THE PREVALENCE, DISTRIBUTION AND RISK OF VIRAL HAEMORRHAGIC FEVERS IN TANZANIA

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EXTENDED ABSTRACT

Viral haemorrhagic fevers (VHFs) are a group of infectious diseases caused by viruses belonging to the *Flaviviridae, Phenuiviridae, Arenaviridae,* and *Filoviridae* families. They are characterized by fever, a constellation of initially nonspecific signs and symptoms, and a propensity for bleeding and shock. The VHFs reported in Sub-Saharan Africa include Crimean-Congo haemorrhagic fever (CCHF), Ebola virus disease (EVD), Lassa fever (LF), Lujo haemorrhagic fever (LUHF), Marburg virus disease (MVD), Rift Valley fever (RVF), and yellow fever (YF). Knowledge surrounding the distribution and risk of VHFs in Tanzania is limited. Accurate epidemiological information of VHFs is critical to implementing appropriate control and prevention strategies. This study aimed to determine the prevalence, risk of introduction, and factors associated with VHF in Tanzania. The findings provided in this thesis consolidate our knowledge of the diseases in Tanzania and the need for improved surveillance, early detection, and prompt response.

The thesis is divided into two major approaches, namely systematic review and research studies. A systematic review was undertaken to analyse EVD outbreak reports, identify challenges and opportunities in detection and response in sub-Saharan Africa and propose effective control approaches.

A research study was conducted to estimate the probability of introducing EVD from DRC to Tanzania. This study involved collection of national data for flights, boats, and car transport schedules from DRC to Tanzania covering a period between May 2018 to June 2019. A population study was carried out in all ecological zones of Tanzania to determine the seroprevalence and associated risk of selected five VHFs. In this epidemiological study, blood samples were collected from households and healthcare facilities in Buhigwe, Kalambo, Kilindi, Kinondoni, Kondoa, Kyela, Mvomero, and Ukerewe districts of Tanzania. The samples were screened for immunoglobulin G (IgG) and M (IgM) antibodies against CCHF, EVD, MVD, RVF, and YF. The prevalence of VHF and malaria co-infections was also determined among participants seeking care from health care facilities. Malaria infections were investigated using rapid diagnostics tests (RDT). In addition, Community knowledge, awareness and practices regarding VHF was assessed in eight districts of Tanzania. Archived sera that tested positive for immunoglobulin IgG antibodies to EBOV and RVFV using a commercially available ELISA were reanalysed using the in-house EBOV whole antigen (WAg) and RVFV inhibition enzyme-linked immunosorbent assay.

The findings of the systematic review indicate that between 1979 and 2020 a total of 34 EVD outbreaks affecting 34,356 cases and causing 14,823 deaths were reported in 11 countries in Sub-Saharan Africa. The overall case fatality rate (95% CI) was 66% (62 to 71) and this did not change substantially over time (OR in 2019 *versus* 1976=1.6 (95% CI 1.5 to 1.8), p<0.001). The challenges to control EVD outbreaks are related to epidemiological, sociocultural and health system factors.

As regards to the risk of introduction of EVD from DRC to Tanzania, the findings indicate that the most likely pathways were the entry of infected humans through air, land, and water travels. The land border crossings were considered the most frequently used means of travel (highest), while water and air travel were deemed less often (high).

High probabilities of introducing EVD from DRC to Tanzania were associated with the viability of the pathogen and low detection capacity at the ports of entry.

The epidemiological survey indicated that the overall, CCHF, EVD, MVD, RVF, and YF seroprevalence were 2.0%, 3.4%, 1.2%, 4.8%, and 1.4, respectively. The highest VHF antibody seroprevalence was recorded in western Tanzania (6.0%), characterized by unimodal rainfall pattern and altitude below 2,300m. The lowest seroprevalence (0.4%) was observed in the semi-arid central Tanzania, characterized by moderate precipitation and a unimodal rainfall pattern. The association between seroprevalence of VHFs and geographical location was found to be significant (P= 0.001). Contact with wild animals (OR = 1.2, CI = 1.3–1.6) and keeping goats (OR = 1.3, CI = 1.5–1.9) were significantly associated with RVF while contact with bats (OR = 1.2, CI = 1.1–1.5) was associated with MVD. The prevalence of VHF and malaria co-infections was 1.9%. The age group 46-60 years had the highest malaria co-infection $p < 0.05$. Over half (57%) of malaria and VHF-infected individuals reported headaches, while over one-third (37%) reported muscle, bone, back, and joint pains (p= 0.001).

All EBOV positive sera turned out to be negative by In-House WAg EBOV ELISA. Two of the nine positive IgG RVF commercial ELISA samples were reactive to the RVF Inhouse ELISA, with inhibitory rates ranging from 66% to 99.6%. A slight agreement was observed between the RVF in-house and the commercial ELISA (kappa value, 0.08; 28%, 11/40 in disagreement).

As regards to community, knowledge, attitudes and practice, slightly over a quarter (29.4%) of the respondents was knowledgeable, 25% had a positive attitude, and 17.9% had unfavourable practice habits. There were increased odds of having poor practice among participants aged 36–45 years (AOR: 3.566, 95% CI: 1.593– 7.821) and those living in Buhigwe, Kilindi and Ukerewe areas (AOR: 2.529, 95% CI: 1.071–6.657; AOR: 2.639, 95% CI: 1.130–7.580 AOR: 2.248, 95% CI: 1.073–3.844) than their peers.

In conclusion, Sub-Saharan Africa faces considerable challenges in EVD control, whereby there are no significant changes in case fatality rates observed during the past four decades. Socioeconomic and cultural factors need to be critically considered to shape the community behaviours that lead to EVD outbreaks.

The risk of introducing EVD from DRC to Tanzania is high. Infected humans arriving via land are the most likely pathway of EVD entry, and therefore, mitigation strategies should be directed towards this pathway.

Although, CCHF, EVD, MVD, and YF outbreaks have not been reported in Tanzania, the viruses are prevalent and vary from one ecological zone to another, with the western part of the country at the highest risk. Co-infections of malaria and viral haemorrhagic fevers are frequent in Tanzania. The rate was higher in the age group 46-60 years. Both malaria and VHF presented with similar symptoms. There were increased odds of having poor practice among participants aged 36–45 years (AOR: 3.566, 95% CI: 1.593– 7.821) and those who reside in Buhigwe, Kilindi and Ukerewe areas (AOR: 2.529, 95% CI: 1.071–6.657; AOR: 2.639, 95% CI: 1.130–7.580 AOR: 2.248, 95% CI: 1.073–3.844) than their peers. According to the study, 29.4% of respondents were knowledgeable, 25% had a positive mindset, and 17.9% had unfavourable practice habits.

We found poor agreement between in-house and commercial ELISA tests for EBOV and fair agreement between in-house and commercial ELISA tests for RVFV, with the commercial ELISA detecting more cases than the validated in-house ELISA. The current study emphasizes the difficulties in diagnosing viral hemorrhagic fever in Tanzania.

Frequent EVD outbreaks in DRC urge the need to intensify the surveillance system, including laboratory screening of suspects, thermal screening of all arrivals from endemic areas, and registering travellers within all the border entry points to prevent disease importation. Equally important, there is a need for strengthening the sensitization and awareness campaigns focusing on cross-border communities. The border healthcare facilities need to be well equipped to ensure a high disease containment capacity. The findings that VHFs are prevalent in Tanzania call for the need to strengthen the surveillance system and management of febrile illnesses in Tanzania. The discrepancy was observed between validated in-house and commercial ELISA tests. Hence, the serological results should be interpreted with caution and this emphasizes the challenges related to the diagnosis of VHFs. Overall, broad-based, one-health approach, effective communication, social mobilisation, and strengthening of the health systems need to be addressed to prevent future outbreaks.

DECLARATION

I, SIMA RUGARABAMU, do hereby declare to the Senate of Sokoine University of Agriculture that, this thesis is my own original work and that it has neither been submitted nor concurrently being submitted for a degree award in any other institution.

Sima Rugarabamu   Date
(PhD Candidate)

The above declaration is confirmed;

Dr. Leonard Mboera
(Supervisor)   Date

Prof. Gerald Misinzo   Date
(Supervisor)

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DEDICATION

This Thesis is dedicated to the Almighty God and all those who have suffered and or died from Viral Haemorrhagic fevers in Africa

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF APPENDICES

LIST OF PAPERS

Paper I: **Rugarabamu S,** Mboera L, Rweyemamu M, et al. Forty-two years of responding to Ebola virus outbreaks in Sub-Saharan Africa: a review.

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Paper II: **Rugarabamu, S**.; George, J.; Mbanzulu, K.M.; Mwanyika, G.O.; Misinzo, G.; Mboera, L.E.G. Estimating Risk of Introduction of Ebola Virus Disease from the Democratic Republic of Congo to Tanzania: A Qualitative Assessment.

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Status: Published in International *Journal of Infectious Diseases (2021), doi: https://doi.org/10.1016/j.ijid.2021.07.006*

Paper IV: **Sima Rugarabamu**, Gaspary O. Mwanyika, Susan F. Rumisha, Calvin Sindato, Hee-Young Lim, Gerald Misinzo, Leonard E.G. Mboera, viral haemorrhagic fevers and malaria co-infections among febrile patients seeking health care in Tanzania.

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Paper V: **Sima Rugarabamu**, Gaspary O. Mwanyikaa, Susan F. Rumisha, Calvin Sindato, Hee Young Lim, Gerald Misinzo and Leonard E.G. Mboera. Community knowledge, attitude and practices regarding zoonotic viral haemorrhagic fevers in five geo-ecological zones in tanzania.

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Paper VI: Sima Rugarabamu, Gerald Misinzo, Leonard E. G. Mboera, Jacquiline Weyer, Hee Young Lim & Janusz Paweska
Comparison of an In-House And a Commercial Enzyme-Linked Immunosorbent Assays for Diagnosis of Ebola Virus Disease and Rift Valley Fever

Status: Manuscript

LIST OF ABBREVIATIONS

CCHF	Crimean-Congo haemorrhagic fever
DRC	Democratic Republic of the Congo
EBOV	Ebola Virus
ELISA	Enzyme-linked immunosorbent assay
EVD	Ebola virus disease
IgG	Immunoglobulin G
IgM	Immunoglobulin M
MVD	Marburg viral disease
NICD	National Institute for Communicable Diseases
PCR	Polymerase Chain Reaction
qRT-PCR	real-time reverse transcription polymerase chain reaction
RVF	Rift Valley fever
SPSS	Statistical Package for Social Sciences
SUA	Sokoine University of Agriculture
VHF	Viral haemorrhagic fever

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Viral haemorrhagic fevers

Viral haemorrhagic fever (VHF) was a term defined by Russian physicians in the 1940s to describe a syndrome comprised of fever, which initially presents non-specific signs and symptoms, and if not self-limited, progresses to bleeding and shock (Moses *et al*., 2012). VHFs are caused by more than 30 viruses from four taxonomic families namely: *Filoviridae, Arenaviridae, Bunyaviridae,* and *Flaviviridae* (Cleri *et al*., 2006). However, not all members of these families are known to cause viral haemorrhagic fevers. The typical characteristics of those causing viral haemorrhagic fever are zoonoses (Racsa *et al*., 2016). Human infection often results in severe or fatal disease, while infection in the host reservoir species is frequently mild or completely asymptomatic (Fox *et al*., 2002). While viruses that cause VHFs are found worldwide, they are relatively restricted by the distribution of their reservoir host species (Cobo *et al*., 2016). These viruses resides either in an animal reservoir host or an arthropod vector, and they are totally dependent on their host for survival. Rodents and arthropods are the main reservoirs for VHFs viruses, although the hosts of some viruses (such as Ebola and Marburg) are still unknown (Charles *et al*., 2001; Pigott *et al*., 2005; Cobo *et al*., 2016). The frequency with which these species interact with humans combined with the number of pathogenic VHF viruses in nature makes them significant threats to human health.

The transmission mode of VHFs is most often through a vector and intermediate host or an animal reservoir. Infection is presumed to most often result from inadvertent contact with virus-contaminated excreta of the reservoir, with inoculation into mucus membranes or broken skin (Leffel *et al*., 2004). Some VHF viruses are arboviruses, spread to humans by mosquitoes or ticks (Nichol *et al*., 2000). Aerosol transmission has also been suggested for some viruses, but there are limited data to confirm or refute this route of exposure reservoir (Nichol *et al*., 2000; Taylor *et al*., 2001). Secondary human-to-human transmission occurs with many VHF viruses, usually through direct contact with contaminated blood or body fluids (Ericsson *et al*., 2001). Infection probably occurs most often through oral or mucous membrane exposure in the context of providing care to sick family members in the community or patients in a healthcare institution or during funeral rituals that entail the touching of the corpse prior to burial. Large outbreaks are almost always the result of amplification in healthcare settings (Nichol *et al*., 2000; Wong *et al*., 2007; Sanjuán *et al*., 2010). The risk of transmission during the incubation period or from asymptomatic persons is negligible, although Studies reported some VHFs transmission due to blood transfusion from an asymptomatic donor (Wong *et al*., 2007).

Although details of the pathophysiology vary with the specific virus, microvascular instability and impaired haemostasis are the consistent hallmarks (Aleksandrowicz *et al*., 2008). The interaction of the virus with immune cells, especially macrophages and endothelial cells, results in cell activation and the unleashing of an inflammatory and vasoactive process consistent with the system (Aleksandrowicz *et al*., 2008; Moraz *et al.,* 2011). The synthesis of cell surface tissue factors triggers the extrinsic coagulation pathway (Aleksandrowicz *et al*., 2008). Impaired haemostasis may entail endothelial cell, platelet, and coagulation factor dysfunction, depending upon the specific infecting virus (Moraz *et al*., 2011).

The virus rapidly clears from the blood upon symptom resolution in survivors, but clearance may be delayed (up to 3 months after acute infection) from a few immunologically protected sites, such as the kidney, gonads, and chambers of the eye (Dean *et al*., 2016). In contrast to the process described above, hantaviruses, yellow fever, and dengue viruses are usually cleared from the blood prior to the most severe phase of the disease (Niedrig *et al*., 2018). Viral haemorrhagic fevers affect in both sex and all age groups, ranging from asymptomatic, mild to severe vascular permeability, shock, multiple organ failure and death (Bausch *et al*., 2009). Incubation period ranging from days to weeks, depending upon the infecting virus, the illness begins with fever and other symptoms, including headache, myalgia, arthralgia, general malaise, anorexia, sore throat, chest or lumbosacral and retrosternal pain (Cleri *et al*., 2006; Moses *et al*., 2012). Gastrointestinal signs and symptoms including abdominal pain and tenderness, vomiting, nausea, and epigastric, diarrhoea, and constipation follow within a few days of illness (Racsa *et al*., 2016). Diarrhoea may become bloody in the severe stages of the disease (Moses *et al*., 2012). Conjunctival injection or haemorrhage is frequent (WHO, 2016). Depending on the specific VHF, skin rash, including morbilliform, maculopapular, petechial, and ecchymotic, may be seen (Borrego *et al*., 2019). For severe cases, patients progress to vascular instability that may be manifested by facial flushing, oedema, bleeding, hypotension, shock, and proteinuria, occurring towards the end of the first week of illness (Pittalis *et al*., 2009; Bente *et al.,* 2009). The likelihood of clinically discernible haemorrhage varies with the infecting virus. Hematemesis, melena, haematochezia, petechiae, purpura, epistaxis, and bleeding from the gums may develop, but haemoptysis and haematuria are infrequent (Jahrling *et al*., 2007; Singh *et al*., 2013).

The non-specific clinical manifestations of VHFs make a clinical diagnosis of single cases extremely difficult, especially in the early stages, as the differential diagnosis is extremely broad. The occurrence of clusters of cases with a compatible clinical syndrome, especially involving healthcare workers, should raise suspicion of disease (Mansfield *et al*., 2015). VHF should be considered among febrile patients with a compatible clinical syndrome and history of travel and exposure, especially if the patient fails to respond to empiric treatment for the usual infectious endemic disease. Although many VHF viruses may be spread person-to-person, secondary attack rates are generally low as long as routine universal precautions are maintained inpatient management (Moses *et al*., 2012). For added safety, specialized VHF precautions are warranted when there is a confirmed case or high index of suspicion. Treatment of VHF is generally supportive, following guidelines for the management of septic shock (Coetzer *et al*., 2015). The antiviral drug ribavirin and treatment with convalescent plasma have demonstrated efficacy in a few VHF cases. With a few exceptions, vaccines for viruses that cause haemorrhagic fever remain unavailable or lack well-documented efficacy (Falzarano *et al.*, 2013).

1.2 Major viral haemorrhagic fevers

Viruses in the genera Ebola virus and Marburg virus in the family Filoviridae cause "classical" haemorrhagic fever disease. Other viral families that cause VHFs include Phenuiviridae (Rift Valley fever virus, Nairoviridae (Crimean-Congo Haemorrhagic Fever virus, and Flaviviridae (Yellow fever virus) (Jahrling *et al*., 2007; Singh *et al*., 2013). All these viruses comprise of RNA genome. RNA genome has plasticity characteristics resulting from error-prone RNA-dependent RNA polymerases (RdRp), allowing for more significant adaptive potential leading to increased fitness selection (Barr *et al*., 2016).

The effect of mutation rates can be seen with a Crimean-Congo Haemorrhagic Fever virus variant, Europe 2, which infects many people in the Balkans, but rarely causes illness (Hua *et al*., 2021). Only mild cases of illness have been associated with Europe 2 strains, while other CCHF virus strains circulating in the same region have caused several thousand severe illnesses (Papa *et al*., 2011; Sidira *et al*., 2012; Sargianou *et al*., 2013). Similar results have been generated with high-fidelity mutants for the RVF virus, resulting in decreased viability in viral progeny (Borrego *et al*., 2019). This implies that reducing genetic diversity harms viral fitness in transmission from vector to host and for replication within the vertebrate host itself (Forester *et al*., 2012).

1.2.1 Filoviruses

Ebola and Marburg viruses are single-stranded, negative-sense, and non-segmented RNA viruses belonging to the family Filoviridae. The family Filoviridae belongs to the order Mononegavirales and contains two genera namely, Ebolavirus and Marburgvirus. The genome of filoviruses is approximately 19 kb long, composed of linear, non-segmented, negative-sense, single-stranded RNA, and contains seven genes that encode structural and non-structural proteins, arranged sequentially in the order NP, viral protein (VP) 35, VP40, glycoprotein (GP), VP30, VP24 and polymerase (L) genes (Figure 1.1).These filoviruses cause haemorrhagic fever in humans and nonhuman primates (Sanchez *et al*., 2007). Most of the known filoviruses are endemic to Africa, with several different virus species belonging to the genus Ebolavirus found in central and western African rain forests, approximately within 10 degrees north and south of the equator (Groseth *et al*., 2007).

The Marburg virus is commonly found in open dry areas of eastern and south-central Africa (Peterson *et al*., 2004). Depending on the virus species and strain, Filoviruses are lethal human pathogens known, with case fatality rates of up to 90% (Pittalis *et al*., 2009; Bente *et al*., 2009). The Marburg virus consists of two viruses, Marburg virus (MARV) and Ravn virus (RAVV). The genus Ebolavirus has five known species namely, Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Taï Forest ebolavirus (TAFV), Bundibugyo ebolavirus (BDBV), Reston ebolavirus (RESTV) and Lloviu virus (Kuhn *et al*., 2010).

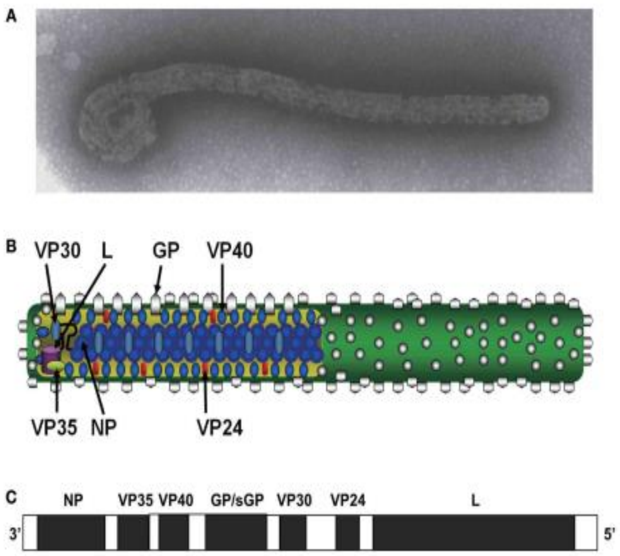

Figure 1.1: Structure of Ebola virus particle and genome organization. (A) Electron micrograph of ebolavirus particle; (B) its schematic diagram and (C) negative-sense genome organization (Takada, 2012).

1.2.2 Bunyaviruses

The predominant vector-borne human pathogen within genus Nairovirus is Crimean-Congo haemorrhagic fever virus which causes a VHF endemic to large regions of Africa, Europe, and Asia (Lasecka & Baron, 2014). CCHF is typically febrile disease with haemorrhagic manifestations such as epistaxis, ecchymoses, and petechial rash. The severe cases of CCHF frequently involve internal haemorrhagic manifestations such as bleeding from the urinary and gastrointestinal tracts. CCHF virus is vectored by ticks of the Hyalomma species, predominately *H. marginatum* and *H. anatolicum* (Ergönül, 2006).

Rift Valley fever virus is a pathogenic virus classified within the Phlebovirus genus and is associated with significant outbreaks in humans and livestock. Itis an important mosquito-borne pathogen endemic to large regions of Africa and the Arabian Peninsula associated with significant neonate mortality and abortions in livestock in addition to a potential viral haemorrhagic fever during human infection (Daubney *et al*., 1932; Coetzer *et al*., 2015; Kwasnik *et al*. 2021). The overall case fatality rate in human is approximately 1-3%, although it can be up to 50% in patients with severe haemorrhagic manifestations (Mansfield *et al*., 2015). The viral genome in this group is approximately (11.9 kb) and consists of three segments designated large (L), medium (M), and small (S), which encode the viral RNA-dependent RNA polymerase, the viral glycoproteins Gn and Gc and non-structural protein NSm, and the viral nucleocapsid protein N and the non-structural virulence protein, NSs, respectively (Havranek *et al*., 2019; Bird *et al*., 2009; Bouloy and Weber, 2010) (Figure 1.2). Together with host proteins, these viral proteins ensure that the virus can replicate its genome during a productive infection (Havranek *et al*., 2019; Bouloy and Weber, 2010). The replication occurs in the cytoplasm of infected cells and virions buds in the Golgi compartment (King *et al*., 2012). Each segment is transcribed during the replication cycle into mRNA and is replicated a complementary RNA (cRNA), or antigenome is synthesized. The cRNA represents the copy of the S ambisense segment act as a template for synthesizing the NSs mRNA.

The protein is then expressed early, a good indication that it has an important role during infection (Pepin *et al.*, 2010; Ikegami *et al.*, 2005). The glycoproteins play an essential role in the penetration of the virus, and their proper processing is crucial for the maturation and budding of the virion (Liu *et al.*, 2008; King *et al.*, 2012). The glycoproteins, being the most exposed components of the virus during infection, are recognized by the immune system and induce the production of neutralizing antibodies, which play a predominant role in protection (Filone *et al.*, 2006, Pepin *et al.*, 2010).

