from flocks with 20–50 animals, and 5 animals (18.3%) were from flocks with more than 50 animals (Table 2).

Table 2: Number of goats included in the study (n=125)

Variables	n	%
Age category (months)		
<12	66	53.25
>12	59	46.75
Sex	n	%
Male	38	30.1
Female	87	69.9
flock Size *	n	%
<20	15	61.7
20-50	5	20.0
>50	5	18.3

4.1.3 Factors Associated with RVFV Seropositivity in Humans and Goats

Human RVFV seropositivity was analyzed for associations with participant age, ITN use within the last 24 hours, positivity for RVFV infection, number of persons living under the same roof, recent travel outside the study site, highest education level of the participant and RVFV infection and seropositivity in goats (Table 3). The results revealed that male sex was significantly associated with RVFV seropositivity (χ^2 =5.351; p=0.030). Similarly, participants aged 50 years and above were more seropositive than their young counterparts were (χ^2 =14.430; p=0.006).

Insecticide-treated net (ITN) use, having more than seven persons living in the same household, and RVFV seropositivity in goats were all significantly associated with higher RVFV seropositivity in humans (χ^2 = 6.003; p = 0.021, χ^2 = 23.213; p = 0.000, and χ^2 = 27.053; p = 0.000, respectively) (Table 3). Among the factors analyzed for possible associations with IgM/IgG RVFV seropositivity in goats, only human IgM/IgG RVFV seropositivity showed a significant association (χ^2 = 27.053; p = 0.000). Additionally, RVFV infection in Aedes *spp.* mosquitoes was significantly associated with human RVFV seropositivity (χ^2 = 7.921; p = 0.040) (Table 3).

Table 3: Factors associated with RVFV seropositivity in humans

Variable	Level	Negative, n (%)	Positive, n (%)	χ^2 (*p*)
Age Group	10-20	18 (94.7)	1 (5.3)	14.430 (0.006)
	21-30	26 (92.9)	2 (7.1)	
	31-40	23 (88.5)	3 (11.5)	
	41-50	19 (82.6)	4 (17.4)	
	>50	51 (67.1)	25 (32.9)	
Bed-Net Use	Yes	24 (64.9)	13 (35.1)	6.003 (0.021)
	No	110 (83.3)	22 (16.7)	
Number of persons in a HH	1-3	45 (90)	5 (10)	23.213 (0.000)
	4-6	74 (85.1)	13 (14.9)	
	7+	17 (50)	17 (50)	
Travel outside site	Yes	48 (82.8)	10 (17.2)	0.521 (0.551)
	No	89 (78.1)	25 (21.9)	
Destination	Urban	22 (88)	3 (12)	2.545 (0.346)
	Peri-urban	10 (90.9)	1 (9.1)	
	Rural	16 (72.7)	6 (27.3)	
Highest Education	No Formal Education	34 (81)	8 (19.8)	0.465 (0.830)
	Primary	91 (78.4)	25 (21.6)	
	tertiary	12 (85.7)	2 (14.3)	
RVFV Infection in goats	Yes	1 (50)	8 (80)	0.800 (1.000)
	No	1 (50)	2 (20)	
RVFV seropositivity in goats	Yes	11 (9.4)	24 (43.6)	27.053 (0.000)
	No	106 (90.6)	31 (56.4)	
Human RVFV PCR	Positive	0 (0)	7 (100)	0.248 (1.000)
	Negative	1 (3.4)	28 (96.6)	

4.1.4 Comparison of Mean IgM/IgG Concentrations in Humans and Goats

Table 4 shows that, age group, bed-net use, number of persons per household, and RVFV seropositivity in goats were significantly associated with RVFV seropositivity in humans. Seropositivity increased with age, peaking at 32.9% in individuals over 50 years old (χ^2 = 14.430; p = 0.006). Bed-net non-users had higher seropositivity (35.1%) compared to users (16.7%) (χ^2 = 6.003; p = 0.021). Households with more than seven persons had the highest positivity rate (50%) (χ^2 = 23.213; p = 0.000). The RVFV seropositivity in goats also showed

a strong association with human seropositivity ($\chi^2 = 27.053$; $p = 0.000$). Other factors such as travel history, destination, education level and RVFV PCR results in humans did not show statistically significant associations (Table 4).