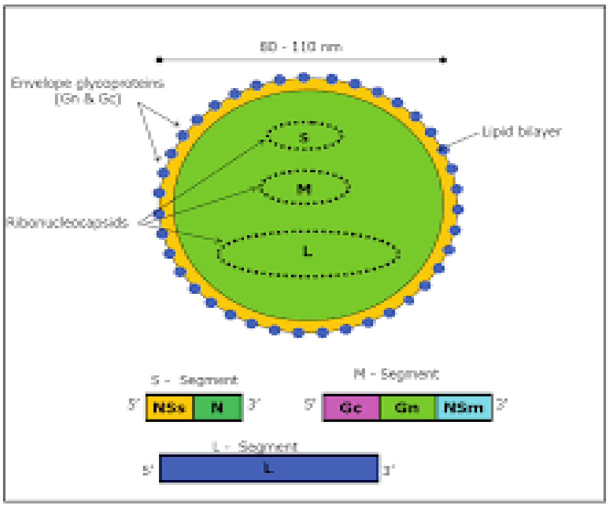

Figure 1.2: Schematic illustration of RVF VIRUS genome containing small (S), medium (M), and large (L) RNA segments and surface glycoproteins (Gn and Gc) incorporated into the lipid bilayer (Nyarobi, 2020)

1.2.3 Flaviviruses

The Flavivirus genus consists of more than 70 small, positive-sense, single-stranded RNA viruses transmitted by arthropods, in particular mosquitoes and ticks (Bryant *et al.*, 2007). These include globally important human pathogens such as West Nile virus, Japanese encephalitis virus, dengue virus, Murray Valley encephalitis virus, tick-borne encephalitis virus, Yellow fever (YF) virus, and Zika virus (Laureti *et al.*, 2018). These viruses are responsible for some of the most severe arbovirus infections affecting humans, pose a serious threat to global health and have the potential to cause severe outbreaks (Messina *et al.*, 2014). YF virus can progress to include haemorrhagic manifestations (Service & Ashford, 2001).

Filoviruses have large ranges of endemicity that correspond with the distribution of the competent mosquito vector. Human disease is characterised by a febrile illness that can progress to include haemorrhagic manifestations. YF virus is a single-stranded, positive-sense RNA virus with a genome of approximately 11 kb (Figure 1.3). Seven lineages have been identified, five in Africa, namely, West Africa I and II, East Africa, East/Central Africa, and Angola, and the other two in the Americas, known as South America I and II (Bryant *et al.*, 2007). Phylogenetic analysis provided evidence that the YF virus circulating in the Americas is derived from a Western African lineage ancestor that emerged in Africa and was imported into the American East Coast from during the slave trade (Vasconcelos *et al*. 2004; Bryant *et al*. 2007; Nunes *et al*., 2012).

YF is native to Africa where it may have emerged around 3000 years ago (Robertson *et al*., 1996). The most feared epidemic occurred in the 15th to 19th centuries, during the large-scale outbreaks in port cities of north and South America, Africa, and Europe caused devastating mortality (Rogers *et al*., 2006). The landmark studies of Walter Reed in 1901 established that the disease was transmissible among humans via *Aedes aegypti* mosquitoes (Reed *et al*., 1901). Within one year of Reed's discovery, the disease was successfully controlled in Cuba as a result of vigilant mosquito control campaigns (Carter *et al*., 1931). Twenty-eight years later, YF remained a significant cause of morbidity and mortality, with an estimated 200,000 cases each year and 30,000 deaths (Robertson *et al*., 1996). Indeed, although a highly effective vaccine is available, epidemiological data suggest an alarming resurgence of virus circulation in West Africa over the last 20 years (Onyango *et al*., 2004).

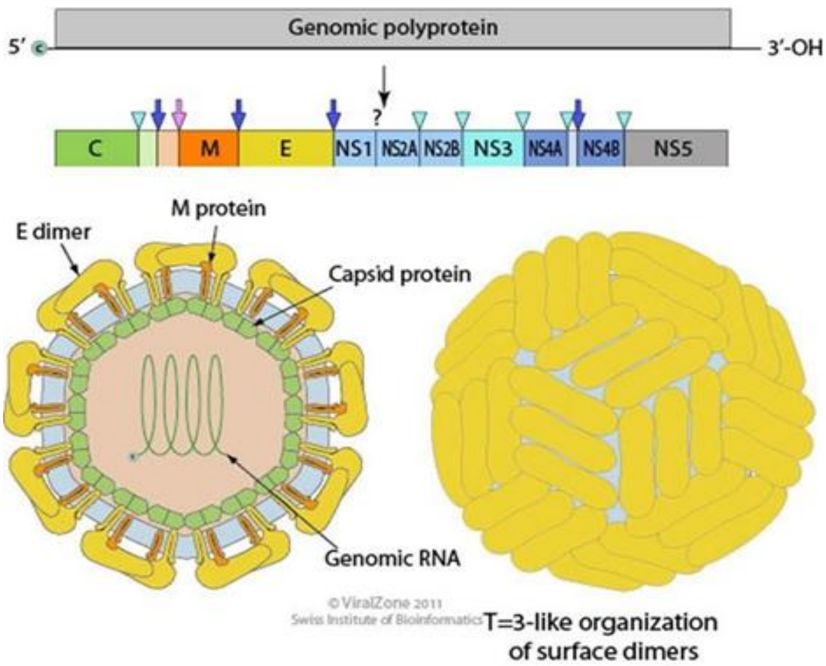

Figure 1.3: Genome composition and virion structure of flaviviruses (Barryatkinson, 2016)

1.3 The global emergence of viral haemorrhagic fevers

The exploitation of new ecological niches by human beings and increased travel and trade has promoted the emergence or re-emergence of VHF around the world (Figure 1.4). Together, VHF diseases affect more than 100 million people around the world and kill more than 60,000 people annually.

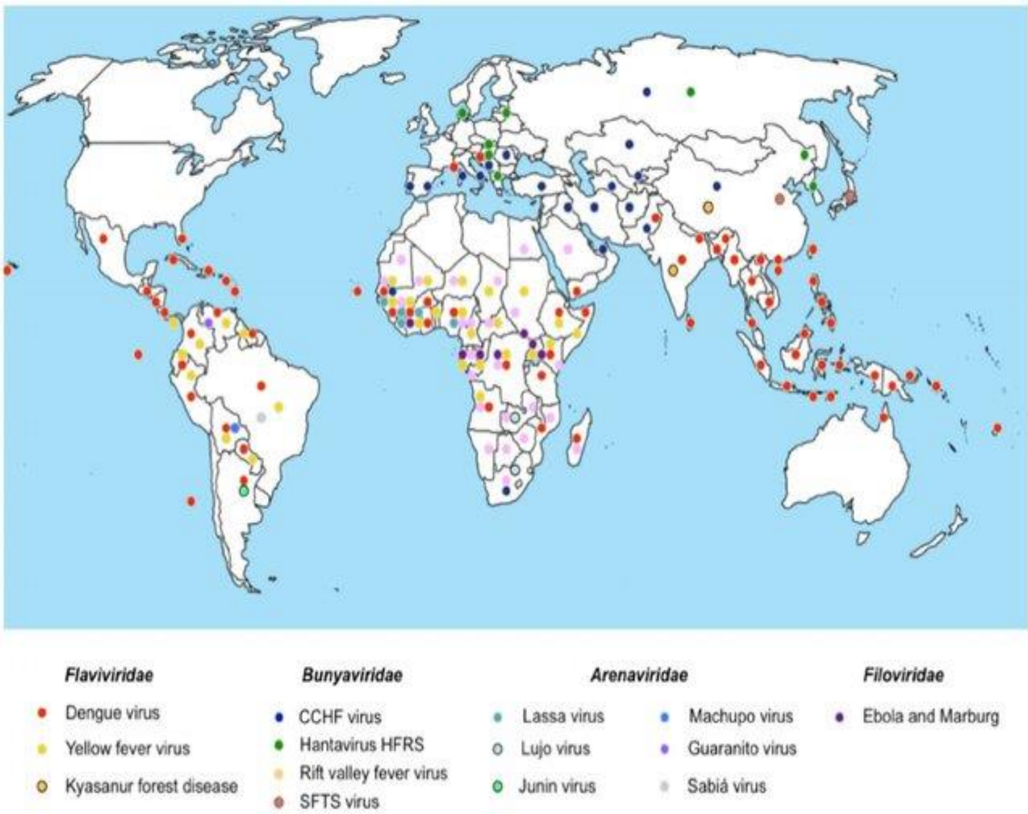

Figure 1.4: Global geographical distribution of viral haemorrhagic fevers (Zapata *et al.,* 2014)

Perhaps the Ebola virus is the most notable example in the 21st Century for the emergence of a novel viral haemorrhagic fever (Ogden *et al*., 2016). Ebola outbreaks were confirmed far back in 1976 (Bowen *et al*., 1977). To date the Ebola outbreaks in Africa have occurred in 2001, 2002, and 2007, 2013, 2014, 2015, 2016, 2018, 2021 (Makiala *et al*., 2018; Nyakarahuka e*t al*., 2020; Rugarabamu *et al.,* 2021). The emergence of VHFs in new territories is a persistent threat (Isaacson *et al*., 2001).

Rift Valley fever was first identified in 1931 on a farm in Kenya's Rift Valley (Daubney *et al*., 1931). Since then, outbreaks have been reported mainly in Africa (Seufi & Galal, 2010), and the Arabian Peninsula (El-Akkad, 1978; Morvan *et al*., 1991; Fontenille *et al*., 1998; Jupp *et al.*, 2002; Diallo *et al*., 2005; Bird *et al*., 2007; Sissoko *et al*., 2009). The spread of RVFV outside its endemic region raises concerns about the threat of its introduction to new geographical areas (Pepin *et al*., 2010).

Of recently, YF outbreaks have been reported in Brazil (Faria *et al*., 2018; Giovanetti *et al*., 2019). In Brazil, the virus spilled over in areas without a history of acquired immunity or mandatory vaccination coverage (Cunha, 2019), causing 2,166 confirmed cases and 751 deaths (Sacchetto *et al*., 2020). Similarly, a concurrent outbreak was reported in Angola and the Democratic Republic of Congo resulting in 884 confirmed cases and 381 deaths (Song *et al*., 2018). Both outbreaks raised fears of initiation of urban transmission, leading to massive immunization drives.

It can be challenging to differentiate between genuine emergence and endemic VHFs that had been previously misdiagnosed. Most of the causative viruses are neither rare nor insignificant pathogens, yet cases are likely misdiagnosed for decades before formal identification. For instance, a trio of Germans reported finding Ebola antibodies in 26 of 433 Liberians (6%) who underwent testing for antibodies to Ebola, Marburg, and Lassa fever viruses in 1982 (Ivanoff *et al*.,1982). By then, the country was considered an Ebola-free zone. In 2013 the first outbreak occurred; although it was not linked to the previous study, this may highlight the complexity of distinguishing between emergence and misdiagnosis (Kaner *et al*., 2016).

1.3.1 Risk Factors of VHFs

Environmental factors leading to disease emergence typically involve a change of conditions allowing the VHF viruses reservoirs such as arthropods and rodents to expand into new territories. Climate change offers a significant risk for expanding potential distribution of many species either in terms of a long-term trend in increased temperatures or an increase in the frequency of abnormal weather events (Gould and Higgs, 2009; Roiz *et al*., 2015). Direct man-made environmental change can also impact ecology and increase interactions between vectors, wild animals, and humans.

Several human factors also have the potential to trigger spill over events. Conflicts and war have a long association with disease emergence for various reasons, mainly due to the movement of people into atypical areas (e.g., temporary settlements for civilians and the movement of troops through rural locations) as well as disruption of the healthcare infrastructure (Ugalde *et al*., 2000). Severe human diseases such as CCHF were initially identified during conflicts (Chumakov, 1945; Lee *et al*., 1978), while disease re-emergence resulting from temporary population displacement is a frequent phenomenon associated with civil unrest and wars (Gayer *et al*., 2007). Similarly, expanding urbanization and land reclamation can introduce many susceptible human hosts into, or close to, pathogens environments (Gould *et al*., 2006).

The trade of livestock offers the opportunity for pathogens to transfer to new locations. The threat of importation of an exotic pathogen via this route is listed as one of the eight plausible scenarios for infectious disease threats to the European Union (Suk & Semenza, 2011). A similar issue related to wildlife trade is presented by the large-scale animal markets common in many areas, especially Asia. These markets can house tens of thousands of animals simultaneously and present opportunities for viruses to transfer into species of mammals not typically encountered as well as providing the potential for viral reassortment with similar species of virus (Parrish *et al*., 2008).

The production and the consumption of animal-based products contribute to an increase in the risks of viral haemorrhagic fevers. The consumption of wild animals (or bushmeat) and animal products such as raw milk and blood is an important driver of VHF emergence (Gonzalenz *et al*., 2015). Wild animals are indeed important reservoirs of VHF, and most of the VHF pathogens originate from wildlife. Animal farming plays a significant role in the emergence and the spread of pathogens, as numerous VHF reach humans through domestic animals (e.g., Rift Valley fever, Crimean-Congo haemorrhagic fevers (Bannister *et al*., 2010). The risk of healthcare and laboratory-acquired infection in handling rare or newly recognized pathogens is well documented (Barry *et al*., 1995; Armstrong *et al*., 1999). Mining, hunting, and intensive farming involve significant risks in exposure to VHF reservoirs through means other than commercial production (Gonzalez *et al*., 2018). Such activities may reduce the likelihood of pathogen introduction through biosecurity intervention but significantly increase the risks of amplification, spread, and the mutation of pathogens once they enter respective facilities (Isaäcson *et al*., 2001; Fhogartaigh *et al*., 2015). In addition, the deforestation associated with the increased production of meat deteriorates natural habitats and biodiversity, thus indirectly increase VHF epidemic events (Gonzalez *et al*., 2018).

Vector survival and VHF virus replication are strongly influenced by climatic conditions such as temperature, humidity and precipitation, with vector activity and virus transmission often being seasonal (Rogers *et al*., 2003). Local vector abundance may further depend on the availability of specific breeding habitats, such as water bodies for mosquito oviposition (Sunish & Reuben, 2001), or specific vertebrate host species for feeding (Estara *et al*., 2014). For example, many tick species have different host preferences at different life stages (Guglielmone *et al*., 2014), and high host densities may greatly increase tick abundance (Wilson, 1985). Further, host migration enables dispersal of both ticks and arboviruses over long distances, often to novel areas (Estara *et al*., 2014). Virus circulation is also influenced by the spatial and temporal variation in the densities of competent vertebrate reservoir hosts. The availability of suitable habitat and/or important host species is often correlated with particular land use categories, such as fragmented and highly mosaic agricultural landscapes (Havelaar *et al*., 2010).

1.4 Vira haemorrhagic fever outbreaks

Outbreaks of VHFs, most notably Ebola virus disease, Marburg virus disease, Yellow fever, Crimean-Congo Haemorrhagic fever and Rift Valley fever, have been well documented across sub-Saharan Africa and have caused widespread morbidity, mortality, and substantial socio-economic losses. There have been 34 confirmed outbreaks of EVD reported since its first identification in 1976 in the Democratic Republic of Congo (Rugarabamu *et al*., 2020), and 14 outbreaks of MVD since its first identification in Germany in 1967 (Langun *et al*., 2019). The largest of EVD outbreaks have occurred in West Africa in 2013–2016 and DRC 2018-2020. The economic impacts of the West African EVD outbreak alone were estimated at over US$2.2 billion in gross domestic product (GDP) from loss of investments, significant drops in private sector growth, lower food and agricultural production, and decreased trade (Huber *et al*., 2014). The costs of the response to the outbreak by the affected countries and the international community exceeded US$3.6 billion (Johnson *et al*., 2017).

RVF and CCHF have also caused widespread outbreaks in human populations across Africa. The most significant socio-economic losses are due to morbidity and mortality of affected livestock. Because of the ecological, zoonotic, and vector-borne nature of their causative viruses, outbreaks tend to primarily affect regions with large livestock populations and countries in which the local economy is highly dependent on livestock and agricultural trade (Muga *et al*., 2015). Of the major outbreaks recorded since 1900s, socio-economic impact has ranged from US$7 to over US$470 million, accounting for losses of over 5% of GDP (Martins *et al*., 2014). Other costs associated with these outbreaks are due to their impact on local and international trade and public health and healthcare expenditures.

Yellow fever is the second most globally distributed arthropod-borne disease after dengue haemorrhagic fever, causing around 200,000 cases per year, with case fatality varying between 15% and 30%. Although there is an effective vaccine against YF, the number of infected people has increased in recent years, especially in urban Africa (Chen *et al*., 2020). This might be attributable to multiple factors including declining population immunity to infection (Barnet *et al*., 2007), increased human activities such as deforestation, urbanization, population movement and climate change (Gardner *et al*., 2010). To date the disease affects more than 170,000 people and caused about 80,000 deaths in Africa (Tini 2014). A total of 32 African countries are located in the so called ''yellow fever belt'' hence making them at risk of yellow fever transmission (Nwaiwu *et al*., 2021). The disease produces extensive epizootics in non-human primates and outbreaks of human cases (Mboussou *et al*., 2019). It is vaccine preventable, with a safe and effective vaccine available since the 1930s that has been introduced to all travellers visiting yellow fever endemic regions. Yellow fever is transmitted by numerous vectors including Aedes mosquitoes. A component of the sylvatic reservoir system is in non-primates, making yellow fever difficult to eradicate.

1.5 Laboratory diagnosis of viral haemorrhagic fevers

If viral haemorrhagic fever is suspected after the initial work-up and laboratory testing, rapid diagnostic laboratory testing is imperative. Rapid access to specialized laboratory testing will vary according to the region and is especially a challenge in resource-limited areas. Unfortunately, few commercial assays are available for the VHFs (Singh *et al*., 2016). The absence of commercial assays poses a significant impediment to patient diagnosis.

The mainstays of diagnosis are the enzyme-linked immunosorbent assay (ELISA), reverse transcriptase-polymerase chain reaction (RT-PCR), and cell culture (Trombley *et al*., 2010). Although extensive validation of these assays has not been conducted, sensitivities and specificities are generally considered over 90%. The immunofluorescent antibody test (IFA) may also be employed but is not as routinely sensitive or specific and is more subjective in its interpretation, varying with the technician's experience (Bausch *et al*., 2000). Post-mortem diagnosis of some VHFs may be established by immunohistochemical staining of formalin-fixed tissue, especially the skin, liver, and spleen (Zaki *et al*., 1999). VHF is usually diagnosed in the acute febrile phase by identifying the virus, virus antigen, or nucleic acid in the specimen (Piggot *et al*., 2005). These measures also provide prognostic value since the high virus, antigen, or nucleic acid levels in the blood correlate with a poor prognosis in most VHFs (Racsa *et al*., 2016). The serum is the most reliable sample to test, but the virus can be variably isolated from throat washings, urine, cerebrospinal fluids, breast milk, and other tissues (Towner *et al*., 2007). ELISA tests are high throughput, and ELISA antigen tests and RT-PCR can usually be done in a few hours. Multiplex PCR assays have been developed to allow for simultaneous testing for the VHFs and the many diseases in the differential diagnosis (Racsa *et al*., 2016). Furthermore, these assays can be performed with inactivated specimens using standard equipment present in most diagnostic laboratories. In contrast, propagation of most VHF viruses in cell culture requires a high containment facility and 2–10 days to detect virus growth, depending on the specific virus and titre in the sample (Kaya *et al*., 2014). ELISAs may lack sensitivity relative to RT-PCR and cell culture (Pretorius *et al*., 2017). Nevertheless, cell culture should not be omitted whenever possible because haemorrhagic fever viruses have occasionally been isolated from specimens for which ELISA antigen, antibody, and RT-PCR tests were negative (Paweska *et al*., 2009). This was attributable to virus concentrations in the test sample that were below the threshold of detection of these assays or the presence of variant or novel viruses that were not detectable with the antigens and primer sets employed (Ippolito *et al*., 2012).

Failure to appreciate the intricacies and limitations of the various laboratory assays has occasionally led to false-negative diagnoses and increased risk of nosocomial transmission (Al-Hamdan *et al*., 2015). If clinical suspicion of VHFs remains high, despite a negative result, tests should be repeated in 1–2 days and, if necessary, again in convalescence. In acute and sub-acute convalescent, VHFs can be diagnosed by identifying IgM and IgG antibodies, respectively, in the blood through ELISA or IFA. Antibody seroconversion (usually interpreted as a fourfold increase in titre) on acute and convalescent serum specimens has also been used to retrospectively diagnose the acute disease when assays to detect virus nucleic acid or antigen is not available (Burrel *et al*., 2017). The demonstration of neutralizing antibodies can increase the specificity of ELISA antibody results. However, neutralization assays are not uniformly standardized and cumbersome to perform, and because they require the use of a live virus, they must be done in a high containment laboratory for most of the VHF viruses; consequently, they are infrequently performed (Oswald *et al*., 2007). The timing of appearance of IgM and IgG antibodies and their duration after the infection has not been systematically studied thus varies with the specific virus. In survivors, IgM generally appears in the first days or weeks of illness and lasts for months after infection, while IgG typically appears during convalescence and is thought to last years, if not decades (Endy *et al*., 2020).

Antibody detection by ELISA is useful for epidemiological surveillance and control programs, import/export certification, early detection of infection, and monitoring immune response in vaccinated humans and animals (Paweska *et al*., 2012). An ELISA can be used to detect the presence of antigens or antibodies in a sample, depending on how the test is designed (Ma & Shieh, 2006; Gaastra, 1984). To detect antibodies using the ELISA test (Figure 1.5), the antigen is coated onto a 96-well plate, and test sera are added. A secondary antibody coupled to an enzyme specific to the tested host species is added to the plate wells. Enzyme substrate is then added, allowing a colour change to be determined by measuring the optical density measured in a spectrophotometer. Depending on the ELISA platform used, either the presence or absence of colour indicates the presence of antibodies (Ma & Shieh, 2006). IgM or IgG antibodies can be detected using isotype-specific secondary antibodies. The antibodies detected may be either neutralizing or non-neutralizing, and the tests cannot differentiate between the two.

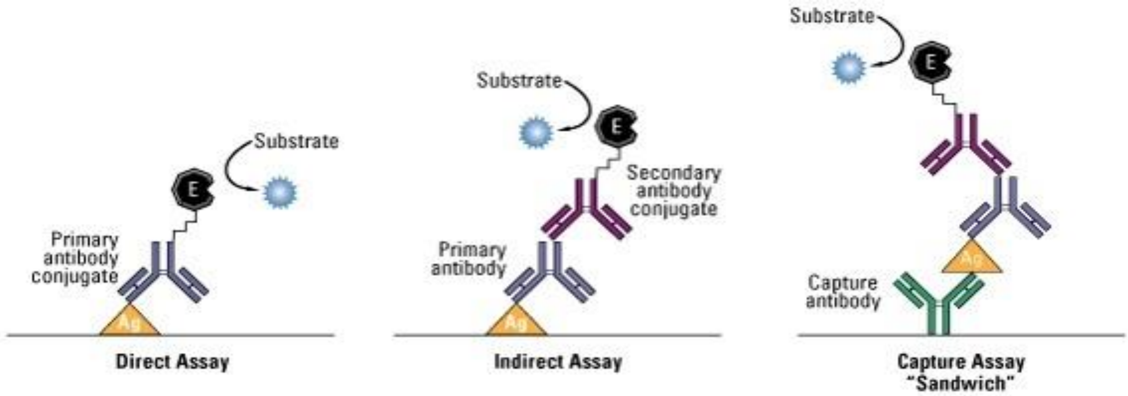

Figure 1.5: Schematic representation of the ELISA test and procedure Provide an appropriate reference or website

1.6 Viral haemorrhagic fever surveillance

While knowledge of the reservoir of VHFs remains insufficient, the distribution of arthropods and rodents is not static. So, information of the suspected cases, potential for vector distribution and spill over events is required in order to develop suitable surveillance strategies, prophylactic treatments, vaccines, travel recommendations, and clinical therapies. Significant emphasis is placed on monitoring epidemics and disease emergence with modern technologies allowing for complex modelling to predict, and potentially prevent, significant epidemics and pandemics (Morse *et al*., 2012).