Table 4: Factors associated with RVFV seropositivity in humans and goats

Variable	Level	Negative, n (%)	Positive, n (%)	χ^2 (p-value)
IgM/IgG Seropositivity in Humans	Positive	11 (9.4)	24 (43.6)	27.053 (0.000)
	Negative	106 (90.6)	31 (56.4)	
RVFV Positivity in Goats	Positive	3 (75.0)	8 (88.9)	0.410 (1.000)
	Negative	1 (25.0)	1 (11.1)	
RVFV Infection in Humans (PCR)	Positive	2 (16.7)	5 (20.8)	0.089 (1.000)
	Negative	10 (83.3)	19 (79.2)	
Sex	Male	17 (34.0)	12 (41.4)	0.430 (0.629)
	Female	33 (66.0)	17 (58.6)	
Age Group (in months)	0–12 months	28 (56.0)	15 (51.7)	0.135 (0.216)
	>12 months	22 (44.0)	14 (48.3)	

4.1.5 Proportion of RVF Seropositivity and RVFV PCR Positivity in Humans, Goats and Mosquitoes

This study demonstrates that goats exhibit the highest levels of Rift Valley Fever Virus (RVFV) exposure and active infection, as indicated by both seropositivity (23.3%) and PCR positivity (4.1%), compared to humans and mosquito vectors. The relatively high seroprevalence in humans (13.2%) alongside detectable PCR positivity (2.6%) suggests ongoing zoonotic transmission. In contrast, Aedes and Culex mosquitoes showed lower PCR positivity rates (2.3% and 1.5%, respectively), underscoring their role as vectors but with limited evidence of widespread infection at the time of sampling. These findings support the hypothesis that goats may serve as key amplifying hosts in RVFV transmission cycles, with implications for One Health-based surveillance and control strategies (Fig. 2)

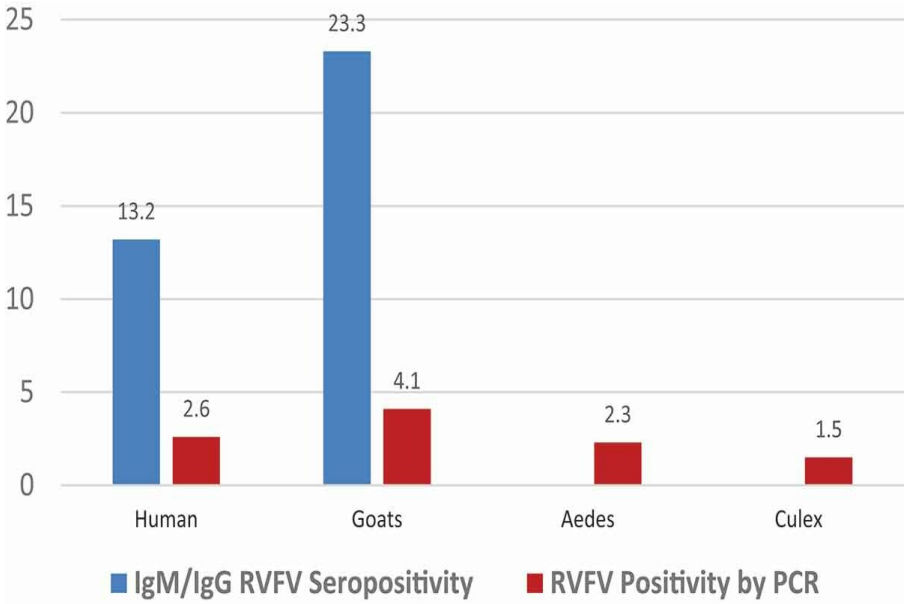

Figure 2: The IgM/IgG seropositivity and PCR positivity for goats, mosquitoes and humans