One potential complicating factor for assessing VHFs is understanding the full spectrum of clinical presentations and the factors influencing these manifestations. For instance, the EVD is widespread across DRC; however, the severity of the disease, and the associated case fatality rate, change markedly depending on the geographic location of the disease locus (Gritsun *et al*., 2003). It is possible that the difference seen could be attributed to the genotypic variation of pathogen or individual host immune interactions as the geographical location changes (Mlera *et al*., 2014). It is important to understand epidemiology variation to ascertain how this impacts disease presentation in different regions.

1.7 Viral haemorrhagic fever in Tanzania

Global emerging pathogens analysis confirms Tanzania as a hotspot for disease emergence (David *et al*., 2004). Historically several high consequence pathogens have been reported, including dengue, chikungunya, CCHF, Rift Valley fever and Zika (Macnamara, 1954; Robinson *et al*., 1955; Swanepoel *et al*., 2007; Klaassen *et al*., 2010). VHF notable yellow fever, Marburg, and Ebola are ubiquitous across a large area in sub-Saharan Africa (Boggild *et al*., 2017). While this is the case, there is no single report on the prevalence of disease to regular hospital visitors, admissions, or the general public in Tanzania, thus resulting in insufficient intervention strategies. The potential impact of VHFs in Tanzania is enormous despite several reports of serious human pathogens that have been studied after their discovery around the globe. Data regarding their distribution in Tanzania is limited to make an accurate assessment of their importance.

1.8 Problem statement, study justification and objectives

1.8.1 Problem statement and justification of the study

VHFs affect more than 100 million people worldwide and cause deaths to 60,000 individuals annually. The emergence of VHF in new territories is a persistent threat, mainly showing extraordinary potential to establish foci of endemicity in regions far removed from historic risk areas and result in a significant burden of disease (Morens, 2004). Of recent, apart from causing morbidity and mortality, the severity of the disease, and the associated case fatality rate, have changed markedly dependent on the geographic location of the disease locus (Gritsun *et al*., 2003). It is possible that the difference is due to the genotypic variation of the pathogens or individual host immune interactions as the geographical location changes (Mlera *et al*., 2014). There is inadequate information for most VHFs in Tanzania. A better, more comprehensive understanding of the ecology, epidemiology, pathogenicity, and genomic factors is essential in the design of interventions measures. There is a need for seroepidemiological studies to provide evidence on the prevalence and distribution of Ebola, Marburg, Rift Valley fever, yellow fever, and Crimean Congo haemorrhagic fever viruses and explain the risk factors. Moreover, it is not known with certainty that co-infection exists with other endemic diseases such as malaria; which are likely to mimic the febrile syndrome, thus causing challenges in differential diagnosis. Moreover, there is a need to investigate how affected communities respond to these outbreaks; doing so will give Tanzania better insight to prevent the occurrence and be a good entry point in case outbreaks occur. Such information would improve public health, allow more sensitive and specific diagnostic testing, and characterize the prevalence and incidence of these viruses in both endemic and nonendemic areas to implement control and prevention strategies. This study provides important information regarding the elements contributing to the risk associated with the introduction of EVD in Tanzania. The information is crucial for preparedness and prevention.

1.8.2 Main objective

To determine the prevalence, distribution and risk of viral haemorrhagic fevers in Tanzania.

1.8.3 Specific objectives

i. To analyse Ebola Virus Disease outbreak reports, determine the case fatality rate pattern and identify challenges and opportunities in its detection and response in Sub-Saharan Africa.
ii. To estimate the risk of introducing EVD from DRC to Tanzania
iii. To determine the seroprevalence of Ebola, Marburg, Lassa, yellow fever, Crimean Congo Haemorrhagic fever and Rift Valley viruses and their associated risk in Tanzania.
iv. To compare the diagnostic performance of a commercial enzyme-linked immunosorbent assay (ELISA) with indirect ELISA assays validated at the National Institute for Communicable Diseases (NICD, South Africa)
v. To determine the prevalence of malaria and VHF infections among patients seeking health care in Tanzania.
vi. To determine community knowledge, attitude and practices regarding VHF in five geo-ecological zones in Tanzania

1.8.4 Hypotheses of the study

Globalization and continuous interactions between humans, animals and the environment facilitate the maintenance and wide distribution of Viral Haemorrhagic fevers across Sub-Saharan Africa.

Ethics consideration

This study was approved Medical Research Coordinating Committee of the National Institute of Medical Research in Tanzania. When applicable, the participant provided informed consent to provide metadata / serum (parent or legal guardian accented for participants under the age of 16 or illiterate).

CHAPTER TWO

Paper one

Forty-Two Years of Ebola Virus Disease Outbreaks in Sub-Saharan Africa: Challenges and Opportunities for Future Prevention and Control

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Paper two

Estimating Risk of the Introduction of Ebola Virus Disease from the Democratic Republic of Congo to Tanzania: A Qualitative Assessment

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Paper Three

Seroprevalence and Associated Risk Factors of Selected Zoonotic Viral Haemorrhagic Fevers in Tanzania

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Paper Four

Viral Haemorrhagic Fevers and Malaria Co-Infections among Febrile Patients Seeking Health Care in Tanzania

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Paper Five

Community knowledge, attitude and practices regarding zoonotic Viral Haemorrhagic fevers in five geo-ecological zones in Tanzania

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Abstract

Background: Viral haemorrhagic fevers cause significant economic and public health impact in Sub-Saharan Africa. Community knowledge, awareness and practices regarding such outbreaks play a pivotal role in their management and prevention. This study was carried out to assess community knowledge, attitude and practices regarding VHF in five geo-ecological zones in Tanzania.

Methods: This cross-sectional study was conducted in Buhigwe, Kalambo, Kyela, Kinondoni, Kilindi, Mvomero, Kondoa and Ukerewe districts representing five geo-ecological zones in Tanzania. Study participants were selected by multistage cluster sampling design. A semi-structured questionnaire was used to collect socio-demographic and information related to knowledge, attitude and practices regarding VHFs. Descriptive statistics and logistic regression were used for the analysis. Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 27.

Results: A total of 2,965 individuals were involved in the study. Their mean age was 35 (SD±18.9) years. Females accounted for 58.2% while males 41.8%. Most of the respondents (70.6%; n=2093) had never heard of viral haemorrhagic fever, and those who have heard, over three quarters (79%) mentioned the radio as their primary source of information. Slightly over a quarter (29.4%) of the respondents were knowledgeable, 25% had a positive attitude, and 17.9% had unfavourable practice habits. The level of knowledge varied between occupation and education levels ($P<0.005$). Most participants were likely to interact with a VHF survivor or take care of a person suffering from VHF (75%) or visit areas with known VHF (73%). There were increased odds of having poor practice among participants aged 36–45 years (AOR: 3.566, 95% CI: 1.593– 7.821) and those living in Western, North-eastern and Lake Victoria zones (AOR: 2.529, 95% CI: 1.071–6.657; AOR: 2.639, 95% CI: 1.130–7.580 AOR: 2.248, 95% CI: 1.073–3.844).

Conclusion: Overall, the knowledge on VHF among communities is low, while a significant proportion of the community is involved in activities that expose them to the disease pathogens in Tanzania. These findings highlight the need for strengthening health educational and promotion efforts on VHF targeting specific populations**.**

Keywords: Knowledge, practice, exposure, transmission, viral haemorrhagic fever, Tanzania

Introduction

Viral haemorrhagic fevers (VHFs) are severe viral infections characterised by multi-organ failure and haemorrhages resulting in high morbidity and mortality rates (Pigott, 2005). They cause severe epidemics resulting in catastrophic situations that can interrupt everyday life, livelihoods or social structure (Bannister *et al.,* 2010; Fhogartaigh & Aarons, 2015). VHFs of public health importance in Sub-Saharan Africa include Ebola Virus Disease (EVD), Marburg Virus Disease (MVD), Crimean-Congo haemorrhagic fever (CCHF), Rift Valley fever (RVF), Lassa and yellow fever (YF) (Bray, 2005; Changula *et al.,* 2014; Iannetta *et al.,* 2019; Mboussou *et al.,* 2019). Available evidence indicates that RVF and CCHF have been reported in Tanzania (Swanepoel *et al.,* 1987; Sindato *et al.,* 2014). Although no clinical cases of EVD, MVD or YF have been reported in the country, a recent study has reported the presence of IgM and IgG antibodies against the viruses across the country (Rugarabamu *et al.,* 2021).

Viruses causing haemorrhagic fever are transmitted to humans when the activities of the human host and infected reservoir hosts or vectors overlap. The exploitation of new ecological niches by human beings and increased travel and trade as well as climate change have promoted the emergence or re-emergence of VHF around the world (Gould & Higgs, 2009; Racsa *et al.,* 2015). Direct man-made environmental change may also impact ecology and increase interactions between vectors, wild animals, and humans (Nava *et al.,* 2017). The consumption of wild animals (or bushmeat) and animal products such as raw milk and blood is an important driver of VHF emergence (Wolfe *et al.,* 2005). Wild animals are indeed important reservoirs of VHF, and most of the VHF pathogens originate from wildlife (Nyakarahuka *et al.,* 2020). Animal farming plays a significant role in the emergence and the spread of VHFs such as Rift Valley fever and Crimean-Congo Haemorrhagic fevers (Bannister *et al.,* 2010). Mining, hunting, and intensive farming involve significant risks in exposure to VHF reservoirs through means other than commercial production (Kurpiers *et al.,* 2016).

Outbreaks of VHF have been reported in communities with limited knowledge and increased exposure practices (Shabani *et al.,* 2015; Raab *et al.,* 2020). Knowledge and practices can be improved by providing appropriate information on disease recognition, transmission, prevention and management (Ibrahim *et al.*, 2016). Disease prevention education and awareness strategy through traditional and social media have significantly improved knowledge and practice regarding Lassa fever in Nigeria (Wogu, 2018). However, there is a marked gap between VHF awareness and exposure practice in some Sub-Saharan Africa countries. Studies have shown that although most people in Sudan, the Democratic Republic of Congo and Uganda were described to be aware of EVD and RVF, their preventative practice levels were poor (De St. Maurice *et al.,* 2018; Wirsiy *et al.,* 2020).

The frequent outbreaks of viral haemorrhagic fevers in Sub-Saharan Africa highlight the importance of developing national strategy for prevention and management of outbreaks. These include the need for strong health education and promotion programmes. So far, only a limited studies on knowledge, attitude, perceptions and practices as regards to VHF have been carried out in Tanzania. Most of them focused on Rift Valley fever (Swai *et al.,* 2010; Chengula *et al.,* 2013; Shabani *et al.,* 2015). Understanding the community knowledge, attitudes, and practices is important in outbreak prevention and control. In recognition of the growing threats of VHFs in the country, this study was carried out to assess the community knowledge, attitudes, and exposure practices on zoonotic viral haemorrhagic fevers in diverse five ecological zones in Tanzania.

Methods

Study areas and design

This community-based cross-sectional study was carried out from April to November 2018. Respondents were selected using a multistage cluster sampling design. The country was divided into five ecological zones based on rainfall pattern, vegetation, land use pattern and altitude. Zone 1 comprised of the western parts of the country with tropical forest, unimodal rainfall pattern, and altitude <2,300m above sea level. In this zone, Buhigwe (in Kigoma Region) and Kalambo (in Rukwa Region) were selected as study districts. Zone 2 included the Southern highlands regions characterised by high precipitations, bimodal rainfall pattern, tropical forest and elevation >2,300 m. In this zone, Kyela district (in Mbeya Region) was selected. Zone 3 comprised of the north-eastern regions, with bimodal rainfall pattern and an elevation of <1,800 m. In this zone, Kinondoni (in Dar es Salaam Region) and Kilindi (in Tanga Region) districts were selected. Zone 4 covered the central regions of the country, characterised by moderate precipitation and unimodal rainfall pattern. In this zone, the selected districts were Mvomero (Morogoro Region) and Kondoa (Dodoma Region). Zone 5 comprised of the Lake Victoria zone, characterised by bimodal rainfall pattern; and Ukerewe (Mwanza Region) was the selected study district. . In each district, three wards and nine villages were selected to account for local ecological biodiversity variations.

Based on the variations on the ecology for each zone, the number of individuals to be included in the study was calculated independently for each zone. Then population-weighted samples were used to split among the districts. The sample size for this study was calculated based on the conservative prevalence of peoples' knowledge about VHF of 50%, a desired absolute precision of 5%, and a confidence level of 95%. The sample size was adjusted for clustering between districts by a design effect factor of 1.5. A contingency of 10% was considered to account for nonresponses, refusal or missingness. The minimum estimated sample size was 2,840 individuals. Within each district, individuals were evenly distributed between sampled wards and villages.

Data collection

A pre-tested semi-structured questionnaire installed in smartphones with digital data collection tool (Karimuribo et al., 2017) was used to collect sociodemographic and other relevant data. The questionnaire consisted of four sections that addressed the (i) socio-demographic information; (ii) participants' knowledge of the VHFs, source of information; (iii) attitudes towards VHF prevention; and (iv) exposure and risk practices related to VHFs. Trained research assistants administered the questionnaires in Kiswahili, the national language in Tanzania. To avoid stigma and improve participation, all interviews were organised privately.

Data management and analysis

Each response was scored 1 for the correct answer while 0 for the wrong/ "don't know" response. Knowledge about VHF was graded on 0 to 3, with 0 being the lowest and 3 being the highest. Participants' scoring from 2 or 3 was considered "good knowledge" while 0 or 1 indicated "poor knowledge". Attitude about VHF score was reversed graded on a scale of 0 to 3, with a score of 0 and 1 indicating "favourable attitude" and a score of

2 or 3 indicating "unfavourable attitude". Finally, practice against VHF was measured on a scale of 0 to 22. Participants who received a score of 11 or higher were deemed to have optimal/good practice, whereas below 11 were considered to have at-risk/poor practice.

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 27.0. Descriptive analysis was performed, and the results were reported as frequencies and percentages. Bivariable and multivariable logistic regression analyses were conducted to determine the relationship between socio-demographic characteristics and knowledge, attitude and practice levels. All variables with a p-value <0.2 from the bivariable analysis were entered into the multivariable model. Possible associations were measured using an adjusted odds ratio (AOR) with 95% CI, and the p-value of less than 0.05 was considered statistically significant.

Results

Socio-demographic characteristics of the respondents

A total of 2,965 participants were interviewed from 24 wards and 48 villages of eight districts. The mean age was 35 (SD±18.9) years. Over half (58.2%) of the study participants were females, and most respondents (61.6%) had primary school education. The majority of the respondents were farmers. Half (60.8%) were farmers (Table 41).

Table 1: Sociodemographic characteristics of the respondents by district

		Zone 1		Zone 2	Zone 3		Zone 4		Zone 5		
Variable	**Response**	**Buhigwe**	**Kalambo**	**Kyela**	**Kinondoni**	**Kilindi**	**Kondoa**	**Mvomero**	Ukerewe	**Total**	**P-value**
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	
Sex	Female	207(56.2)	209(59.2)	207(55.2)	221(60.2)	203(54.4)	223(59%)	225(64%)	231(65%)	1726(58.2%)	
	Male	161(43.8)	164(40.8)	168(44.8)	146(39.8)	170(45.6)	148(41%)	142(36%)	140(35%)	1239(41.8%)	0.00
Age	18–35	132(33.6)	142(39.6)	142(37.8)	134(36.5)	141(37.8)	135(36.3)	135(36.7)	135(36.4)	1096(37.0%)	
	36–45	126(34.2)	123(33.1)	125(33.4)	125(34.0)	124(33.2)	126(34.0)	122(33.2)	126(33.9)	997 (33.6%)	
	46- 65	110(32.2)	108(29.1)	108(28.2)	108(29.5)	108(29)	110(29.7)	110(30.1)	110(29.7)	872(29.4%)	0.00
Education	None	128(34.7)	107(28.8)	84(22.4)	42(11.5)	131(35.1)	76(20.4)	87(23.7)	80(21.5)	759(25.6%)	
	Primary	225(61.1)	231(62.7)	213(56.7)	226(61.5)	208(55.8)	242(65.3)	236(64.4)	256(69.2)	1826(61.6%)	
	≥Secondary	20(5.6)	32(8.5)	85(25.2)	103(30.7)	49(12.9)	53(14.3)	56(15.3)	47(12.4)	458(15.4%)	0.00
Occupation	Farmer	251(68.1)	278(74.6)	254(67.7)	277(21.1)	59(75.0)	228(61.2)	201(57.6)	240(64.6)	1803(60.8%)	
	Employed	15(4.2)	26(6.8)	40(10.7)	25(52.0)	72(19.5)	38(10.1)	38(10.2)	57(15.4)	461(15.6%)	0.02
	Student	25(6.9)	6(1.7)	29(7.7)	35(9.6)	19(5.2)	45(12.2)	31(8.5}	40(10.8)	228(7.7%)	
	Others	77(20.8)	63(16.9)	52(13.9)	65(17.3)	38(10.3)	60(16.3)	87(23.7)	34(9.2)	473(15.9%)	

None = did not obtained formal education

Knowledge about VHF

Table 2 shows the responses of participants on knowledge on VHF. Only about a third (29.4%) of the study participants had previously heard about VHF. Ebola was the VHF known by the majority (52.6%) of the respondents. Rift valley fever, CCHF, Yellow fever were least mentioned VHF diseases. Other diseases mentioned were Dengue (10%), Chikungunya (5%) and malaria (2.2%). According to 79% of the respondents, radio was the main source of information about VHFs. The majority (81.8%) of the respondents were of the opinion that VHF is preventable (Table 4.2).

Table 2: Knowledge of study participants about VHF

Variable	Category	Frequency	Percentage (%)
Heard of VHF (N = 2965)	Yes	872	29.4
	No	2093	70.6
VHF disease heard (N=872)**	Ebola	460	52.6
	Rift Valley Fever	112	12.8
	Yellow Fever	44	5.0
	Crimean-Congo Haemorrhagic Fever	88	10.1
	Others	150	17.2
	Don’t know	23	2.6
Source of Information (N=872)**	Television	213	24.4
	Radio	688	79.0
	Family	45	5.1
	Friend	22	2.5
	School	180	20.6
	Newspaper	75	8.6
Know how VHF can be prevented (N=872) **	Yes	714	81.9
	No	158	18.1

Multiple responses were allowed

Factors associated with knowledge

Bivariable analysis revealed that education and occupation were highly associated with the knowledge index. The multivariable regression analysis indicated that occupation and education were the two independent predictors of the knowledge index. The employed participants (AOR: 5.82, 95% CI: 1.27–6.48) were more likely to have better knowledge than other participants. Respondents with highest education level had a great understanding of the VHFs compared to their counterparts (AOR: 2.03, 95% CI: 1.27–6.48). Respondents' knowledge did not statistically vary between zones (Table 3.3).

Table 3: Association between knowledge level and socio-demographic characteristics

Variable		Bivariate Analysis Odds ratio (95% CI)	P-value	Multivariate Analysis Adjusted odds ratio (95% CI)	P-value
Sex	Female	1			
	Male	1.097 (0.438–2.745)	0.734		
Age (years)	18–35	1			
	36–45	1.061 (0.410–2.743)	0.903		
	46- 65	1.026 (0.361–2.918)	0.952		
Education	None	1		1	
	Primary	1.02(0.63-1.42)	0.229	1.02(0.71-1.36)	0.243
	≥Secondary	2.06(1.53-2.86)	0.024*	2.03(1.39-2.98)	0.0023*
Occupation	Farmer	3.000(0.968-6.302)	0.027*	1.818 (0.357–9.255)	0.375
	Employed	4.286(1.223-5.022)	0.022*	5.82(1.27–6.48)	0.021*
	Student	0.079(0.009–0.672)	0.020*	0.090(0.008–1.023)	0.054
	Others	1		1	
Zones	Western	1.061 (0.410–2.743)	0.229		
	Southern Highlands	2.842 (0.326–3.792)	0.228		
	North-eastern	1.026 (0.361–2.918)	0.264		
	Central	1.928 (0.493–3.485)	0.962		
	Lake Victoria	1.913 (0.827–4.422)	0.224		

**p-value<0.05*

Attitude toward VHF

The disease fatality was unknown to almost over half of the study participants. Three quarters (75%) of the respondents said would either interact or take care of a person suffering from VHF. A significant proportion of respondents (73%) said that they would visit regions known to experience VHF outbreaks (Table 4. 4).

Table 4: Respondents Attitudes toward VHF (n=872)

Variable	Response	Frequency	Percentage
Living with a person who suffered VHF	Yes	654	75.0
	No	201	23.1
	I don’t know	17	1.9
Taking care of a person with VHF	Yes	654	75.0
	No	183	21.0
	Don’t Know	35	4.0
Travel to regions with VHF	Yes	637	73.0
	No	192	22.0
	Don’t know	44	5.0

Factors associated with attitude

Age, and sex were the two independent predictor variables of attitude towards VHF. Male participants (AOR: 2.72, 95% CI: 1.07–6.89) were more likely to have an unfavourable attitude than females. Participants aged 36 years and above had significantly less favourable attitude than their peers (AOR: 2.95, 95% CI: 1.25–8.27). There was no association between either education level, occupation or zone and attitude (Table 4.5).

Table 5: Association between attitude and socio-demographic characteristics

Variable		Bivariate Analysis Odds ratio (95% CI)	p-value	Multivariate Analysis Adjusted odds ratio (95% CI)	p-value
Sex	Female	1		1	
	Male	2.168 (1.98–5.05)	0.050*	2.72 (1.07–6.89)	0.048*
Age (years)	18–35	1		1	
	36–45	2.600 (1.988–6.769)	0.040*	2.95 (1.25–8.27)	0.038*
	46- 65	1		1	
Education	None	1			
	Primary	1.376 (0.438–4.020)	0.078		
	≥Secondary	0.951 (0.457–2.466)	0.945		
Occupation	Farmer	1.832 (0.805–4.167)	0.149		
	Employed	1	1		
	Student	0.976 (0.356–2.408)	0.770		
	Others	0.876 (0.211–2.312)	0.650		
Zones	Western	1.034 (0.309–2.651)	0.242		
	Southern Highlands	2.542 (0.126–7.692)	0.326		
	North-eastern	1.032 (0.341–2.618)	0.264		
	Central	1.528 (0.493–4.465)	0.663		
	Lake Victoria	1.712 (0.727–3.422)	0.424		

VHF exposure and risk practices

About half (49.7%) of participants admitted to have had received a tick bite, removed a tick and /or crushed a tick with their bare hands during a period of six month before the study. About half (47.9%) of the respondents reported having received a mosquito bite during the previous week. The majority (64%) of the participants were using mosquito nets to protect themselves from mosquito bites. Other exposure practices identified included keeping animals within the household (35.4%), contact with wild animals or rodents (26.6%), consumption of raw milk (24.5%), frequent visits of outdoor recreation facilities (22.4%) and contact with bats (15.9%) (Figure 4.1). Overall, 22% of the participants scored below the mean. The mean exposure practice score was 9.70 (SD = 1.17) out of a maximum of 22 points, indicating a low level of exposure practice.

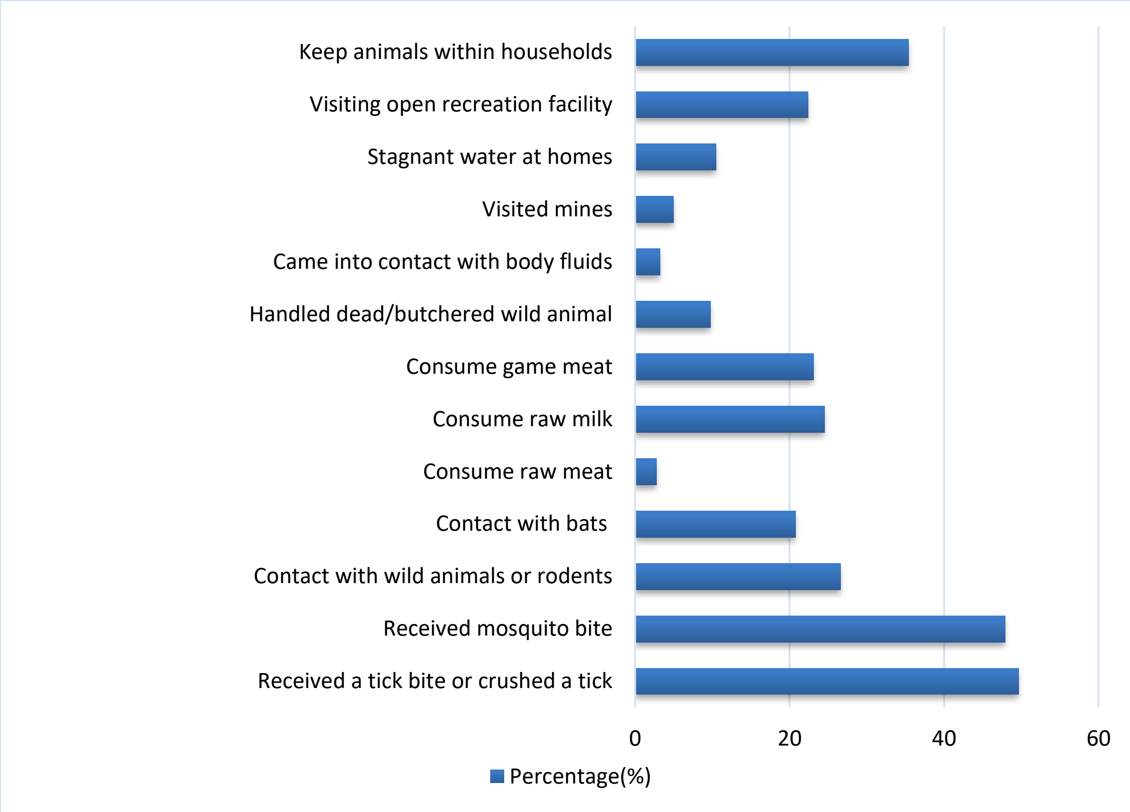

Figure 4.1: Percentage distribution of exposure practise scores

Participants' practices toward VHF exposure

Sex, age and residence of the respondents were significantly associated with the high-risk practices. There were increased odds of VHF exposure and risk practices among males (AOR: 2.95, 95% CI: 1.25– 6.27), participants aged 36–45 years (AOR: 3.58, 95% CI: 1.59– 7.82) and those who reside in Western, North-eastern and Lake Victoria zone (AOR: 2.53, 95% CI: 1.07–6.66; AOR: 2.64, 95% CI: 1.13–7.58 AOR: 2.25, 95% CI: 1.07–3.84) than their peers in the other zones (Table 4.6).

Table 6: Association between practise and socio-demographic characteristics

Variable		Bivariate Analysis Odds ratio (95% CI)	P-value	Multivariate Analysis Adjusted odds ratio (95% CI)	P-value
Sex	Female	4.08 (0.47– 5.43)	0.202	4.82(0.38-6.59)	0.228
	Male	2.95 (1.27–5.27)	0.005*	2.95(1.25–6.27)	0.046*
Age (years)	18–35	1.04 (0.36–3.06)	0.938	1.34 (0.13–1.45)	0.300
	36–45	4.08 (1.47– 5.43)	0.040*	3.58 (1.59–7.82)	0.006*
	46- 65	2.95 (1.26–5.27)	0.005*	2.89 (0.09-6.023)	0.052
Education	None	1			
	Primary	1.376 (0.438–4.020)	0.078		
	≥Secondary	1.133 (0.262–3.99)	0.063		
Occupation	Farmer	1.832 (0.805–4.167)	0.449		
	Employed	1	1		
	Student	0.98 (0.36–2.41)	0.770		
	Others	0.88 (0.21–2.31)	0.650		
Zone	Western	2.44 (1.05–5.66)	0.038 *	2.53 (1.17–6.66)	0.039*
	Southern Highlands	2.35 (0.23–4.49)	0.465	2.07(0.44-2.85)	0.865
	North-eastern	2.74 (1.14–6.58)	0.024 *	2.64(1.13–7.58)	0.022*
	Central	1.01 (0.22 –2.32)	0.3440	1.00(0.09-4.67)	0.388
	Lake Victoria	2.32 (1.11–3.94)	0.035*	2.25(1.97–3.84)	0.024*

*P-value<0.05

Discussion

This study aimed to determine the level of community knowledge and practices in Tanzania following the increased spread of VHFs in Sub-Saharan countries. Only about a quarter of the respondents have heard of VHFs. About three quarters of the respondents were not aware of VHFs, indicating that majority of the people in Tanzania are not familiar with the diseases. The level of awareness observed in the current study is lower than that reported in previous study among the pastoral community in previous studies in Tanzania (Shaban *et al.,* 2015) and the general population in Uganda (Nyakarahuka *et al.,* 2017), Kenya (Abdi *et al.,* 2015) and DRC (Oppenheim *et al.,* 2019). Except for Rift Valley Fever, which most pastoral communities in Tanzania have herd of (Shabani *et al.,* 2015) most likely due to previous outbreaks (Sindato *et al.,* 2014), most other VHF have not been reported in the country hence not been given much attention in community awareness programmes.

The most commonly known VHF among the community in this study was Ebola. The knowledge about Ebola was likely to be associated with the fact it has occurred in the Democratic Republic of Congo during this study period, and several national and international communication channels have raised awareness about the disease (Mayhew *et al.,* 2021). In a similar study in West Africa, most members of the community could identify EVD as the most known VHF (Raab *et al.,* 2020). These findings suggest that knowledge about EVD is the general consequence of heightened awareness that the recent outbreaks in West Africa and DRC have created around the globe. EVD has sharpened people's knowledge and practice because it has dominated sub-Saharan

Africa's daily lives for so long (Masumbuko *et al.,* 2019). From our study, most of those who knew VHF named mass media, specifically radio, as their main sources of information. Similar findings have been reported in previous studies in Tanzania (Chengula *et al.,* 2013; Shaban *et al.,* 2015) and elsewhere in Africa (Nettey *et al.,* 2016). This indicates that mass media channels, are important sources of information. Three-quarters of the study participants had unfavourable attitudes toward VHF. This could be attributed to the fact that the majority of them had limited knowledge about the diseases. Our findings contrast with those previously reported from VHF endemic regions (Raab *et al.,* 2020).

This study found that occupation and education were important determinants of good VHF knowledge. A similar finding was reported from Tanzania, Sierra Leone, Kenya and Uganda (Abdi *et al.,* 2015; Nyakarahuka *et al.,* 2017; Jalloh *et al.,* 2017. Employed participants were more likely to have good knowledge. Respondents who had education were more knowledgeable of the disease than their peers. This finding is concurrent with a study conducted in Guinea where educated people had better knowledge on EVD than those not educated (Raab *et al.,* 2020). In a study in Ghana, people with no education, those not working were reported to be the least knowledgeable in EVD (Nettey *et al.,* 2016). Educational level, monthly income has been reported to be significantly associated with practices as regards to infectious disease prevention and control (Wake, 2020).

Males and adults 36–45 years old and people living in Western, North-eastern and Lake Victoria districts were at higher odds of having poor practice as regards to VHFs. The possible reason for this might be because participants in this group are in the adulthood stage, which allows them to be socially proactive and on-demand of exposure practice such as taking care of sick, hunting or mining. The finding also indicates that the areas with odds of exposure practise have characterisation of risk of occurrence of mosquito- and tick-borne viral diseases in Tanzania (Mweya *et al.,* 2016; Vairo *et al.,* 2016; Mwanyika et al., 2021). Moreover, a recent serological study in Tanzania has shown that the districts in the three zones to have relatively higher prevalence of VHF antibodies (Rugarabamu *et al*., 2021).

The findings of this study have highlighted other exposure practices that would put the community at risk of VHF. These include tick bites, crushing ticks with bare hands, mosquito bites, and contact with wild animals including rodents, birds and bats. Other identified practices were handling and butchering of wild animals, contact with the body fluids, or direct contact with dead animals/ individuals. Even though few respondents practised this, it takes only a single person to be exposed to a virus before the disease would spread to the community. Thus, when it comes to VHF transmission and spread low percentage of risk practice would sound large for an epidemic to occur. Educational programs should encourage avoiding exposure practices that would put a community at risk of disease. In addition, a heightened awareness of VHF may lead to more rapid identification of outbreaks and may result in decreased transmission.

Our study showed that about one-fifth of respondents consumed raw meat, raw milk, raw blood, game (including primate) meat, which are among the risk practices for the occurrence of RVF and EVD (De St. Maurice *et al.,* 2018). Some studies have reported exposure to raw milk, raw meat or blood as a potential route for RFV transmission (Olive *et al.,* 2016; Mutua *et al.,* 2017; Grossi-Soyster *et al.,* 2019). Animal products, regardless of status, is an important part of the diet in Tanzania. Milk and meat are mostly raw, fermented, and rarely boiled (Århem, 1989). Overall, the findings emphasise the importance of educational campaigns and endless activities towards behavioural change in communities living in risk areas.

Conclusions

Based on the findings, it can be concluded that there is a low level of community knowledge regarding VHF despite higher exposure risk behaviour in Tanzania. Only about a third of the study participants had previously heard about VHF; and the majority have heard of Ebola. Occupation and education were the most important predictors of the knowledge index. Respondents with highest education level had a great understanding of the VHFs than those with low education levels. Male participants were more likely to have an unfavourable attitude than females. Sex, age and residence of the respondents were significantly associated with the high-risk practices. These findings call for heightening VHF awareness and prevention campaigns in Tanzania.

Declarations

Ethics approval and consent to participate

This study was approved by the Tanzania Medical Research Coordinating Committee of the National Institute for Medical Research (Reference: NIMR/HQ/R.8a/Vol. IX/2724). Written informed consent was obtained from each participant. All procedures performed in the study were in accordance with the ethical standards of this institution as well as with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Participation in the study was voluntary. Informed consent was obtained from all individual participants included in the study. The interviewer obtained informed consent by a written signature from participants (or their parent or legal guardian in the case of illiterate participants) prior to each interview. To ensure data confidentiality and anonymity, data were anonymized and irreversibly de-identified to protect patients, healthcare providers, hospital and community privacy.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Competing interests

All authors declare no conflicts of interest.

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Authors' contributions

S.R. and GOM carried out data collection and analysis. A first draft of the manuscript was prepared by S.R. and LEGM, then further revised by CS, SFR, GOM, H-YL, GM. All authors reviewed the manuscript.

Data Availability Statement

The datasets generated for this study are available on request to the corresponding author.

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Conflict of interest

The authors declare that they conducted the research in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

Abdi, I. H., Affognon, H. D., Wanjoya, A. K., Onyango-Ouma, W. and Sang, R. (2015). Knowledge, attitudes and practices (KAP) on Rift Valley fever among pastoralist communities of Ijara District, North Eastern Kenya. *PLoS Neglected Tropical Diseases* 9(11): 39 – 42.

Århem, K. (1989). Maasai food symbolism: The cultural connotations of milk, meat, and blood in the pastoral Maasai Diet. *Anthropos* 84(1/3):1–23.

Bannister, B. (2010). Viral haemorrhagic fevers imported into non-endemic countries: risk assessment and management. *British Medical Bulletin* 95(1): 193–225.

Bray, M. (2005). Pathogenesis of viral haemorrhagic fever. *Current Opinion in Immunology* 17(4): 399 – 403.

Changula, K., Kajihara, M., Mweene, A. S. andTakada, A. (2014). Ebola and Marburg virus diseases in Africa: Increased risk of outbreaks in previously unaffected areas? *Microbiology and Immunology* 58(9): 483 – 491.

Chengula, A. A., Mdegela, R. H. and Kasanga, C. J. (2013). Socio-economic impact of Rift Valley fever to pastoralists and agro-pastoralists in Arusha, Manyara and Morogoro regions in Tanzania. *Springer Plus* 2: 549 – 557.

De St. Maurice, A., Nyakarahuka, L., Purpura, L., Ervin, E., Tumusiime, A. Balinandi, S. (2018). Rift Valley Fever: A survey of knowledge, attitudes, and practice of slaughterhouse workers and community members in Kabale District, Uganda. *PLoS Neglected Tropical Diseases* 12(3): 175 – 187.

Fhogartaigh, C. N. and Aarons, E. (2015). Viral haemorrhagic fever. *Clinical Medicine* 15(1): 61–66.

Gould, E. A. and Higgs, S. (2009). Impact of climate change and other factors on emerging arbovirus diseases. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103(2): 109–121.

Grossi-Soyster, E. N., Lee, J., King, C. H. and LaBeaud, A.D. (2019). The influence of raw milk exposures on Rift Valley fever virus transmission. *PLoS Neglected Tropical Diseases* 13(3): 1 – 28.

Iannetta, M., Di Caro, A., Nicastri, E., Vairo, F., Masanja, H. and Kobinger, G. (2019). Viral Hemorrhagic Fevers other than Ebola and Lassa. *Infectious Diseases Clinics of North America* 33: 977 – 1002.

Ibrahim, A. A. and Elshafie, S. S. (2016). Knowledge, awareness, and attitude regarding infection prevention and control among medical students: a call for educational intervention. *Advances in Medical Education and Practices* 7: 505 – 510.

Jalloh, M. F., Sengeh, P., Monasch, R., Jalloh, M. B., DeLuca, N. and Dyson, M. (2017) National survey of Ebola-related knowledge, attitudes and practices before the outbreak peak in Sierra Leone: August 2014. *BioMed Journal Global Health* 2: 285 – 296.

Karimuribo, E. D., Mutagahywa, E., Sindato, C., Mboera, L., Mwabukusi, M., Kariuki, N. M. (2017). A Smartphone App (AfyaData) for Innovative One Health Disease Surveillance from Community to National Levels in Africa: Intervention in Disease Surveillance. *JMIR Public Health Surveillance* 3(4): 1 – 12.

Masumbuko, C. K., Underschultz, J. and Hawkes, M. T. (2019). Social resistance drives persistent transmission of Ebola virus disease in Eastern Democratic Republic of Congo: A mixed-methods study. *PLoS One* 14(9): 1 – 30.

Mayhew, S. H., Kyamusugulwa, P. M., Kihangi, B. K., Richards, P., Kiyungu, C. and Balabanova, D. (2021). Responding to the 2018-2020 Ebola Virus Outbreak in the Democratic Republic of the Congo: Rethinking Humanitarian Approaches. *Risk Management and Healthcare Policy* 14: 1731–1747.

Mboussou, F., Ndumbi, P., Ngom, R., Kamassali, Z., Ogundiran, O. and Van Beek, J. (2019). Infectious disease outbreaks in the African region: an overview of events reported to the World Health Organization in 2018. *Epidemiology and Infection* 147: 1 – 16.

Mutua, E. N., Bukachi, S. A., Bett, B. K., Estambale, B. A. and Nyamongo, I. K. (2017). We do not bury dead livestock like human beings": community behaviors and risk of Rift Valley fever virus infection in Baringo County, Kenya. *PLoS Neglected Tropical Diseases* 11(5): 1 – 22.

Mwanyika, G. O., Sindato, C., Rugarabamu, S., Rumisha, S. F., Karimuribo, E. D., Misinzo, G. (2021).Seroprevalence and associated risk factors of chikungunya, dengue and Zika in Tanzania. *International Journal of Infectious Diseases* 111: 271 – 280.

Mweya, C. N., Kimera, S. I., Stanley, G., Misinzo, G. and Mboera, L. E. G. (2014). Climate change influences the potential distribution of infected Aedes aegypti co-occurrence with dengue epidemics risk areas in Tanzania. *PLoS One* 11(9): 1 – 16.

Nava, A., Shimabukuro, J. S., Chmura, A. A. and Bessa, S. L. (2017). The Impact of global environmental changes on infectious disease emergence with a focus on risks for Brazil. *Institute for Laboratory Animal Research Journal* 58(3): 393–400.

Nettey, O. E. A., Enuameh, Y. A., Zandoh, C., Anane, E. A., Abukari, M., Agbokey, F. (2016). Knowledge, attitudes and preventive practices on ebola virus disease in the Kintampo Districts of Ghana. *Health* 8(14): 1–10.

Nyakarahuka, L., Skjerve, E., Nabadda, D., Sitali, D.C., Mumba, C. and Mwiine, F. N. (2017). Knowledge and attitude towards Ebola and Marburg virus diseases in Uganda using quantitative and participatory epidemiology techniques. *PLoS Neglected Tropical Diseases* 11(9): 1 – 36.

Nyakarahuka, L., Schafer, I.J, Balinandi, S., Mulei, S., Tumusiime, A. and Kyondo, J. (2020). A retrospective cohort investigation of seroprevalence of Marburg virus and ebolaviruses in two different ecological zones in Uganda. *BioMed Central Infectious Diseases* 20: 461 – 472.

Olive, M. M., Chevalier, V., Grosbois, V., Tran, A., Andriamandimby, S. F. and Durand, B. (2016). Integrated analysis of environment, cattle and human serological data: risks and mechanisms of transmission of Rift Valley fever in Madagascar. *PLoS Neglected Tropical Diseases* 10(7): 1 – 21.

Oppenheim, B., Lidow, N., Ayscue, P., Saylors, K., Mbala, P., Kumakamba, C. and Kleinman, M. (2019). Knowledge and beliefs about Ebola virus in a conflict-affected area: early evidence from the North Kivu outbreak. *Journal of Global Health* 9(2): 1 – 31.

Pigott, D. C. (2005). Hemorrhagic fever viruses. *Critical Care Clinics* 21(4): 765–785.

Raab, M., Pfadenhauer, L. M., Millimouno, T. J., Hoelscher, M. and Froeschil, G. (2020). Knowledge, attitudes and practices towards viral haemorrhagic fevers amongst healthcare workers in urban and rural public healthcare facilities in the N'zérékoré prefecture, Guinea: a cross-sectional study. *BioMed Central Public Health* 20: 296 – 318.

Racsa, L. D., Kraft, C. S., Olinger, G. G. and Hensley, L. E. (2016). Viral Hemorrhagic Fever Diagnostics. *Clinical infectious Diseases* 62(2): 214–219.

Rugarabamu, S., Mwanyika, G. O., Rumisha, S. F., Sindato, C., Lim, H. Y. and Misinzo, G. (2021) Seroprevalence and associated risk factors of selected zoonotic viral hemorrhagic fevers in Tanzania. *International Journal of Infectious Diseases* 109: 174 – 181.

Shabani, S. S., Ezekiel, M. J., Mohamed, M. and Moshiro, C. S. (2015). Knowledge, attitudes and practices on Rift Valley fever among agro-pastoral communities in Kongwa and Kilombero districts, Tanzania. *BioMed Central Infectious* Diseases 15: 363 – 385.

Sindato, C., Karimuribo, E. D., Pfeiffer, D. U., Mboera, L. E. G., Kivaria, F. and Dautu, G. (2014). Spatial and temporal pattern of Rift Valley fever outbreaks in Tanzania; 1930 to 2007. *PLoS One* 9(2): 1 – 17.

Swai, E., Schoonman, L. and Daborn, C. (2010). Knowledge and attitude towards zoonoses among animal health workers and livestock keepers in Arusha and Tanga, Tanzania. *Tanzania Journal of Health Research* 12: 282 – 288.

Swanepoel, R., Shepherd, A. J., Leman, P. A., Shepherd, S. P. and McGillivray, G. M. 1987). Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. *American Journal of Tropical Medicine and Hygiene* 36(1): 120 – 132.

Vairo, F., Mboera, L. E. G., De Nardo, P., Oriyo, N. M., Meschi, S. and Rumisha, S. F. (2016). Clinical, virologic, and epidemiologic characteristics of Dengue Outbreak, Dar es Salaam, Tanzania, 2014. *Emerging Infectious Diseases* 22 (5), 895-899.

Wake, A. D. (2020). Knowledge, attitude, practice, and associated factors regarding the novel coronavirus disease 2019 (COVID-19) Pandemic. *Infection and Drug Resistance* 13: 3817–3832.

Wirsiy, F. S., Boock, A. U. and Akoachere, J. F. T. K. (2021). Assessing the determinants of Ebola virus disease transmission in Baka Community of the Tropical Rainforest of Cameroon. *BioMed Central Infectious Diseases* 324: 1 – 22.

Wogu J. O. (2018). Mass media awareness campaign and the prevention of the spread of Lassa fever in the rural communities of Ebonyi State, Nigeria: An impact evaluation. *Journal of Public Health in Africa* 9(3): 882 – 884.

Wolfe, N. D., Daszak, P., Kilpatrick, A. M. and Burke, D. D. (2005). Bushmeat hunting, deforestation, and prediction of zoonoses emergence. *Emerging Infectious Diseases* 11(12): 1822 – 1827.

Paper Six

Comparison of an In-House And a Commercial Enzyme-Linked Immunosorbent Assays for Diagnosis of Ebola Virus Disease and Rift Valley Fever

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Abstract

Background: Infection with Ebola or Rift Valley viruses can result into haemorrhagic disease and death. A number of serosurveys have reported exposure to Rift valley virus (RVFV) in human populations across Tanzania and a recent study found evidence of antibodies to Ebola virus (EBOV) in the western Tanzania. Various serological diagnostic tools have been used to detect exposure to RVFV and EBOV, but the agreement between these tools has not been critically evaluated. The aim of this study was to compare the diagnostic performance of a commercial enzyme-linked immunosorbent assay (ELISA) with indirect ELISA assays validated at the National Institute for Communicable Diseases (NICD, South Africa) for the detection of IgG antibodies against RVFV and EBOV in human sera.

Methods: This laboratory-based study used human sera collected in Tanzania. Archived sera that tested positive for immunoglobulin IgG antibodies to EBOV and RVFV using a commercially available ELISA (Rugarabamu *et al*., 2021) were reanalysed using the in-house EBOV whole antigen (WAg) and RVFV inhibition enzyme-linked immunosorbent assay (Paweska *et al*., 2005; Paweska *et al*., 2019).