4.2 Discussion

The main aim of this study was to examine the degree of exposure to RVFV in goats and humans. This study also sought to isolate RVFV from humans, goats and mosquitoes and to determine whether *Aedes* spp. and *Culex* spp. are the key RVFV vectors. Although no RVF outbreak has been reported in Tanzania since 2006--2007, antibodies to RVFV and the virus have been detected in humans and goats in the Lower Moshi area. The findings from this study indicate that 13.2% and 23.3% of the tested humans and goats, respectively, had circulating antibodies against RVFV. The findings emphasize active or postexposure to RVFV during IEPs, as previously reported by some studies across the geoecological zones of Tanzania (Bell *et al.,* 2005; Lumley *et al.,* 2017; Maciel-de-Freitas *et al.,* 2006; Wensman *et al.,* 2015).

In this study, goats had higher exposure rates to RVFV than did humans. *Aedes* spp., the major vector for RVFV, is known to have bimodal daily feeding behavior, with both exophoric and exophilic behaviors, and they feed on a wide range of mammalian hosts (Ahmed *et al.,* 2018). Consequently, this behavior can be implicated as a key behavior because of its role as a vector for many zoonotic infections. Despite its preference for human hosts (Ahmed *et al.,* 2018), goats are more seropositive than humans are. The transmission of RVFV is not absolutely

dependent on the presence of vector mosquitoes. Direct human contact with infected animal tissues has been reported as a significant factor for its transmission from animals to humans (Chengula *et al.,* 2014; Heinrich *et al.,* 2012; Matiko *et al.,* 2018).

The RVFV RNA has been detected in humans, goats and mosquitoes. Goats presented the highest infection rate of 4.1%, followed by humans (2.6%). Viral RNA was also detected in 2.3% and 1.5% of the tested *Aedes* spp. and *Culex* spp. mosquito pools, respectively. This study was conducted to elucidate the maintenance mechanisms of RVFV by investigating both the exposure and infection rates in mammalian and arthropod vectors. This is the first study conducted in Tanzania to report RVFV diagnosis in humans, animals and mosquitoes simultaneously in the same area. Many of the previous studies that aimed to understand the epidemiology of RVFV in Tanzania primarily focused on sero-epidemiology, particularly in livestock such as cattle. However, few integrated both serological and molecular approaches, and several of those attempting molecular detection failed to identify RVFV RNA in either mammalian hosts or arthropod vectors. This study fills that gap by combining sero- and molecular-epidemiological methods to provide a more comprehensive understanding of RVFV transmission dynamics.

Although the interactions of arboviruses and their vectors are complex and their epidemiology is poorly understood, The present findings support the hypothesis that, during IEPs, RVFV is likely maintained by localized low-level transmission between mosquito vectors and mammalian hosts without any noticeable clinical symptoms (Lorenzo *et al.,* 2015; Matiko *et al.,* 2018; Terasaki & Makino, 2015). Evidence for RVFV transmission during IEPs has previously been reported among livestock and wild animals in Tanzania and elsewhere (Nyarobi, 2020; Olovsson, 2019; Sanderson *et al.,* 2020); however, this study focused on one health approach to detect RVFV during IEPs in humans, small ruminants (goats) and mosquito vectors to identify potential reservoirs of RVFV. Although goats were more seropositive for RVFV than were humans, paired comparisons of the mean anti-RVFV IgG/IgM concentrations revealed no difference between humans and goats.

Some factors, including sex, living in a household, being older than 50 years, not using an insecticide-treated bed net, and higher RVFV seropositivity in goats, were significantly associated with seropositivity for RVFV in humans. The high RVFV seropositivity in males could be attributed to the nature of their daily activities. The RVFV seropositivity in humans was consequently associated with seropositivity in goats. Males seem to be more active

outdoors for various subsistence animal-related activities, including farming and grazing, which bring them into frequent contact with RVFV-susceptible or RVFV-infected animals. This finding stresses the need for continued education to create awareness to take protective measures, especially among rural and agro-pastoral communities that are more prone to zoonotic diseases such as RVF.