Results: All EBOV positive sera turned out to be negative by In-House WAg EBOV ELISA. Two of the nine positive IgG RVF commercial ELISA samples were reactive to the RVF In-house ELISA, with inhibitory rates ranging from 66% to 99.6%. A slight agreement was observed between the RVF in-house and the commercial ELISA (kappa value, 0.08; 28%, 11/40 in disagreement).

Conclusion: We found poor agreement between in-house and commercial ELISA tests for EBOV and fair agreement between in-house and commercial ELISA tests for RVFV, with the commercial ELISA detecting more cases than the validated in-house ELISA. The current study emphasizes the challenges in diagnosing viral hemorrhagic fever in Tanzania.

Keywords: Rift valley fever, Ebola virus disease, Tanzania, serologic tests, ELISA,

Background

Viral haemorrhagic fevers (VHFs) are a group of zoonotic diseases caused by several distinct groups of viruses from the families *Flaviviridae, Phenuiviridae, Arenaviridae, and Filoviridae.* The VHFs reported in Sub-Saharan Africa include Crimean-Congo haemorrhagic fever (CCHF), Ebola virus disease (EVD), Lassa fever (LF), Lujo haemorrhagic fever (LUHF), Marburg virus disease (MVD), Rift Valley fever (RVF), and yellow fever (YF) (Bray, 2005; Changula *et al*., 2014; Iannetta *et al*., 2019; Mboussou *et al*., 2019). The World Health Organization considers CCHF, EVD, MVD, and RVF among the top 10 pathogens that pose the most significant risk to public health (WHO, 2018). A number of countries in Africa have experienced sporadic and large outbreaks of VHFs in recent years (Ericsson, 2001; Nicastri *et al*., 2019; Iannetta *et al*., 2019; Rugarabamu *et al*., 2020).

When VHF is suspected, prompt diagnostic laboratory testing is imperative (Pang *et al*., 2013). Rapid access to specialized laboratory testing varies according to region and is especially a challenge in resource-limited areas (Racsa *et al*., 2016). The enzyme-linked immunosorbent assay (ELISA) and reverse transcription polymerase chain reaction (RT-PCR) are mainstays of EVD and RVF diagnosis (Niikura *et al*., 2001). Although extensive validation of these assays has not been conducted, sensitivities and specificities are generally considered to be over 90% (Racsa *et al*., 2016). Unfortunately, the specificity of ELISA diagnostic assays for VHFs is challenged by high degree of cross reactivity due to the similarity that exists between pathogens (Gomez *et al* 2008; Sakamoto *et al*., 2018). On the other hand, PCR-based diagnostics are sensitive and can be rapidly be developed for emerging or mutating viruses, but they are beyond the resources and capability at a level lower than the national level in most low-and middle-income countries. The feasibility of an ELISA for the detection of antibodies to VHF has been reported (Swanepoel *et al*., 1986; Paweska *et al*., 1995). Most countries have access to commercial kits, however most of kits have not been adequately validated (Hur *et al*., 2020; Nyakarahuka *et al*., 2020). This is largely due to the difficulties of obtaining panels of clinical materials sufficient for assessing their effectiveness (Tighe *et al*., 2015).

A number of serosurveys have reported exposure of RVFV in human populations across Tanzania (Ahmed *et al*., 2018; Matiko *et al*., 2018; Budodo *et al*., 2020; Medard *et al*.,2021; Rugarabamu *et al*., 2021, Sindato *et al*., 2022). Very recently, a study that investigated seroprevalence and risk factors for a selection of zoonotic viral haemorrhagic fevers in Tanzania and found evidence of IgG antibodies to EBOV in the western zone of Tanzania (Rugarabamu *et al*., 2021).

Assay comparisons are in high demand due to the reported increase in the magnitude of exposure to viral haemmorhagic fever viruses research results. The Centre for Emerging Zoonotic and Parasitic Diseases at the National Institute for Communicable Diseases (NICD) in Johannesburg developed and tested point-of-care (POC) rapid diagnostic tests (RDTs) based on derived antigen for the detection of antibodies to EBOV and RVFV in humans (Paweska *et al*., 2005; Paweska *et al*., 2019). The cut-off values for these assays were chosen to be 95% accurate. The ELISA sensitivity was approximately 99%. These assays are useful for reporting diagnostic results, comparing patient results between diagnostic laboratories, evaluating ELISA efficacies, determining seroprevalence rates, infection risk population studies, and determining the occurrence of asymptomatic infections (Thomas *et al*., 1999; Furuyama *et al*., 2020). VHFs' serological diagnosis and epidemiological investigations are facing challenges of limited diagnostic capacities. Low diagnostic capacity affects the interpretation of the findings of such epidemiological surveys and, hence, results in inadequate infection control practices. The objective of this study was to compare the diagnostic performance of a commercial enzyme-linked immunosorbent assay (ELISA) with indirect ELISA assays validated at the National Institute for Communicable Diseases (NICD, South Africa) for the detection of IgG antibodies against RVFV and EBOV in human sera.

Materials and Methods

Source of sera

A panel of serum samples obtained from individuals in Buhigwe, Kalambo, Kyela, Kilindi, Kinondoni, Kondoa, Mvomero, and Ukerewe districts in Tanzania and archived at Sokoine University of Agriculture University of Agriculture, Morogoro was used. Aliquots of the sera were previously tested for the presence of human immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies against CCHF, EVD, MBV, RVF, and YF using commercial ELISA kits (Rugarabamu *et al*., 2021). These sera were reanalysed using the in-house EBOV whole antigen (WAg) and RVFV inhibition enzyme-linked immunosorbent assay (Paweska *et al.,* 2005; Paweska *et al.,* 2019).

Sample and data collection

As part of standard testing, validated NICD in-house Indirect Enzyme-Linked Immunosorbent Assays for the detection of antibodies to EBOV and RVFV were performed on sera chosen based on clinical and laboratory findings from archival records. Two groups of sera were formed. Group 1 included both positive and negative EVD sera. Group 2 consists of sera from both positive and negative RVF. The NICD in-house ELISA testing was carried out at the Special Viral Pathogens Laboratory (SVPL), Centre for Emerging Zoonotic and Parasitic Diseases (CEZPD), as previously described (Paweska *et al*., 2005; Paweska *et al*., 2019).

Laboratory procedures

EBOV detection

Microtiter 96-well plates (Maxisorb Immunoplates, Nunc, Roskilde, Denmark) for Whole Antigen ELISAs were coated with EBOV WAg and corresponding control antigen. WAg and control antigens were added to the rows of the top half of the plate (rows A–D: 1–12) and the bottom half of the plate (rows E–H: 1–12), respectively. Antigens were diluted in phosphate PBS (30 ml of antigen and control in 6 mls of Phosphate-Buffered Saline (PBS). Each 96-well plate had four replicates of positive serum control (C++), two replicates of negative serum control (C−) and two replicates of conjugate control (Cc). For WAg, C++ was added to wells A 1–2 and B 1–2 coated with virus antigen and to corresponding wells E 1–2 and F 1–2 coated with a control antigen. C was added to C 1–2 and G 1–2, and Cc (1 ml milk in PBS) was added to D 1–2 and H 1–2. The remaining wells (A–D 3–12 and E–H 3–12) were used to test 20 test sera in duplicate, with test serum no. 1 added to wells A3 and B3 and corresponding E3 and F3; the remaining test sera were added in the same manner. All reagents were added to the immunoplates at a volume of 100 µL/well unless otherwise stated. Passive adsorption onto ELISA plates was performed at 4°C overnight and all subsequent incubations (except for substrate addition) were performed at 37°C in a humidified chamber for 1 hour.

Following coating, plates were washed three times with 300 µL of PBS containing 0.1% Tween 20. The same washing procedure followed each subsequent stage of ELISA. Plates were blocked with 200 µL 10% non-fat milk powder in PBS. After incubation, plates were washed, and control and test sera diluted 1:400 in 2% non-fat milk powder in PBS (diluent buffer) were added. Each test serum, negative control serum and conjugate control was tested in duplicate, and positive control was tested in quadruplicate. Following incubation with the sera, the plates were washed and horse raddish-conjugated goat anti-human IgG (Invitrogen, ThermoFisher Scientific, USA). After incubation, plates were washed and 2, 2'-azinodiethylbenzothiazoline sulfonic acid (ABTS) peroxidase (Seracare Lifesciences, Milford, MA, USA) substrate added to the wells. Plates were incubated in a dark at room temperature (±22 °C) for 30 minutes. Reactions were stopped by the addition of 1% sodium dodecyl sulphate and the optical densities (OD) readings were measured at 405 nm. OD readings were converted into a percentage of the positive internal control serum (PP) using the equation as previously described (Paweska *et al.,* 2005). Briefly, a specific activity of each serum (net OD) was calculated by subtracting the non-specific background OD in the wells with control antigen from the OD in wells with virus antigen. The mean net OD readings were converted to PP using the formula: PP = (mean net of test serum/mean net OD of positive control) × 100. The cut off for positive samples' PP values was ≥13, with sensitivity and specificity being 96.4% and 99.6% respectively (Paweska *et al*., 2018).

RFV detection

Inhibition ELISA was used to test for RVF using a protocol as previously described (Paweska *et al*., 2005). Plates were coated overnight at 4°C with 100 µl polyclonal sheep anti-RVF capture antibody diluted at 1:500 in PBS. After incubation, plates were washed three times with the washing buffer (0,1% Tweeen-20 in PBS) and incubated with 10% skim milk in PBS in a moist chamber for 1 hour at 37°C. Plates were washed 3 times with the washing buffer and duplicate volumes of 100 µl of test and control sera (NICD-SPU) diluted 1:400 added in rows A–D; 1–12 respectively to the corresponding wells in the bottom half of the plate (rows E–G: 1–12). After incubation at 37°C for 1 h and washing 6 times with washing buffer, 100 µl of the virus (NICD-SPU) and control antigen (NICD-SPU) diluted 1:200 in PBS containing 2% skim milk were added to both the rows of the top half of the plate (rows A–D: 1–12) and of the bottom half of the plate (rows E–G: 1–12) respectively. Plates were incubated for 1 h at 37°C, washed 3 times with the washing buffer and mouse anti-RVF serum diluted 1:3,000 added to each well of the plate.

Plates were incubated again for 1 h at 37°C, washed 3 times with the washing buffer and goat anti-mouse IgG conjugated with peroxidase diluted 1:4,000 added to each well for 1,h at 37°C. Plates were washed 6 times with the washing buffer and the reaction was developed by the addition of ABTS (KPL Laboratories, Inc.). After incubation in a dark at room temperature for 30 min the reaction was stopped by the addition of 100 µl 1% SDS. Optical densities (OD) were determined at 405 nm. A specific activity of each serum (net OD) was calculated by subtracting the non-specific background OD in the wells with control antigen from the specific OD in wells with virus antigen. The mean OD readings for replicate tests were converted to a percentage inhibition (PI) value using the equation: [(100 – (mean net OD of test sample / mean net OD of negative control) x 100]. The cut off for positive samples' PP values was ≥60%, with sensitivity and specificity being 96.4% and 99.6% respectively (Paweska *et al*., 2005).

Statistical analysis

The data were entered in Excel (Microsoft Office Excel, 2012). A descriptive statistical analysis was performed and the agreement between test results was calculated. The level of agreement was expressed by means of the Cohen kappa statistic (McHugh *et al.,* 2012), using the following classification: “slight” (kappa value: 0.01–0.20), “fair” (0.21–0.40), “moderate” (0.41–0.60), “substantial” (0.61–0.80), or “almost perfect” agreement (>0.81) (Julius *et al*., 2005).

Ethical considerations

This study was approved by the Medical Research Coordinating Committee of the National Institute of Medical Research in Tanzania (Reference No. NIMR/HQ/R.8c/ Vol.1/1612). Written informed consent was obtained from all subjects ≥18 years, and consent was obtained from parents or guardians of the respective individuals <18 years old following subject’s assent.

Results

The 24 commercially positive ELISA sera (14 RVF and 10 EBOV) had an average age of 15 ± 9 years (Table 5.1). More than half were female (16/24, 66.6%), and the majority were farmers (37.5%). Most of positive sera were obtained from health facilities. Overall, 75% (n = 18) were found to have fever during the clinical examination. A total of five (14%) individuals tested positive for malaria.

Table 1: Sociodemographic characteristics of commercial ELISA positive samples examined at the Special Viral Pathogens Laboratory (SVPL), Centre for Emerging Zoonotic and Parasitic Diseases (CEZPD), (n = 24)

Variable		**RVF**		**EVD**	
		IgG	**IgM**	**IgG**	**IgM**
Age (years)	0-15	1	-	1	1
	16–30	3	2	2	1
	31–45	2	1	2	1
	46- 60	1	1	1	-
	61-75	1	1	1	-
	≥ 76	1	-	-	-
Sex	Female	5	4	5	2
	Male	4	1	2	1
Education level	None	3	2	3	2
	Primary	3	2	3	1
	Secondary	2	1	1	1
	College	1	-	-	-
Occupation	Livestock keeping	4	1	-	-
	Business	1	1	2	-
	Crop farming	3	2	3	1
	Employed	-	-	-	-
	Student	1	1	-	1
	Others	-	-	1	1
Residence	Buhigwe	1	1	6	2
	Kinondoni	2	2	0	0
	Mvomero	2	1	0	0
	Kyela	0	1	0	0
	Kalambo	1	0	1	1
	Kondoa	1	0	0	0
	Kilindi	0	0	0	0
	Ukerewe	2	0	0	0
Sampling location	Household	2	2	2	1
	Facility	7	3	5	2
Current fever	Yes	6	3	5	3
	No	3	2	2	0
Malaria	Positive	2	0	2	1

Key: RVF= Rift Valley fever; EVD= Ebola Virus Disease

The results for the tested sera with both inhouse and commercial ELISA are shown in Table 2 None of the tested sera were positive for the In-House whole antigen EBOV ELISA. These sera included seven sera which were IgG positive, three of which had the highest OD reading; three sera which were IgM positive by the commercial ELISA; and 10 sera, five from those tested negative and five from other VHF positive samples. The in-house RVF-ELISA identified two positive IgG seropositive results in 22.2% of cases (2/9) (Table 2).

Table 2: Serological results of serum samples with in-house and commercial ELISA

EBOV ELISA		RVF ELISA	
Inhouse	Commercial	Inhouse	Commercial
-	-	+	+
-	+	+	+
-	-	-	-
-	+	-	-
-	+	-	-
-	+	-	+
-	-	-	+
-	+	-	+
-	+	-	+
-	-	-	-
-	-	-	+
-	-	-	-
-	-	+	NT
-	+	+	NT
-	-	-	-
-	+	-	-
-	-	-	-
-	+	-	-
-	+	-	-
-	-	-	+
-	-	-	+
0/20 (0%)	10/20 (50%)	2/40 (5%)	9/40 (23%)

Key: NT- Not tested - = Negative += Positive

For the sera tested, only a slight agreement was observed between the RVF in-house and the commercial ELISA (kappa value, 0.08, mainly due to positive result in the in-house Ab-ELISA and in the commercial Ab-ELISA (28%, 11/40 in disagreement). Kappa value could not be established since the positive value for In-house EBOV was zero however there was agreement of 52% between Wag EBOV in-house and the commercial ELISA mainly due to negative result in the in-house ELISA and the commercial ELISA.

Table 3: Distribution of EBOV in-house ELISA percentage positivity value for the tested samples

Sample ID	Mean	Median	Percentage Positivity
C++	1.06175	1.072	100.9654
C-	-0.283		-26.6541
CC	-0.001		-0.09418
BUH 077*	0.007		0.659289
KLM 476	-0.009		-0.84766
BUH 279	-0.026		-2.44879
KLN282	-0.007		-0.65929
KLM 261*	0.0205		1.930775
BUH 032*	0.007		0.659289
BUH 444	-0.003		-0.28255
BUH 406	-0.003		-0.28255
BUH 212	-0.001		-0.09418
BUH 181	-0.0035		-0.32964
BUH 332	-0.019		-1.7895
BUH 383	-0.106		-9.98352
BUH 211	-0.022		-2.07205
BUH 107	-0.0445		-4.19119
BUH 334	-0.03		-2.82552
KYL 008	-0.039		-3.67318
UKW 107	-0.012		-1.13021
KIN 432	-0.09		-8.47657
KIN 354	-0.0015		-0.14128
KYL 001	-0.0235		-2.21333

Key: *= Samples with highest OD reading on commercial ELISA BUH = Buhigwe district KLM =Kalambo District KYL = Kyela District UKW = Ukerewe District KIN=Kinondoni district KYL = Kyela District

Three of the samples with the highest optical density OD readings on commercial ELISA (BUH 032, BUH 077, and KLM 261) tests also had the highest percentage positivity readings on Wag ELISA (Table 3). The agreement between three samples with a high OD ratio was fair, with a kappa value of 0.35 (76% of agreement).

Discussion

Our study observed the difference between commercial and in-house ELISA assays. For example, the in-house RVF-ELISA identified two positive IgG seropositive results, 22.2% of cases, whereas none of the tested sera were positive for In-House Wag EBOV ELISA. These observations may suggest that the tests perform consistently across the different groups with precise estimates of very high specificities but with more uncertainty in these populations. The results of this study are in line with diagnostic assay assessment studies by others (Toni *et al*., 2016; Nyakarahuka *et al*., 2020). However, there potential inflation of the standard errors between the two kits (Thompson & Van den Bruel, 2011; Umemneku *et al*., 2019).

WAg test in this study could not detect any EBOV which were previously detected by commercial ELISA. The most probable explanation for this discrepancy is likely to be the nature of the used antigens (Reverberi & Reverberi, 2007). In this study, the commercial test used less antigen than the in-house ELISA and, hence, this could have affected its capacity to detects distinct subsets of infected animals (Anderson *et al*., 2019). On the other hand, considering the specificities of the in-house ELISA *versus* commercial ELISA (Paweska *et al*., 2019) and the cross-reactivity with other viruses, the possibility of false results that might overestimate the seroprevalence among the study populations cannot be completely ruled out. To minimise the probability of cross reactivity, the commercial test used was the My BioSource EV-ELISA, which uses inactivated and homogenized EBOV and was reported to detect 2 of 3 EVD survivors and the same 2 out of 10 EVD contacts tested positive while the control HCWs remained negative (Herrera *et al*., 2018).

In this study, in-house validated assay was utilised, which is essential for reporting diagnostic or research results. The diagnostic decision limit, or cut-off, represents a serological assay test value that is used to dichotomize negative and positive results, and by inference, to define the infection status of an individual against a specific pathogen of disease (Olsen *et al*., 2022). Data used to determine the cut-off may be affected by several factors, including the distribution of covariate factors (genetic, nutritional, geographical, and infection stage).

Three participants with fever who reacted with a commercial kit showed a significant deviation from the negative control of the specific plate by having a high PP value. Although this is below the cut-off point and considered negative, given the magnitude of febrile illnesses of unknown origin that are reported (Crump *et al*., 2013), it remains subjective and calls for continued surveillance and intense epidemiological investigations. It is equally critical to evaluate the pathogenicity and the likelihood that unidentified, serologically cross-reacting, and non-pathogenic members of the filo virus families may be present in Tanzania. Particularly given that this is only observed in regions with close interactions to outbreak areas and associated risk factors (Rugarabamu *et al*., 2021).

Discrepancies in positivity rates could reflect several factors. But it is also obvious that the type of assay and methods lead to result bias and therefore probably detect a distinct subset of infected humans at different evolutionary moments of infection (van Belkum *et al.,* 2007). This could explain the high positive rate that was detected among participant from hospital facilities who had fever compared to those from the household. The current methodological differences available between countries and the constraints of the access to diagnostic kit imposes limitations. This explorative study demonstrated that it is possible to improve detection and confirmation of VHF.

Conclusions

The findings of this study indicate differences between commercial and in-house ELISA in the sample tested for EBOV and RVF. The results indicated that there was poor agreement between in-house and commercial ELISA tests for EBOV and fair agreement between in-house and commercial ELISA tests for RVFV, with the commercial ELISA detecting more cases than the validated in-house ELISA. The current study emphasizes the difficulties in diagnosing viral hemorrhagic fever in Tanzania. The in-house protocols described here can be recommended as a screening test for the identification of viral haemorrhagic fevers in Tanzania since they were developed specifically with virus antigens which could explain the disparate results with the commercial test, although further evaluation of commercial test performance is needed. The current study emphasizes the challenges related to the diagnosis of viral hemorrhagic fever in Tanzania.

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References

Ahmed, A., Makame, J., Robert, F., Julius, K. and Mecky M. (2018). Seroprevalence and spatial distribution of Rift Valley fever infection among agro-pastoral and pastoral communities during Interepidemic period in the Serengeti ecosystem, northern Tanzania. *BioMed Central Infectious Diseases* 18: 276 -289.

Andersson, A. M., Aspán, A., Wisselink, H. J., Smid, B., Ridley, A. and Pelkonen, S. (2019). A European inter-laboratory trial to evaluate the performance of three serological methods for diagnosis of Mycoplasma bovis infection in cattle using latent class analysis. *BioMed Central Veterinary Research* 15(1): 369 - 376.

Boyle, D. S., Lehman, D. A., Lillis, L., Peterson, D., Singhal, M. and Armes, N., (2013). Rapid detection of HIV-1 proviral DNA for early infant diagnosis using recombinase polymerase amplification. *Clinical Infection Diesels* 4(135): 1 – 13.

Bray, M. (2005). Pathogenesis of viral haemorrhagic fever. *Current Opinion in Immunology* 17(4): 399 – 403.

Budodo, R. M., Horumpende, P. G., Mkumbaye, S. I., Mmbaga, B. T., Mwakapuja, R. S. and Chilongola, J. O. (2020). Serological evidence of exposure to Rift Valley, Dengue and Chikungunya Viruses among agropastoral communities in Manyara and Morogoro regions in Tanzania: A community survey. *PLoS Neglected Tropical Diseases* 14(7): 1 – 6.

Changula, K., Kajihara, M., Mweene, A. S. and Takada, A. (2014). Ebola and Marburg virus diseases in Africa: Increased risk of outbreaks in previously unaffected areas? *Microbiology and Immunology* 58(9): 483 – 491.

Cross, R. W. and Ksiazek, T. G. (2017) Production of antigens for ELISA. *Ebolaviruses. Methods in Molecular Biology* 1628: 353 –362.

Crump, J. A., Morrissey, A. B., Nicholson, W. L., Massung, R. F., Stoddard, R. A. and Galloway, R. L. (2013). Etiology of severe non-malaria febrile illness in Northern Tanzania: a prospective cohort study. *PLoS Neglected Tropical Diseases* 7(7): 23 – 28.

Ericsson, C. D., Stetten, R. and Isaäcson, M. (2001) Viral Hemorrhagic Fever Hazards for Travelers in Africa. *Clinical Infectious Diseases* 33(10): 1707–1712.

Furuyama, W. and Marzi, A. (2020). Development of an Enzyme-Linked Immunosorbent Assay to Determine the Expression Dynamics of Ebola Virus Soluble Glycoprotein during Infection. *Microorganisms* 8(10):15 – 35.

Gómez-Morales, M. A., Ludovisi, A., Amati, M., Cherchi, S., Pezzotti, P. and Pozio, E. (2008). Validation of an enzyme-linked immunosorbent assay for diagnosis of human trichinellosis. *Clinical Vaccine Immunology* 15(11): 1723 – 1729.

Herrera, B. B., Hamel, D. J., Oshun, P., Akinsola, R., Akanmu, A. S and Chang, C. A. (2018). A modified anthrax toxin-based enzyme-linked immunospot assay reveals robust T cell responses in symptomatic and asymptomatic Ebola virus exposed individuals. *PLoS Neglected Tropical Diseases* 12(5): 5 – 30.

Hur Kyu, H., Park, K., Lim, Y., Jeong, Y. S., Sung, H. and Mi-Na, K. (2020). Evaluation of four commercial kits for SARS-CoV-2 real-time reverse-transcription polymerase chain reaction approved by emergency-use-Authorization in Korea. *Frontiers in Medicine* 7: 35 – 42.

Iannetta, M., Di, C. A., Nicastri, E., Vairo, F., Masanja, H. and Kobinger, G. (2019) Viral Hemorrhagic Fevers other than Ebola and Lassa. *Infectious Disease Clinics of North America* 33: 977 – 1002.

James, A. S., Todd, S., Pollak, N. M., Marsh, G. A. and Macdonald, J. (2018). Ebolavirus diagnosis made simple, comparable and faster than molecular detection methods: preparing for the future. *Virology Journal* 15(1): 75 – 85.

Julius, S. and Chris, C. (2005). Wright, The Kappa statistic in reliability studies: *Use, Interpretation, and Sample Size Requirements, Physical Therapy* 85(3): 257–268,

Matiko, M. K., Salekwa, L. P., Kasanga, C. J., Kimera, S. I., Evander, M. and Nyangi, W. P. (2018). Serological evidence of inter-epizootic/interepidemic circulation of Rift Valley fever virus in domestic cattle in Kyela and Morogoro, Tanzania. *PLoS Neglected Tropical Disease* 12(11): 1 – 31.

Mboussou, F., Ndumbi, P., Ngom, R., Kamassali, Z., Ogundiran, O. and Van Beek, J. (2019.) Infectious disease outbreaks in the African region: an overview of events reported to the World Health Organization in 2018. *Epidemiology and Infections* 147: 1 – 12.

McHugh ML. (2012). Interrater reliability: the kappa statistic. *Biochem Med* (Zagreb). 22(3): 276 – 282.

Kumalija, M. S., Chilongola, J. C., Budodo, R. M. Horumpende, P. G., Mkumbaye, S. I., Vianney, J. M., Mwakapuja, R. S. and Mmbaga, B. T. (2021). Detection of Rift Valley Fever virus inter-epidemic activity in Kilimanjaro Region, North Eastern Tanzania, *Global Health Action* 14: 1 – 16.

Nicastri, E., Kobinger, G., Vairo, F., Montaldo, C., Mboera, L. E. G. and Ansunama, R. (2019). Ebola Virus Disease: Epidemiology, Clinical Features, Management, and Prevention. *Infectious Disease Clinics of North America* 33(4): 953 – 976.

Niikura, M., Ikegami, T., Saijo, M., Kurane, I., Miranda, M. E. and Morikawa, S. (2001). Detection of Ebola viral antigen by enzyme-linked immunosorbent assay using a novel monoclonal antibody to nucleoprotein. *Journal Clinical Microbiology* 39(9): 3267 – 3271.

Nyakarahuka, L., Schafer, I. J. and Balinandi, S. (2018). A retrospective cohort investigation of seroprevalence of Marburg virus and ebolaviruses in two different ecological zones in Uganda. *BioMed Central Infectious Disease* 20: 461 – 472.

Olsen, A., Nielsen, H. V., Alban, L., Houe, H., Jensen, T. B. and Denwood, M. (2022) Determination of an optimal ELISA cut-off for the diagnosis of *Toxoplasma gondii* infection in pigs using Bayesian latent class modelling of data from multiple diagnostic tests. *Preventive Veterinary Medicine* 201: 105 – 116.

Pang, Z. and Li, D. X. (2013). [Laboratory diagnosis of viral hemorrhagic fevers]. *Bing Du Xue Bao* 29(3): 349 – 356.

Paweska, J. T., Moolla, N., Storm, N., Msimang, V., Conteh, O., Weyer, J. and Vuren, P. J. V. (2019). Evaluation of diagnostic performance of three Indirect Enzyme-Linked Immunosorbent Assays for the detection of IgG antibodies to Ebola Virus in human sera. *Viruses* 11(8): 678 – 689.

Paweska, J. T., Burt, F. J. and Swanepoel, R. (2005). Validation of IgG-sandwich and IgM-capture ELISA for the detection of antibody to Rift Valley fever virus in humans. *Journal of Virological Methods* 124: 173–181.

Qian, J., Boswell, S. A., Chidley, C., Chidley, C., Lu, Z., Pettit, M. E. and Gaudio, B. L. (2020). An enhanced isothermal amplification assay for viral detection. *Nature Communications* 11(1): 5920 – 5942.

Racsa, L. D., Kraft, C. S., Olinger, G. G. and Hensley, L. E. (2016). Viral Hemorrhagic Fever Diagnostics. *Clinical Infection Disease* 62(2): 214 – 219.

Reverberi, R. and Reverberi, L. (2007) Factors affecting the antigen-antibody reaction. *Blood Transfusion* 5(4): 227 – 240.

Rugarabamu, S., Mboera, L., Rweyemamu, M., Mwanyika, G., Lutwama, J. and Paweska,

J. (2020). Forty-two years of responding to Ebola virus outbreaks in Sub-Saharan Africa: a review. *British Medical Journal Global Health* 5: 19 – 55.

Rugarabamu, S., Mwanyika, G. O., Rumisha, S. F., Sindato, C., Lim, H. Y., Misinzo, G. and Mboera, L. E. G. (2021). Seroprevalence and associated risk factors of selected zoonotic viral hemorrhagic fevers in Tanzania. *International Journal of Infectious Diseases* 109: 174 – 181.

Sakamoto, S., Putalun, W. and Vimolmangkang, S. (2018). Enzyme-linked immunosorbent assay for the quantitative/qualitative analysis of plant secondary metabolites [published correction appears. *Journal of Natural Medicine* 72(1): 32 – 42.

Sun, N., Wang, Y., Yao, X., Chen, F., Gao, D. and Wang, W (2019) Visual signal generation for the detection of influenza viruses by duplex recombinase polymerase amplification with lateral flow dipsticks. *Analytical and Bioanalytical Chemistry* 411: 3591–3602.

Teoh, B. T., Sam, S. S., Tan, K. K., Danlami, M. B., Shu, M. H. and Johari, J. (2015). Early detection of dengue virus by use of reverse transcription-recombinase polymerase amplification. *Journal of Clinical Microbiology* 53: 830–837.

Thomas, G., Ksiazek, C. P., Rollin, P. E., Petr B. Jahrling, P. B. and C. J. P. (1999). ELISA for the Detection of Antibodies to Ebola Viruses, *The Journal of Infectious Diseases* 179: 192 – 198.

Thompson, M. and Van den Bruel, A. (2011). *Sources of Bias in Diagnostic Studies. Diagnostic Tests Toolkit*. John Wiley and Sons Blackwell, New York. pp. 26–33.

Tighe, P. J., Ryder, R. R., Todd, I. and Fairclough, L. C. (2015). ELISA in the multiplex era: potentials and pitfalls. *Proteomics. Clinical Applications* 9(4): 406 – 422.

Toni, R., Romy, K., Halas, H., Elisa, P., Duraffour, S., Günther, S. and Ölschläger, S. (2016). Evaluation of real star reverse transcription–polymerase chain reaction kits for filovirus detection in the laboratory and field. *The Journal of Infectious Diseases* 214(3): 243 – 249.

Umemneku, C. M., Wilson, K., Graziadio, S., Vale, L. and Allen, A. J. (2019) Diagnostic test evaluation methodology: A systematic review of methods employed to evaluate diagnostic tests in the absence of gold standard - An update. *PLoS One* 14(10): 1 – 32.

van Belkum, A., Tassios, P. T., Dijkshoorn, L., Haeggman, S. Cookson, B. and Fry, N.K.. (2007) Guidelines for the validation and application of typing methods for use in bacterial epidemiology. *Clinical Microbiology and Infection* 13(3): 1 – 46.

Wu, H., Zhao, P., Yang, X., Li, J., Zhang, J. and Zhang, X. (2020). A recombinase polymerase amplification and lateral flow strip combined method That Detects Salmonella enterica serotype typhimurium with no worry of primer-dependent artifacts. *Frontiers in Microbiology* 11: 1015 – 1020.

CHAPTER THREE

3.0 General Discussion conclusions and Recommendations

3.1 General Discussion

The systematic review analysed the trend in EVD during the 40 years and found no statistically significant change over time in case fatality rates. The findings indicate a substantial heterogeneity between outbreaks. However, in most outbreaks more than half of the individuals who contracted EVD were likely to die from the disease. This could mean that the severity of ebolaviruses has not changed or little has been improved in the management of the EVD cases. The current surveillance system for outbreak detection and response requires immediate confirmation of positive cases (Hamblion *et al*., 2018; Broni *et al.* 2020). However, in most Sub-Saharan Africa, the health systems have inadequate capacities for early detection and prompt response (Olumade *et al*., 2020).

Current EVD control measures such as case management, vaccination, active surveillance, case identification and isolation, and strategic community engagement have helped to contain outbreaks. However, many people still die, and more epidemics are likely to occur in the previously affected and new geographical areas. It is therefore important that studies on the role of reservoir in the transmission of EVD are undertaken. There is also a need to accelerate research and development for novel diagnostic tools, drugs, and safe and efficacious vaccines. The findings of this review advocate for the need to develop a strategy to reduce transmission between human beings and wildlife through a shared commitment as part of the solution to the future control of EVD outbreaks. Overall, the success of EVD control requires a balance between bioscience, and socio-anthropological sciences to facilitate early detection and response to unusual disease events.

To establish the possibilities of introduction of EVD into Tanzania, a situation analysis was carried out as part of this study. High probabilities of introducing EVD from DRC to Tanzania through the assessed pathways were associated with the viability of the pathogen and low detection capacity at the ports of entry. This study identified that both DRC and Tanzania use thermal scans for screening. The effectiveness of this method can be challenged by the fact that peak viremia or assumed infectivity occurred 2-8 days of disease onset, time which coincides with the severity of symptoms (Eichner *et al*., 2011; Rajak *et al*., 2015; Jacob *et al*., 2020). A person with an asymptomatic infection and in incubation period does not appear to be infectious in that case patient isolation within eight days of infection can limit transmission (Breeching, 2014).

The risk of transmission during the incubation period or from asymptomatic persons is negligible. However high risk of silent spread by asymptomatic persons has been speculated (Moyen *et al*., 2015). Infected persons who remain asymptomatic play a significant role in the recent epidemics (Mbala *et al*., 2017). Because of the high risk of silent spread by asymptomatic persons, it is imperative that testing programmes are supplemented with conventional diagnostic testing to both symptomatic and asymptomatic persons from outbreak region.

This study suggests that both active screening and movement monitoring of arrivals should go together to limit exposure and spread from DRC to Tanzania. Case detection could be a useful strategy for delaying the entry of EVD into Tanzania and limiting the opportunity for the virus to spread. To effectively intensify screening without causing much burden to the health system, provision of information to arriving travelers, and effective communication with local communities and health personnel, are measures considered effective disease control currently in Tanzania.

Epidemiological studies were carried out to establish the prevalence of VHF in Tanzania. This thesis provides findings of the first serological survey of five VHFs in different ecological zones in Tanzania. Results of this study indicated that VHF infections are prevalent among human populations in Tanzania. The highest VHF antibody seroprevalence was recorded in western Tanzania while the lowest seroprevalence was in the semi-arid central Tanzania. Serological evidence of RVF and CCHF has been reported consistently for high-risk groups in Tanzania's diverse ecological zones (Swanepoel *et al*., 1987, Matiko *et al.,* 2018). Antibodies observed in this study imply that RVF and CCHF antibodies are still circulating from decades age (Sindato *et al.,* 2010, Paweska *et al.,* 2018).

There is a high probability that the virus is circulating or hiding in the reservoirs silently, with the potential to cause n epidemics. This is also true for antibodies to Ebola and Marburg virus observed in this zone. Both Ebola and Marburg viruses are endemic to Africa mostly in areas with forest and rainfall, the important features for bats survival (Luis *et al*., 2013; Schuh *et al*., 2017). Most of these seropositivities were detected in Buhigwe one of the study risk samples within the zone. The district is located near DRC a country that historically had several EVD outbreak epidemics. DRC have recently reported several regions and neighbourhood with Ebola infection without clinical symptoms (Glennon *et al*., 2019; Goldstein, 2020). The observed seropositivity of EVD and MBV in this study support this and other reports elsewhere in Africa which observed the absence of severe clinical manifestations associated with asymptomatic or mild infections, suggesting subclinical forms of the diseases does occur (Boisen *et al*., 2015; Brainard *et al.,* 2016; Budodo *et al*., 2020). Multiple factors have been implicated in the high seroprevalence of VHFs observed in this study zone.

The prevalence rate of antibodies against YFV in the areas without reported outbreak was reported in a previous study conducted in eastern and central Africa whereby there was a slightly higher seroprevalence that was unexpected and explained by the higher rate of daily commuters from neighbouring countries, migration of refugees, and seasonal movement of pastoralists in search of pasture (Lilay *et al*., 2017; Asebe *et al*., 2021).

The findings of this study indicate that contact with wild animals and keeping goats were significantly associated with RVF seropositivity. Domestic and wild animals may play a role as maintenance hosts of the RVF virus and serve as sources of the virus during inter-epidemic periods contributing to sporadic outbreaks (Matiko *et al*., 2018; Rright *et al*., 2019; Lubisi *et al*., 2020). On the other hand, contact with bats was a potential risk for MBV seropositivity as reported previously by other studies (Monath, 1999; Towner *et al*., 2007). Several studies in Africa have reported the close interaction of non-human primates and bats during outbreaks of MVD (Adjemian *et al*., 2011; Wood *et al*., 2012; Green, 2012).

In this thesis, in-house a whole EBOV antigen (WAg) ELISA and RVF inhibition ELISA were used to retest commercial ELISA positive EBOV and RVF samples. In sera that were EBOV positive by ELISA, commercial kits were negative by the in-house evaluated ELISA. One third of the samples that were reactive to the RVF ELISA commercial kit were reactive.

The results of these approaches may appear contradictory, given the uniqueness of the tests used. Previous studies comparing the performance of in-house ELISA with commercial ELISA to detect EBOV confirmed inherent differences amongst assay systems (Sanches *et al*.,1993; Paweska *et al*., 2019) and thus advised that binding-antibody levels should be expressed in relative rather than absolute terms (Natesan *et al*., 2016). In addition, the binding of antibodies to cellular contaminants present in EBOV-infected cell lysates may lead to cross-reactivity, resulting in reduced specificity (Nakayama *et al*., 2010; Paweska *et al*., 2018). The viral inactivation protocols evaluated in the commercial kit used indicate that they do not alter detectable levels of anti-EBOV-antibodies, thus providing a safe and reliable testing platform (Herrera *et al*., 2018). The observations made in this study require confirmation using the entire arsenal of diagnostic methods. If, in this case, no evidence is found to refute the cross-reactions, they are either transferred to the category of false positives, or are explained by the positions of classical epidemiology (Van Steelandt *et al*., 2017). At the same time, there are more and more facts that show that the amino acid sequences of nucleoprotein, NP of the Marburg and Ebola viruses have a high degree of homology not only with themselves but also with other viruses (Sanches *et al*., 1993). Given the magnitude of febrile illnesses of unknown origin that are reported in Tanzania (Crump *et al*., 2013), it remains subjective and calls for continued surveillance and intense epidemiological investigations. It is equally critical to evaluate the pathogenicity and the likelihood that unidentified, serologically cross-reacting, and non-pathogenic members of the filovirus families may be present in Tanzania. This is especially noteworthy given that it is only observed in regions with close interactions to outbreak areas and associated risk factors (Rugarabamu *et al*., 2021).

The object of this study was to confirm or exclude the presence of cross-reacting antibodies induced and, in the case of a positive response, to approach the elucidation of the mechanism of the appearance of such antibodies. The other explanation for this observation could mean antigen-capture ELISA methods are more sensitive but prone to false positives and false negatives (Bannister, 2010). To avoid this challenge, results need to be interpreted with caution until confirmation with gold standards is done.

Concurrent malaria and VHF infections were detected in about 2% of the febrile individuals in Tanzania. VHF such as RVF and CCHF, and malaria are known to occur in Tanzania (Mohammed *et al*., 2010; Ahmed *et al*. 2018), allowing concurrent infections in patients as previously reported in endemic regions (Waxman *et al*., 2017). The presence of antibodies against RVF, CCHF, EVD, MVD and malaria in this study was highly unexpected. The results are alarming because if sublethal VHF infections commonly occur, they are likely to be missed in co-infections with malaria. The serological results are based on PoC tests, which also have limitations associated with the possibility that one or more as-yet-unknown viral haemmorhagic fever viruses are circulating among the human populations sampled in this study, producing antibodies cross-reactive. The results of this study should be interpreted with caution. However, useful information gathered on the presence of VHF and malaria co-infections could indicate the need to improve viral haemorrhagic fever detection in febrile patients.

This study discovered a disparity in knowledge levels in a region where several disease awareness campaigns have been implemented. The results could indicate a continued need for more information and, indeed, specify the areas in which more information is needed. The fact that the information being demanded is of a very basic nature (preventive and protective measures, nature of the disease, modes of transmission, signs and symptoms, and causes), clearly points to some areas that need strengthening. VHF messages in information, education, and communication (IEC) materials (IEC, radio broadcast, and inter-personal communication) must be refined, particularly on VHF causes, transmission, and preventive measures, as well as reinforcing signs and symptoms, while simultaneously improving the quality and content of training provided to mobilizers and radio broadcasters to effectively deliver those messages. More information is also needed to facilitate appropriate care because of the changing nature of the disease. Risk Communication and Community Engagement (RCCE) should probably engage high level administrative, religious and political leaders to participate in prevention, and help raise not only community knowledge but more on behavioral change that will impact on good practice.

In conclusion, Research output has facilitated scientific response to the viral haemorrhagic fever pandemic. The findings of this study fit into a number of key areas that were it is incompletely understood and helps to highlight the benefits gained. The evidence of risk of disease entry from the outbreak region following the occurrence of an outbreak in a neighboring country using real data highlights the importance of cross-border monitoring in the prevention of disease occurrence.

Evidence from KAP studies established that population levels of knowledge were low and remained even with the previous explained campaigns, which indicates the need to redesign and strategise education models that will ensure effectiveness and efficient information for prevention and control. Serological studies are important for epidemiology and surveillance, and they help in assessing risk factors that can feed into control and prevention strategies. However, as a result of the previous outbreaks, available tests have been developed to detect antibodies focusing on already known species. There is a significant challenge if expression differs depending on the biological environment within hosts. In addition, the lack of validated multiplex assays for testing viral haemorrhagic fevers and the laboratory facilities for validation is a big challenge. Developing capacity in Tanzania will greatly help in providing a detailed epidemiological understanding of VHFs in the country.

3.2 Conclusions

From the results of this thesis, it can be concluded that Sub-Saharan Africa faces considerable challenges in EVD clinical management, prevention, and control which may increase the risk of disease in non-outbreak reported regions.

In Tanzania, the exposure and risk of viral hemorrhagic fevers have not been fully evaluated or exploited. It is clear that viral hemorrhagic fevers are a growing threat and may contribute significantly to the number of febrile illnesses in the country. The potential for improving management and control lies in facilitating health facility effectiveness through improved transfer of knowledge on general management, disease control, and the provision of effective diagnostic tools, in addition to periodic assessment of the identification, management, and prevention constraints in the system.
Risk Communication and Community Engagement (RCCE) should probably engage at-risk communities' including administrative, religious, and political leaders to participate in prevention and help raise not only community knowledge but more on behavioural change

that will impact good practice. The risk of introducing EVD from DRC to Tanzania in this thesis is high. Infected humans arriving via land are the most likely pathway of EVD entry, and therefore, mitigation strategies should be directed towards this pathway.

Highest VHF seroprevalence was found in western Tanzania which is at high risk of introduction from DRC. Frequent EVD outbreaks in closer regions in DRC urge the need to intensify the surveillance system, including screening and registering travellers within all the border entry points to prevent disease importation.

Viral hemorrhagic fevers due to CCHF, EVD, MVD, RVF, and YF viruses are prevalent in Tanzania and vary from one ecological zone to another. While RVF antibodies were found in all eight districts, CCHF was detected in six districts, and EVD, MVD, and YF in four districts. Contact with wild animals and keeping goats were significantly associated with RVF seropositivity, while contact with bats was a potential risk for MVD seropositivity. This study provides evidence of exposure to CCHF, EVD, MVD, RVF, and YF in Tanzania. Considering the detection of both IgM and IgG positivity for the viruses in this study, it can be concluded that these viruses were circulating very recently with a high probability of active circulation.

Seroprevalence data show that co-infections of malaria and VHF are also prevalent in Tanzania. The co-infection rate was higher among the older individuals. These findings therefore suggest that case management of febrile illnesses should be based on adequate laboratory diagnosis. The knowledge of VHF among the studied communities is low, while a significant proportion of the community is involved in activities that expose them to the disease pathogens. The findings therefore, highlight the need for reapproaching health education and promotion efforts on VHF targeting specific populations.

This study observed poor agreement between the in-house and commercial ELISA tests, whereas the commercial ELISA seemed to detect more cases than the validated in-house ELISA. The discrepancy observed highlights the potential inflation of the standard errors when the different assays are used. The current study emphasizes the challenges related to the diagnosis of viral hemorrhagic fever in Tanzania. These findings suggest there is a need to improve the detection of viral haemorrhagic fever in Tanzania.

3.3 Recommendations

The limitation of this study, however, is the discrepancy observed between commercial and in-house ELISA. In previous studies, the phenomenon of cross-immunoreactivity among pathogens of viral, bacterial, and other infections within the same family is beyond doubt, and if such cross-activity is manifested in pathogens from different families such data require confirmation using the entire arsenal of diagnostic methods. If, in this case, no evidence is found to refute the cross-reactions, they are either transferred to the category of false positives or are explained by classical epidemiological positions. Although this study detects antibodies generated against recombinant Ebola virus protein GP, we cannot be sure that these signals are generated against only Ebola virus. The WAg test in this study could not detect any EBOV, which had previously been detected by commercial ELISA. We strongly recommend other diagnostic techniques such as neutralization tests to confirm which VHF species involved or if indeed these reactions seen are against targeted viral hemorrhagic fever viruses, not other pathogens or biological mimicry.

We know, for example, that Tanzania is one of the hotspot areas for viral hemorrhagic fever, and the western part of the country has a high prevalence of Ebola virus antibodies. The region bordering DRC has the possibility of similar ecosystems and risk of disease introduction, so the signal we are seeing could be due to a virus. The results obtained could be interpreted positively because it means that infection or exposure by one virus species could lead to infection by another. The Center Point of these uncertainties in the findings highlights the true VHF diagnostic challenge. Because many species have the potential to cross-react with each other, especially when the target analyte is IgG, there is a need to explore other methods such as the Luminex multiplex assay that can detect multiple analytes. Finding true antigens against viral hemorrhagic fever could increase our knowledge of viral hemorrhagic fever in the country for better improvement of management and control. Since the majority of the sampled participants were asymptomatic at the time of sampling with no previous reports of sickness, RTPCR would be a helpful technique to use.