The study site is characterized by features that support vector mosquito breeding and intimate human–animal interactions. With the paucity of data on RVFV infection in areas near the study area, the detection of antibodies to RVFV in humans and goats and the detection of RVFV in humans, goats and mosquitoes in the study area suggest that the site is a potential RVF hotspot. The dominant pastoral grazing system in the study area and surrounding areas manifests unlimited movements of livestock as a result of environmental degradation of the wetland; overstocking and overgrazing increase the likelihood of introducing the disease into new areas.

The absence of clinical manifestations among livestock and humans in the study area, which could be a consequence of herd immunity, seems to have escaped the knowledge of the veterinary and public health authorities, raising concerns about the available local and national capacity for preparedness and response machinery against zoonotic infections with the potential to cause fatal epidemics. Thus, there is a critical need for improved surveillance of RVF transmission through the detection of RVFV activity among humans, livestock, and vector mosquitoes.

In this study, goats had higher exposure rates to RVFV than did humans. Although *Ae. aegypti*, the major vector for RVFV, is known to be anthropophilic in nature, it has also been reported to have both exophagic and exophilic feeding behaviors (Delatte *et al.,* 2010; Matiko *et al.,* 2018). Consequently, this behavior can be implicated as a key behavior because of its role as a vector for many zoonotic infections. Despite its preference for human hosts, the present study reported higher RVF seropositivity in goats. The transmission of RVFV is not absolutely dependent on the presence of vector mosquitoes. Direct human contact with infected animal tissues has been reported as a significant actor in transmission from animals to humans (Nyakarahuka *et al.,* 2018; Ponlawat & Harrington, 2005).

Not all of the human participants in this study were directly involved in activities that increase their direct contact with infected tissues, such as infected aborted fetuses and those working in slaughterhouses, which could partly explain the lower seropositivity to RVFV in humans than in goats. Furthermore, much more intense interactions between RVFV vectors in mosquitoes

and goats exist in the studied agropastoral community, exposing goats to higher RVFV exposure and infection rates. Clinically, goats could be the main source of RVFV infection in humans, rather than vice versa.

Since passive surveillance of RVF is challenging in the absence of clinical features among humans and livestock, active surveillance is recommended, and where resources may be limited, targeted surveillance in high-risk areas (hot spots) will help prevent future RVF outbreaks.

CHAPTER FIVE

CONCLUSION AND RECOMENDATIONS

5.1 Conclusion

The present study reveals the presence of anti-RVFV antibodies in both humans and goats and more importantly, the detection of RVFV RNA in humans, goats and mosquitoes within an area characterized by ecological features conducive to mosquito breeding. Data collected during the dry season, corresponding to an inter-epidemic period (IEP), suggest that the Lower Moshi area is a potential hotspot for Rift Valley Fever (RVF). This finding indicates that Lower Moshi may serve as a reservoir and source of RVFV transmission to other regions. These insights underscore the need for enhanced surveillance, early warning systems, and targeted vector control measures as part of preparedness and response strategies in RVF-prone areas.

5.2 Recommendations

Based on the study findings, it is recommended to incorporate disease screening during IEPs to identify potential hotspots affecting domestic animals and human beings; promote animal vaccination and vector control in these areas; enhance community awareness through training, mass media and outreach; and most importantly, adopt the One Health approach in RVFV surveillance as a strategic priority for zoonotic disease control in Tanzania.

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RESEARCH OUTPUTS

(i) Publications

Kumalija, M. S., Chilongola, J. O., Budodo, R. M., Horumpende, P. G., Mkumbaye, S. I., Vianney, J. M., Mwakapuja, R. S., & Mmbaga, B. T. (2021). Detection of Rift Valley fever virus inter-epidemic activity in Kilimanjaro Region, North Eastern Tanzania. *Global Health Action, 14*(1), 1957554. https://doi.org/10.1080/16549716.2021.1957554

(ii) Poster Presentation