From the present study, it is evident that A novel approach to preventing a future outbreak in Sub-Saharan Africa should include a broad-based, one-health approach; effective communication; social mobilization; and strengthening health systems. The same is true for Tanzania, public awareness and sensitization about EVD and other potentially infectious diseases, with a focus on cross-border communities, are needed. Healthcare facilities need to be well-equipped with measures to ensure a high disease containment capacity. Evidence of exposure and risks identified in this study calls for the need to strengthen the surveillance system and management of febrile illnesses in Tanzania. Health systems should be strengthened for differential diagnosis of viral hemorrhagic fevers to improve clinical management and patient outcomes. At-risk communities should be engaged to raise community knowledge and behavioral change that will impact good practice. Further serological studies in Tanzania to determine antibody characteristics of the human and animal populations, isolate and identify viruses, and elucidate vertebrate host and vector characteristics are desirable. Genomic surveillance of VHF circulating in Tanzania should be instituted by utilizing currently available cost-effective technologies and designing and developing nucleic acid detection assays that will allow rapid epidemiological and clinical detection of a pathogen to confirm infection.

References

Abdi, I. H., Affognon, H. D., Wanjoya, A. K., Onyango-Ouma, W. and Sang, R. (2015). Knowledge, attitudes and practices (KAP) on Rift Valley fever among pastoralist communities of Ijara District, North Eastern Kenya. *PLoS Neglected Tropical Diseases* 9(11): 39 – 48.

Aleksandrowicz, P., Wolf, K., Falzarano, D., Feldmann, H., Seebach, J. and Schnittler, H. (2008). Viral haemorrhagic fever and vascular alterations. *Hämostaseologie* 28(2): 77 – 84.

Århem, K. (1989). Maasai Food Symbolism: The cultural connotations of milk, meat, and blood in the pastoral maasai diet. *Anthropos* 84(1/3): 1 – 23.

Armstrong, L. R., Dembry, L. M. and Rainey, P. M. (1999). Management of a Sabia virus-infected patient in a US hospital. *Infection Control Hospital Epidemiology* 20: 176 – 82.

Bannister, B. (2010). Viral haemorrhagic fevers imported into non-endemic countries: risk assessment and management. *British Medical Bulletin* 95: 193 – 225.

Barr, J. N. and Fearns, R. (2016). Genetic Instability of ribonucleic acid Viruses. *Genome Stability* 2016: 21 – 35.

Barry, M., Russi, M., Armstrong, L., Geller, D., Tesh, R. and Dembry, L. (1995). Treatment of a laboratory-acquired Sabiá virus infection. *New England Journal Medicine* 333(5): 317–318.

Bausch, D. G., Rollin, P. E., Demby, A. H., Coulibaly, M., Kanu, J. and Conteh, A. S. (2000). Diagnosis and clinical virology of Lassa fever as evaluated by enzyme-linked immunosorbent assay, indirect fluorescent-antibody test, and virus isolation. *Journal of Clinical Microbiology* 38(7): 2670–2677.

Bente, D., Gren, J., Strong, J. E. and Feldmann, H. (2009). Disease modelling for Ebola and Marburg viruses. *Disease Models and Mechanism* 2: 12 – 17.

Bird, B. H., Ksiazek, T. G., Nichol, S. T. and Maclachlan, N. J. (2009). Rift Valley fever virus. *Journal American Veterinary Medicine Association* 234(7): 883 – 893.

Boggild, A. K. and Liles, W.C. (2017). Travel-acquired illnesses associated with Fever. *The Travel and Tropical Medicine Manual* 2017: 271 – 299.

Borrego, B., de Ávila, A. I., Domingo, E. and Brun, A. (2019). Lethal mutagenesis of Rift Valley fever virus-induced by Favipiravir. *Antimicrobial Agents and Chemotherapy* 63(8): 1 – 19.

Bouloy, M. and Weber, F. (2010). Molecular biology of rift valley Fever virus. *The Open Virology Journal* 4: 8–14.

Bowen, E. T., Lloyd, G., Harris, W. J., Platt, G.S., Baskerville, A. and Vella, E. E. (1977). Viral haemorrhagic fever in southern Sudan and northern Zaire. *Lancet* 1: 571 – 573.

Bray, M. (2005). Pathogenesis of viral haemorrhagic fever. *Current Opinion in Immunology* 17(4): 399 – 403.

Bryant, J. E., Holmes, E. C. and Barrett, A. D. T. (2007). Out of Africa: A molecular perspective on the introduction of yellow fever virus into the Americas. *PLoS Pathogen* 3(5): 668 – 673.

Burrell, C. J., Howard, C. R. and Murphy, F. A. (2017). Laboratory diagnosis of virus diseases. *Fenner and White's Medical Virology* 2017: 135 – 154.

Carter, H. R. (1931). *Yellow Fever: An Epidemiological and Historical Study of Its Place of Origin.* The Williams and Wilkins Company, Baltimore. 308pp.

Casey, R. M., Harris, J. B., Ahuka-Mundeke, S., Dixon, M. G., Kizito, G. M. and Nsele, P. M. (2019). Immunogenicity of fractional-dose vaccine during a yellow fever outbreak – final report. *New England Journal Medicine* 381: 444 – 454.

Changula, K., Kajihara, M., Mweene, A. S. and Takada, A. (2014). Ebola and Marburg virus diseases in Africa: Increased risk of outbreaks in previously unaffected areas? *Microbiology and Immunology* 58(9): 483 – 491.

Chen, L. H. and Wilson, M. E. (2020) Yellow fever control: current epidemiology and vaccination strategies. *Trop Dis Travel Med Vaccine* 6:1–10.

Chengula, A. A., Mdegela, R. H. and Kasanga, C. J. (2013). Socio-economic impact of Rift Valley fever to pastoralists and agro-pastoralists in Arusha, Manyara and Morogoro regions in Tanzania. *Springer Plus* 2: 549 – 562.

Chumakov, M. P. (1979) Crimean hemorrhagic fever [In Russian]. In: *Viral hemorrhagic fevers. Meditsina I zdravookhranenie.* (Edited by Chumakov, M. P.), *Moscow*. pp. 10–33.

Cleri, D. J., Ricketti, A. J., Porwancher, R. B., Ramos-Bonner, L. S. and Vernaleo, J. R. (2006). Viral hemorrhagic fevers: Current status of endemic disease and strategies for control. *Infectious Disease Clinics of North America* 20(2): 359– 393.

Cobo, F. (2016). Viruses causing hemorrhagic fever. Safety laboratory procedures. *The Open Virology Journal* 10: 1–9.

Coetzer, J. and Tustin, R. (2004). *Infectious Diseases of Livestock*. Oxford University Press, Cape Town. pp. 374–382.

Cunha, M. S., da Costa, A. C., de Azevedo Fernandes, N. C. C., Guerra, J. M., Dos Santos, F. C. P. and Nogueira, J. S. (2019). Epizootics due to yellow fever virus in Sao Paulo state, Brazil: viral dissemination to new areas (2016–2017). *Science Report* 9(474): 1 – 13.

Daubney, R. and Hudson, J. R. (1932). Rift Valley fever. *Lancet* 1: 611–612.

De St. Maurice, A., Nyakarahuka, L., Purpura, L., Ervin, E., Tumusiime, A. and Balinandi, S. (2018) Rift Valley Fever: A survey of knowledge, attitudes, and practice of slaughterhouse workers and community members in Kabale District, Uganda. *PLoS Neglected Tropical Diseases* 12(3): 175 – 184.

Dean, N. E., Halloran, M. E., Yang, Y. and Longini, I. M. (2016). Transmissibility and Pathogenicity of Ebola Virus: A systematic review and meta-analysis of household secondary attack rate and asymptomatic infection. *Clinical Infectious Diseases* 62(10): 1277–1286.

Diallo, M., Nabeth, P., Ba, K., Sall, A. A. and Ba, Y. (2005). Mosquito vectors of the 1998–1999 outbreak of Rift Valley Fever and other arboviruses (Bagaza, Sanar, Wesselsbron and West Nile) in Mauritania and Senegal. *Medical and Veterinary Entomology* 19: 119 – 126.

El-Akkad, A. M. (1978). Rift Valley fever outbreak in Egypt. *Journal of Egypt Public Health Asso*ciation 53(4): 123 – 128.

Endy, T. P. (2020). Viral febrile illnesses and emerging pathogens. *Hunter's Tropical Medicine and Emerging Infectious Diseases* 2020:325–350.

Ergönül, O. (2006). Crimean-Congo haemorrhagic fever. *Lancet Infection Disease* 6: 203–214.

Ericsson, C., Steffen, R. and Isaäcson, M. (2001). Viral hemorrhagic fever hazards for travellers in Africa. *Clinical Infectious Diseases* 33(10): 1707–1712.

Estrada-Peña, A. and de la Fuente, J .(2014). The ecology of ticks and epidemiology of tick-borne viral diseases. *Antivirus Research* 108:104–28.

Faburay, B., Wilson, W., McVey, D. S., Drolet, B. S., Wingert, H., Madden, D., Young, A., Ma, W. and Richt, J. A. (2013). Rift Valley fever virus structural and nonstructural proteins: recombinant protein expression and immunoreactivity against antisera from sheep. *Vector-borne and Zoonotic Diseases Larchmont* 13(9): 619 – 629.

Falzarano, D. and Feldmann, H. (2013). Vaccines for viral hemorrhagic fevers–progress and shortcomings. *Current Opinion in Virology* 3(3): 343–351.

Faria, N. R., Kraemer, M. U. G., Hill, S. C., Goes de Jesus, J., Aguiar, R. S. and Iani, F. C. M., (2018) Genomic and epidemiological monitoring of yellow fever virus transmission potential. *Science* 361: 894–899.

Fhogartaigh, C. N. and Aarons, E. (2015). Viral haemorrhagic fever. *Clinical Medicine* 15(1): 61–66.

Filone, C. M., Heise, M., Doms, R. W. and Bertolotti-Ciarlet, A. (2006). Development and characterization of a rift valley fever virus cell-cell fusion assay using alphavirus replicon vectors. *Virology* 356: 155 – 164.

Fontenille, D., Traore-Lamizana, M., Diallo, M., Thonnon, J., Digoutte, J. P. and Zeller, H. G. (1998). New vectors of Rift Valley fever in West Africa. *Emerging Infectious Diseases* 4: 289– 293.

Forrester, N. L., Guerbois, M., Seymour, R. L., Spratt, H. and Weaver, S. C. (2012). Vector-borne transmission imposes a severe bottleneck on an ribonuclec acid virus population. *PLoS Pathogens* 8(9): 1 – 12.

Fox, J. G., Newcomer, C. E. and Rozmiarek, H. (2002). Selected Zoonoses. *Laboratory Animal Medicine* 2002: 1059 –1105.

Gaastra, W. (1984). Enzyme-linked immunosorbent assay. *Methods Molecolour Biology* 1: 349 – 355.

Gardner, C. L. and Ryman, K. D. (2010). Yellow fever: A remerging threat. *Clinics in Laboratory Medicine* 30(1): 237– 260.

Gayer, M., Legros, D., Formenty, P. and Connolly, M. A. (2007). Conflict and emerging infectious diseases. *Emerging Infection Dis*ease 13(11): 1625 – 1631.

Giovanetti, M., de Mendonca, M. C. L., Fonseca, V., Mares-Guia, M. A., Fabri, A., Xavier, J. (2019). Yellow fever virus re-emergence and spread in southeast Brazil, 2016–2019. *Journal of Virol*ogy 94(19): 1 – 12.

Gonzalez, J. P., Souris, M. and Valdivia-Granda, W. (2018). Global Spread of Hemorrhagic Fever Viruses: Predicting Pandemics. *Methods in Molecular Biology* 1604: 3–31.

Gould, E. A. and Higgs, S. (2009). Impact of climate change and other factors on emerging arbovirus diseases. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 103(2): 109 – 121.

Gould, E. A. and Solomon, T. (2008). Pathogenic flaviviruses. *Lancet* 371: 500–509.

Gritsun, T. S., Frolova, T. V., Zhankov, A. I., Armesto, M., Turner, S. L., Frolova, M. P., Pogodina, V. V., Lashkevich, V. A. and Gould, E. A. (2003). Characterization of a Siberian virus isolated from a patient with progressive chronic tick-borne encephalitis. *Journal of Virology* 77: 25 – 36.

Groseth, A., Feldmann, H. and Strong, J. E. (2007). The ecology of the Ebola virus. *Trends Microbiology* 15(9): 408 – 416.

Grossi-Soyster, E. N., Lee, J., King, C. H. and LaBeaud, A. D. (2019). The influence of raw milk exposures on Rift Valley fever virus transmission. *PLoS Neglected Tropical Diseases* 13(3): 58 – 73.

Guglielmone, A. A. and Nava, S. (2014) Names for Ixodidae (Acari: Ixodoidea): valid, synonyms, incertae sedis, nomina dubia, nomina nuda, lapsus, incorrect and suppressed names—with notes on confusions and misidentifications. *Zootaxa* 3767(1): 1 – 256.

Havelaar, A. H., Van Rosse, F., Bucura, C., Toetenel, M. A., Haagsma, J. A. and Kurowicka, D. (2010). Prioritizing emerging zoonoses in the Netherlands. *PLoS One* 5: 1 – 9.

Heinrich, N., Saathoff, E., Weller, N., Clowes, P., Kroidl, I. and Ntinginya, E. (2012) High seroprevalence of Rift Valley fever and evidence for endemic circulation in Mbeya region, Tanzania, in a cross-sectional study. *PLoS Neglected Tropical Diseases* 6: 1 – 10.

Higgs, S., Schneider, B. S., Vanlandingham, D. L., Klingler, K. A. and Gould, E. A. (2005). Nonviremic transmission of West Nile virus. *Proceeding National Academic Science* 102: 8871 – 8874.

Hua, B. L., Scholte, F. E., Ohlendorf, V., Kopp, A., Marklewitz, M. and Drosten, C. (2020). A single mutation in Crimean-Congo hemorrhagic fever virus discovered in ticks impairs infectivity in human cells. *eLife* 9: 1 – 18.

Iannetta, M., Di Caro, A., Nicastri, E., Vairo, F., Masanja, H. and Kobinger, G. (2019). Viral Hemorrhagic Fevers other than Ebola and Lassa. *Infectious Diseases Clinics of North America* 33: 977 – 1002.

Ibrahim, A. A. and Elshafie, S. S. (2016) Knowledge, awareness, and attitude regarding infection prevention and control among medical students: A call for educational intervention. *Advances in Medical Education and Practices* 7: 505 – 510.

Ikegami, T., Peters, C. J. and Makino, S. (2005). Rift valley fever virus nonstructural protein NSs promotes viral RNA replication and transcription in a minigenome system. *Journal of Virology* 79(9): 5606–5615.

Ippolito, G., Feldmann, H., Lanini, S., Vairo, F. Di Caro, A., Rosaria, M. (2012). Viral hemorrhagic fevers: advancing the level of treatment. *BioMed Central Medicine* 10(1): 1 – 31.

Isaäcson, M. (2001). Viral hemorrhagic fever hazards for travelers in Africa. *Clinical Infection Disease* 33: 1707 – 1712.

Ivanoff, B., Duquesnoy, P., Languillat, G., Saluzzo, J. F., Georges, A., Gonzalez, J. P. and McCormick, J. (1982). Haemorrhagic fever in Gabon. Incidence of Lassa, Ebola and Marburg viruses in Haut-Ogooué. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 76(6): 719 – 720.

Jahrling, P. B., Marty, A. M. and Geisbert, T. W. (2007). Viral hemorrhagic fevers. In: *Medical Aspects of Biological Warfare Office of the Surgeon General*. (Edited by D. E. Lounsbury, D. E. and Dembek, Z. F.), Textbooks of Military Medicine, Washington DC. pp. 271 – 310.

Jalloh, M. F., Sengeh, P., Monasch, R., Jalloh, M. B., DeLuca, N. and Dyson, M. (2017) National survey of Ebola-related knowledge, attitudes and practices before the outbreak peak in Sierra Leone: August 2014. *BioMed Journal Global Health* 2: 120 – 132.

Jup, P. G., Kemp, A., Grobbelaar, A., Lema, P., Burt, F. J. and Alahmed, A. M. (2002). The 2000 epidemic of Rift Valley fever in Saudi Arabia: mosquito vector studies. *Medical and Veterinary Entomology* 16: 245 – 252.

Kaner, J. and Schaack, S. (2016). Understanding Ebola: the 2014 epidemic. *Global Health* 12: 53 – 64.

Karimuribo, E .D., Mutagahywa, E., Sindato, C., Mboera, L., Mwabukusi, M. and Kariuki, N. M. (2017). A Smartphone App (AfyaData) for Innovative One Health Disease Surveillance from Community to National Levels in Africa: Intervention in Disease Surveillance. *Journal of Medical Internet Research Public Health Surveillance* 3(4): 105 – 121.

Kaya, S., Elaldi, N., Kubar, A., Gursoy, N., Yilmazi, M. and Karakuz, G. (2014). Sequential determination of serum viral titers, virus-specific IgG antibodies, and TNF-α, IL-6, IL-10, and IFN-γ levels in patients with Crimean-Congo hemorrhagic fever. *BioMed Central Infection Disease* 14: 416 – 432.

King, A. M., Adams, M. J., Carstens, E. B. and Lefkowitz, E. J. (2012). Virus taxonomy. *International Committee on Taxonomy of Viruses* 46(1): 486 – 487.

Kraemer, M. U. G., Faria, N. R., Reiner, R. C., Golding, N., Nikolay, B. and Stasse, S. (2017). Spread of yellow fever virus outbreak in Angola and the Democratic Republic of the Congo 2015-16: A odelling study. *Lancet Infection Disease* 17: 330 – 338.

Kuhn, J. H., Becker, S., Ebihara, H., Geisbert, T. W., Johnson, K. M. and Kawaoka, Y. (2010). Proposal for a revised taxonomy of the family Filoviridae: classification, names of taxa and viruses, and virus abbreviations. *Archieve Virology* 155(12): 2083 – 2103.

Kurpiers, L. A., Schulte-Herbrüggen, B., Ejotre, I. and Reeder, D. M. (2016). Bushmeat and emerging infectious diseases: Lessons from Africa. In: Problematic Wildlife. (Edited by Angelici, F.), Springer, Cham. pp. 507 – 551.

Kwaśnik, M., Rożek, W. and Rola, J. (2021). Rift valley fever – a growing threat to humans and animals. *Journal of Veterinary Research* 65(1): 7–14.

La Ruche, G. Y., Souarès, A., Armengaud, F., Peloux-Petiot, P., Delaunay, P., Desprès, A. (2010). First two autochthonous dengue virus infections in metropolitan France. *European Surveillance* 15(39): 19676 – 19683.

Languon, S. and Quaye, O. (2019). Filovirus disease outbreaks: A chronological overview. *Virology* 21(10):1 – 12.

Lasecki, L. and Baron, M. D. (2014). The molecular biology of nairoviruses, an emerging group of tick-borne arboviruses. Archive Virology 159: 1249–1265.

Laureti, M., Narayanan, D., Rodriguez-Andres, J., Fazakerley, J. K. and Kedzierski, L. (2018). Flavivirus Receptors: Diversity, Identity, and Cell Entry. *Frontiers in Immunology* 9: 2180 – 2191.

Lee, V. H. and Kemp, G. E. (1970). Congo virus: experimental infection of *Hyalomma rufipes* and trans- mission to a calf. *Bulletin Entomology Society Nigeria* 2: 133 –135.

Leffel, E. K. and Reed, D. S. (2004). Marburg and Ebola viruses as aerosol threats. (angol nyelven). *Biosecurity Bioterror* 2 (3): 186 – 191.

Ma, H. and Shieh, K. (2006). ELISA Technique. *Nature and Science* 4(2): 34 – 70.

Macdonald, G. (1957). *The Epidemiology and Control of Malaria. Oxford University Press*, London. 201pp.

Macnamara, F. N. (1954). Zika virus: A report on three cases of human infection during an epidemic of jaundice in Nigeria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 48: 139–145.

Makiala, S., Mukadi, D., De Weggheleire, A., Muramatsu, S., Kato, D., Inano, K. (2019). Clinical Evaluation of QuickNaviTM-Ebola in the 2018 Outbreak of Ebola Virus Disease in the Democratic Republic of the Congo. *Viruses* 11(589): 1 – 7.

Mansfield, K. L., Banyard, A. C., McElhinney, L., Johnson, N. and Horton, D. L. (2015) Rift Valley fever virus: A review of diagnosis and vaccination, and implications for emergence in Europe. *Vaccine* 33(42): 5520 – 5531.

Martins, S. B., Häsler, B. and Rushton, J. (2014). Economic aspects of zoonoses: Impact of zoonoses on the food industry. *Springer Science* 8(22): 1107–1126.

Masumbuko, C. K., Underschultz, J. and Hawkes, M. T. (2019). Social resistance drives persistent transmission of Ebola virus disease in Eastern Democratic Republic of Congo: A mixed-methods study. *PLoS One* 14(9): 104 – 123.

Mayhew, S. H., Kyamusugulwa, P. M., Kihangi Bindu, K., Richards, P., Kiyungu, C. and Balabanova, D. (2021). Responding to the 2018-2020 Ebola Virus Outbreak in the Democratic Republic of the Congo: Rethinking Humanitarian Approaches. *Risk Management and Healthcare Policy* 14: 1731–1747.

Mboussou, F., Ndumbi, P., Ngom, R., Kamassali, Z., Ogundiran, O. and Van Beek, J. (2019). Infectious disease outbreaks in the African region: an overview of events reported to the World Health Organization in 2018. *Epidemiology and Infection* 147: 1 – 8.

Messina, J. P., Brady, O. J., Scott, T. W., Zou, C., Pigott, D. M. and Duda, K. A. (2014) Global spread of dengue virus types: mapping the 70-year history. *Trends Microbiology* 2: 138 – 146.

Mlera, L., Melik, W. and Bloom, M. E. (2014). The role of viral persistence in flavivirus biology. *Pathogens and Disease* 71(2): 137–163.

Moraz, M. L. and Kunz, S. (2011). Pathogenesis of arenavirus hemorrhagic fevers. *Expert Review Anti Infection Ther Anti-infective Therapy* 9(1): 49 – 59.

Morens, D., Folkers, G. and Fauci, A. (2004). The challenge of emerging and re-emerging infectious diseases. *Nature* 430: 242 –249.

Morse, G., Salyers, M. P., Rollins, A. L., Monroe-DeVita, M. and Pfahler, C. (2012). Burnout in mental health services: a review of the problem and its remediation. *Administration and Policy in Mental Health* 39(5): 341–352.

Morvan, J., Saluzzo, J. F., Fontenille, D., Rollin, P. E. and Coulanges, P. (1991). Rift Valley fever on the east coast of Madagascar. *Research in Virology* 142(6): 142 – 475.

Moses, L. M. and Peterson, A. T. (2012). Environmental aspects of the viral hemorrhagic fevers. In: Friis R, editor. Praeger Handbook of Environmental Health. Santa Barbara: *Praeger* 34(1): 133 – 161.

Muga, G. O., Onyango-Ouma, W., Sang, R. and Affognon, H. (2015). Sociocultural and economic dimensions of Rift Valley fever. *American Journal of Tropical Medicine and Hygiene* 92(4): 730–738.

Mutua, E. N., Bukachi, S. A., Bett, B. K., Estambale, B. A. and Nyamongo, I. K. (2017). We do not bury dead livestock like human beings: community behaviors and risk of Rift Valley fever virus infection in Baringo County, Kenya. *PLoS Neglected Tropical Diseases* 11(5): 82 – 97.

Mwanyika, G. O., Mboera, L. E. G., Rugarabamu, S., Ngo, B., Sindato, C., Lutwama, J. J., Paweska, J.T. and Misinzo, G. (2021). Dengue Virus Infection and Associated Risk Factors in Africa: A Systematic Review and Meta-Analysis. *Viruses* 13(4): 536 – 547.

Mwanyika, G. O., Sindato, C., Rugarabamu, S., Rumisha, S. F., Karimuribo, E. D., Misinzo, G. (2021) Seroprevalence and associated risk factors of chikungunya, dengue and Zika in Tanzania. *International Journal of Infectious Diseases* 111: 271 – 280.

Mweya, C. N., Kimera, S. I., Stanley, G., Misinzo, G. and Mboera, L. E. G. (2014). Climate change influences the potential distribution of infected Aedes aegypti co-occurrence with dengue epidemics risk areas in Tanzania. *PLoS One* 11(9): 49 – 62.

Nava, A., Shimabukuro, J. S., Chmura, A. A. and Bessa, S. L. (2017). The Impact of global environmental changes on infectious disease emergence with a focus on risks for Brazil. *Institute for Laboratory Animal Research Journal* 58(3): 393 – 400.

Nettey, O. E. A., Enuameh, Y. A., Zandoh, C., Anane, E. A., Abukari, M. and Agbokey, F. (2016) Knowledge, attitudes and preventive practices on Ebola Virus Disease in the Kintampo Districts of Ghana. *Health* 8(14): 34 – 52.

Nichol, S. T., Arikawa, J. and Kawaoka, Y. (2000). Emerging viral diseases. *Proceedings of the National Academy of Sciences* 97: 12411 – 12412.

Niedrig, M., Patel, P. and El Wahed, A. A. (2018). Find the right sample: A study on the versatility of saliva and urine samples for the diagnosis of emerging viruses. *BioMed Central Infection Disease* 18: 707 – 718.

Nunes, M.R., Palacios, G., Cardoso, J. F., Martins, L. C., Sousa, E. C. Lima, C. P. (2012). Genomic and phylogenetic characterization of Brazilian yellow fever virus strains. *Journal Virology* 86(24):13263 – 13271.

Nwaiwu, A. U., Musekiwa, A. and Tamuzi, J. L. (2021). The incidence and mortality of yellow fever in Africa: A systematic review and meta-analysis. *Biomed Med Central I Infectious Disease* 21(1):1089 – 1112.

Nyakarahuka, L., Schafer, I. J, Balinandi, S., Mulei, S., Tumusiime, A. and Kyondo, J. (2020). A retrospective cohort investigation of seroprevalence of Marburg virus and ebolaviruses in two different ecological zones in Uganda. *BioMed Central Infectious Diseases* 20: 461 – 483.

Nyakarahuka, L., Skjerve, E., Nabadda, D., Sitali, D. C., Mumba, C. and Mwiine, F. N. (2017). Knowledge and attitude towards Ebola and Marburg virus diseases in Uganda using quantitative and participatory epidemiology techniques. *PLoS Neglected Tropical Diseases* 11(9): 59 – 67.

Nyarobi, M. J. (2020). The epidemiology of Rift Valley fever in northern Tanzania. Thesis for Award of PhD Degree at University of Glasgow, United Kingdom, 160pp.

Ogden, N. H., Abdel-Malik, P. and Pulliam, J. (2017). Emerging infectious diseases: prediction and detection. *Canada Communicable Disease Report* 43(10): 206–211.

Olive, M.M., Chevalier, V., Grosbois, V., Tran, A., Andriamandimby, S. F. and Durand, B. (2016). Integrated analysis of environment, cattle and human serological data: Risks and mechanisms of transmission of Rift Valley fever in Madagascar. *PLoS Neglected Tropical Diseases* 10(7): 27 – 48.

Onyango, C. O., Grobbelaar, A. A., Gibson, G. V., Sang, R. C., Sow, A. and Swanepoel, R. (2004). Yellow Fever Outbreak, Southern Sudan, 2003. *Emerging Infectious Diseases* 10(9): 1668 – 1670.

Oppenheim, B., Lidow, N., Ayscue, P., Saylors, K., Mbala, P., Kumakamba, C. and Kleinman, M. (2019). Knowledge and beliefs about Ebola virus in a conflict-affected area: early evidence from the North Kivu outbreak. *Journal of Global Health* 9(2): 311 – 332.

Oswald, W. B., Geisbert, T. W., Davis, K. J., Geisbert, J. B., Sullivan, N. J. and Jahrling, P. B. (2007). Neutralizing antibody fails to impact the course of Ebola virus infection in monkeys. *PLoS Pathogens* 3(1): 342 – 268.

Papa, A., Velo, E., Kadiaj, P., Tsioka, K., Montana, A., Kota, M. and Bino, S. (2017). Crimean-Congo hemorrhagic fever virus in ticks collected from livestock in Albania. *Infection Genetics and Evolution* 54: 496 – 500.

Parrish, C. R., Holmes, E. C., Morens, D. M., Park, E. C., Burke, D. S. and Calisher, C. H. (2008). Cross-species virus transmission and the emergence of new epidemic diseases. *Microbiology and Molecular Biology Reviews* 72(3): 457–470.

Paweska, J. T., Sewall, N. H., Ksiazek, T. G., Blumberg, L. H., Hale, M. J. and Lipkin, W. I. (2009). Nosocomial outbreak of novel arenavirus infection, southern Africa. *Emerging Infectious Diseases* 15(10): 1598–1602.

Pepin, M., Bouloy, M., Bird, B. H., Kemp, A. and Paweska, J. (2010). Rift Valley fever virus (Bunyaviridae: Phlebovirus): an update on pathogenesis, molecular epidemiology, vectors, diagnostics, and prevention. *Veterinary Research* 41(6): 61 – 115.

Peterson, A.T., Lash, R., Carroll, D. and Johnson, K. (2006). Geographic potential for outbreaks of Marburg hemorrhagic fever. *American Journal of Tropical Medicine and Hygiene* 75(1): 9 – 15.

Pigott, D. C. (2005). Hemorrhagic fever viruses. *Critical Care Clinics* 21(4): 765 –783.

Pittalis, S., Fusco, F. M., Lanini, S., Nisii, C., Puro, V. and Lauria, F. N. (2009). Case definition for Ebola and Marburg haemorrhagic fevers: A complex challenge for epidemiologists and clinicians. *New Microbiol*ogy 32 (4): 359 – 367.

Pretorius, M. and Venter, M. (2017). Diagnosis of Viral Infections. *Viral Infections in Children* 1: 151–182.

Raab, M., Pfadenhauer, L. M., Millimouno, T. J., Hoelscher, M. and Froeschil, G. (2020) Knowledge, attitudes and practices towards viral haemorrhagic fevers amongst healthcare workers in urban and rural public healthcare facilities in the N'zérékoré prefecture, Guinea: A cross-sectional study. *BioMed Central Public Health* 20: 296 – 312.

Racsa, L. D., Kraft, C. S., Olinger, G. G. and Hensley, L. E. (2016). Viral Hemorrhagic Fever Diagnostics. *Clinical Infectious Diseases* 62(2): 214–219.

Reed, W., Carroll, J. and Agramonte, A. (1901). Experimental yellow fever. *American Medicine* 2:15–23.

Robertson, S. E., Hull, B. P., Tomori, O., Bele, O. and LeDuc, J. W. (1996). Yellow fever: A decade of reemergence. *Journal of the American Medical Association* 276: 1157–1162.

Robinson, M.C. (1955). An epidemic of virus disease in southern province, Tanganyika territory, in 1952–1953. Clinical features. *Transactions of the Royal Society of Tropical Medicine and Hygiene 49*: 28 – 38.

Rogers, D. J., Wilson, A. J., Hay, S. I. and Graham, A. J. (2006). The global distribution of yellow fever and dengue. *Advance Parasitology* 62:181 – 220.

Rogers, D. J. and Randolph, S. E. (2003). Studying the global distribution of infectious diseases using GIS and RS. *National Review Microbiology* 1: 231–237.

Rugarabamu, S., Mboera, L. E. G., Rweyemamu, M. M., Mwanyika, G., Lutwama, J. and Paweska, J. T. (2020). Forty-two years of responding to Ebola virus outbreaks in Sub-Saharan Africa: a review. *BioMed Journal Global Health* 5: 1 – 23.

Rugarabamu, S., Mwanyika, G. O., Rumisha, S. F., Sindato, C., Lim, H. Y. and Misinzo, G. (2021) Seroprevalence and associated risk factors of selected zoonotic viral hemorrhagic fevers in Tanzania. *International Journal of Infectious Diseases* 109: 174 – 181.

Sacchetto, L., Drumond, B. P., Han, B. A., Nogueira, M. L. and Vasilakis, N. (2020). Re-emergence of yellow fever in the neotropics – quo vadis?. *Emerging Topics in Life Sciences* 4(4): 399 – 410.

Sanchez, A., T. W. Geisbert, T. W. and Feldmann, H. (2007). Filoviridae: Marburg and Ebola Viruses. *Emerging Viruses* 2: 1409 – 1444.

Sanjuán, R., Nebot, M.R., Chirico, N., Mansky, L. M. and Belshaw, R. (2010). Viral mutation rates. *Journal Virol*ogy 84: 9733 – 9748.

Sargianou, M., Panos, G., Tsatsaris, A., Gogos, C. and Papa, A. (2013). Crimean-Congo hemorrhagic fever: seroprevalence and risk factors among humans in Achaia, western Greece. *International Journal of Infectious Diseases* 17: 1160–1165.

Service, M. W. and Ashford, R. W. (2001). *Encyclopedia of Arthropod-Transmitted Infections of Man and Domesticated Animals. Encyclopedia of Arthropod-Transmitted Infections of Man and Domesticated Animals* Commonwealth for Agriculture Bureau International, Wallingford, Oxon, UK. 9pp.

Shabani, S. S., Ezekiel, M. J., Mohamed, M. and Moshiro, C. S. (2015) Knowledge, attitudes and practices on Rift Valley fever among agro-pastoral communities in Kongwa and Kilombero districts, Tanzania. *BioMed Central Infectious Diseases* 15: 363 – 385.

Sidra, P., Maltezou, H. C., Haidich, A. B. and Papa, A. (2012). Seroepidemiological study of Crimean-Congo haemorrhagic fever in Greece. *Clinical Microbiology and Infection* 18I: 16–19.

Sindato, C., Karimuribo, E. D., Pfeiffer, D. U., Mboera, L. E. G., Kivaria, F. and Dautu, G. (2014). Spatial and temporal pattern of Rift Valley fever outbreaks in Tanzania; 1930 to 2007. *PLoS One* 9(2): 1 – 18.

Singh, S. K. and Ruzek, D. (2013). *Viral Hemorrhagic Fevers*. Springer and Taylor and Francis Press, England. 42pp.

Song, R., Guan, S., Lee, S.S., Chen, Z., Chen, C. and Han, L. (2018). Late or lack of vaccination linked to the importation of yellow fever from Angola to China. *Emerging Infectious Disease* 24: 1383–1386.

Souza, R. P., Foster, P. G., Sallum, M. A., Coimbra, T. L., Maeda, A. Y. and Silveira, V. R. (2010). Detection of a new yellow fever virus lineage within the South American genotype I in Brazil. *Jounal of Medicine Virology* 82(1): 175 –185.

Sufi, A. M. and Galal, F. H. (2007). Role of Culex and Anopheles mosquito species as potential vectors of rift valley fever virus in Sudan outbreak, 2007*. BioMedCentral Infection Dis*ease 11: 10 – 65.

Suk, J. E., Paez Jimenez, A., Kourouma, M., Derrough,, T., Baldé, M., Honomou, P. and Kolie, N. (2016). Post-Ebola Measles Outbreak in Lola, Guinea. *Emerging Infectious Disease* 22(6): 1106 – 1108.

Sunish, I. P. Reuben, R. (2001). Factors influencing the abundance of Japanese encephalitis vectors in rice fields in India-I. Abiotic. *Medicine Veterinary Entomology* 15: 381 – 392.

Swai, E., Schoonman, L. and Daborn, C. (2010). Knowledge and attitude towards zoonoses among animal health workers and livestock keepers in Arusha and Tanga, Tanzania. *Tanzania Journal of Health Research* 12: 282 – 288.

Swanepoel, R., Shepherd, A. J., Leman, P. A., Shepherd, S. P. and McGillivray, G. M. (1987). Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. *American Journal of Tropical Medicine and Hygiene* 36(1): 120 – 132.

Swanepoel, R., Shepherd, A. J., Leman, P. A., Shepherd, S. P., McGillivray, G. M. and Erasmus, M. J. (1987). Epidemiologic and clinical features of Crimean-Congo hemorrhagic fever in southern Africa. *American Journal Tropical Medicine Hygiene* 36(1): 120 – 132.

Towner, J. S., Pourrut, X., Albariño, C. G., Nkogue, C. N., Bird, B. H. and Grard, G. (2007). Marburg Virus Infection Detected in a Common African Bat. *PLoS One* 2(8): 764 – 776.

Trombley, A. R., Wachter, L., Garrison, J., Buckley-Beason, V. A., Jahrling, J. and Hensley, L. E. (2010). A comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and New World hantaviruses. *American Journal Tropical Medicine Hygiene* 82(5): 954 – 960.

Ugalde, A., Selva-Sutter, E., Castillo, C., Paz, C. and Cañas S. (2000) Conflict and health: The health costs of war: can they be measured? Lessons from El Salvador. *BioMed Journal* 321(7254): 169 – 172.

Vairo, F., Mboera, L. E. G., De Nardo, P., Oriyo, N. M., Meschi, S. and Rumisha, S. F. (2016) Clinical, virologic, and epidemiologic characteristics of Dengue Outbreak, Dar es Salaam, Tanzania, 2014. *Emerging Infectious Diseases* 22(5): 895 – 899.

Wake, A. D. (2020) Knowledge, Attitude, Practice, and Associated Factors Regarding the Novel Coronavirus Disease 2019 (COVID-19) *Pandemic. Infection and Drug Resistance* 13: 3817 – 3832.

Wilson, M. E. (1995). Travel and the emergence of infectious diseases. *Emerging Infection Disease* 1(2): 39–46.

Wilson, M. L., Adler, G. H. and Spielman, A. (1985). Correlation between abundance of deer and that of the deer tick, *Ixodes dammini* (Acari: Ixodidae). *Annual Entomology Society Am*erican 78: 172 – 176.

Wirsiy, F. S., Boock, A. U. and Akoachere, J. F. T. K. (2021). Assessing the determinants of Ebola virus disease transmission in Baka Community of the Tropical Rainforest of Cameroon. *BioMed Central Infectious Diseases* 21: 324 – 342.

Wogu J. O. (2018). Mass media awareness campaign and the prevention of the spread of Lassa fever in the rural communities of Ebonyi State, Nigeria: An impact evaluation. *Journal of Public Health in Africa* 9(3): 179 – 184.

Wolfe, N. D., Daszak, P., Kilpatrick, A. M. and Burke, D. D. (2005). Bushmeat hunting, deforestation, and prediction of zoonoses emergence. *Emerging Infectious Diseases* 11(12): 1822 – 1827.

Wong, S., Lau, S., Woo, P. and Yuen, K. Y. (2007). Bats as a continuing source of emerging infections in humans. *Review Medicine Virology* 17: 67–91.

Zaki, S. R., Shieh, W. J., Greer, P. W., Goldsmith, C. S., Ferebee, T.and Katshitshi, J. (1999). A novel immunohistochemical assay for detecting Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. Commission de Lutte contre les Epidémies à Kikwit. *Journal Infection Diseases* 179(1): 36 – 47.

APPENDICES

Appendix 1: Structured questionnaire

Assessment of risk factors associated with increased probability of viral haemorrhagic fever virus exposure in Tanzania

Interviewer names____

A. Identification Information *(To be filled by Interviewer)*	
A.1 Region:	
A.2 District done	
A.3 Village/Street	
A.4 Date of interview/sample collection (dd/mm/yyyy):	[__][__]/[__][__]/[__][__][__][__]
A.5Place of sample/data collection	1. Household [___] 2. Facility [___]
A.6. If A.5 = "2"; mention the name and level of facility	6.0. Facility name_______________________________ Level: 6.1. Dispensary [___], 6.2. Health Centre [__], 6.3. Hospital [__]
B. Participant details *(To be filled by Interviewer)*	
B.0 Participant ID (**Take from names sheet**; use it to label the sample). The first three letters represent the district name ***(Refer A.2)*** followed by three numbers representing the ID of participant).	[__][__][__] [__][__][__]
B.1 Age (years) / (months)	[__][__][__] [_]
B.2 Sex	1. Male [__] 2. Female [__]
B.3 Place of Living (Village/street)	
B.4 How long have you lived in this village/street?	Years [___] Months [____]
B.5 Occupation (multiple selection)	1=Livestock farming 2=Crop farming 3=Fishing 4=Hunting 3=Informal sector (Daily labored/self-employed) 4=Formal employment (Public service, /private sector/NGOs) 6=Student (primary/secondary) 7=Business/trader 8=Mining 8=Others, specify _ __ ____________
B.6 Highest Educational level of the respondent	1= Primary school; 2=Secondary school 3= College/University; 4=Vocational training school; 5= Adult education; 6=None
C. Travel history (*to be filled by Interviewer)*	

C.1 On average how many times have you been outside your region during the past 12 months?	1=none 2= weekly 2=monthly 3=quarterly
C.2 Please, mention the countries other than Tanzania where you have been to during the past 12 months	0. Never been outside Tanzania 1.______________________________ 2._______________________________ 3.________________________________
D. Clinical Assessment	
Present Illness	**Write notes, if any**
For the past one months: D.1.Ever suffered from fever? Yes [__] No [__] NK[__] D.2.History of fever persisting after using antimalarials Yes [__] No [__] NK[__]	
D.3 Headache	1 [ ] Yes 2 [ ] No ______________________________
D.4 Rash	1 [ ] Yes 2 [ ] No ______________________________
D.5 Muscle, bone, back or joint pain	1 [ ] Yes 2 [ ] No ______________________________
D.6 Bleeding from natural body openings	1 [ ] Yes 2 [ ] No ____________________________
D.7 Nausea	1 [ ] Yes 2 [ ] No ____________________________
D.8 Vomiting	1 [ ] Yes 2[ ] No ______________________________
D.9 Bruising	1 [ ] Yes 2 [ ] No ______________________________
D.10 Fatigue	1 [ ] Yes 2 [ ] No ____________________________
D.11 Stomach ache	1 [ ] Yes 2 [ ] No ____________________________
D.12 Red spots (skin/eyes/mucosa)	1 [ ] Yes 2 [ ] No ____________________________
D.13. Jaundice	1 [ ] Yes 2 [ ] No ____________________________
D.14 Others, specify	1 [ ] Yes 2 [ ] No _____________________________
E. Exposure risk practices (*To be filled by interviewer*)	
During the past three months; has participant:	
E.1 Have you Visited caves?	[ ] Yes [ ] No [ ] NK
E.2 Have you Visited mines?	[ ] Yes [ ] No [ ] NK
E.3 Do you have piped water at home? E.4 Do you frequently visit recreation facilities? E.5 Do you use mosquito net? E.6 Do you use mosquito repellants? E.7 Are there some stagnant water bodies at home?	[ ] Yes [ ] No [ ] NK [ ] Yes [ ] No [ ] NK [ ] Yes [ ] No [ ] NK [ ] Yes [ ] No [ ] NK [ ] yes [ ] No [ ]NK

Appendix 2: Informed Consent Seroprevalence of Viral Haemorragic fevers in Tanzania: Strengthening Scientific capacity for Surveillance and Response

SOKOINE UNIVERSITY OF AGRICULTURE (SUA)
SACIDS Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Eastern and Southern Africa
College of Veterinary Medicine and Biomedical Sciences
P.O Box 3015, Chuo Kikuu, Morogoro, Tanzania
Tel: +255 23 264 0037; +255 787 011 677

Participant's Consent Statement
The following statement will be read to all individuals asked to participate in the survey.
My name is ………………………………………, and I am working for the National Institute for Medical Research (Tanzania)/Sokoine University of Agriculture/Muhimbili University of Health and Allied Sciences in collaboration with the Korean National Health Institute. We are carrying out a study to establish the risk of Viral Haemorrhagic Fevers in Tanzania. It is your free choice to be part of your study. The results of the survey will lead to a better understanding of the risk of VHF in our country.

A trained laboratory technologist will describe to you about the collection of blood and urine for testing of VHF viruses. He/she will seek your permission to collect the specimen from you. She/he will ask for 5mls-10mls of blood which he/she will take from your hand using a syringe and needle. The blood specimen will be taken to a laboratory at Sokoine University of Agriculture in Morogoro and tested for VHF viruses. If it is found to be positive then the specimen will be shipped to a laboratory in Korea for further testing and approval. The results of the test will be kept confidential.

There is the possibility of mild discomfort, bruising and very rarely infection at the site where the blood is taken. But, should you be injured as a direct result of participating in this survey, you will be provided with medical care at a local public health facility at no cost.

You are free to choose to be part of this survey. However, if you accept to take part in this study, there will be no payment to you. The facts about you from this survey will be kept confidential as directed by the Laws of the United Republic of Tanzania. No names will be used on any of the survey reports, publications or presentations. Only we, the researchers, will ever see the surveys with people's names. If you choose not to participate in this study, that is fine too. You will not be treated differently by the health personnel in this area. You may ask the researchers any questions you have at any time.

Do you wish to participate? YES;   NO (Please circle)
Signature ________________________
If you have any questions regarding this research, you may ask the research staff or contact Dr. Leonard Mboera, Leader, Emerging and Vector-borne Diseases, Southern African Centre for Infectious Disease Surveillance, P.O. Box 3297, Morogoro, Tanzania; Telephone: +255 754 314701; E-mail: lmboera@gmail.com

If you have questions about your rights as participant in this research, please contact the chairperson of the National Health Research Ethics Review Committee of the National Institute of Medical Research, at 255 22 2121400.

Appendix 3: Kiambatanisho 1a: Tamko La Ridhaa Utafiti Kuhusu Magonjwa Yasababishayo Uvujaji Wa Damu Mwilini Nchini Tanzania: Kujenga Uwezo Wa Ufuatiliaji Na Udhibiti

	SOKOINE UNIVERSITY OF AGRICULTURE (SUA) **SACIDS Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Eastern and Southern Africa** **College of Veterinary Medicine and Biomedical Sciences** P.O Box 3015, Chuo Kikuu, Morogoro, Tanzania Tel: +255 23 264 0037; +255 787 011 677	

Taarifa ifuatayo itasomwa kwa kila mshiriki (umri miaka ≥18) katika utafiti huu.
Mini ni ……………………………, kutoka Taasisi ya Taifa ya Utafiti wa Magonjwaya Binadamu/ Chuo Kikuu cha Sokoine cha Kilimo/ Chuo Kikuu cha Tiba na Sayansi Shirikishi cha Muhimbil. Tunafanya utafiti kuhusu uwepo na kiwango cha maambukizi ya magonjwa ya virusi yanayosababisha uvujaji wadamu katika binadamu. Magonjwa hayo ni pamoja na Homa ya Manjano, Homa ya Bonde la Ufa. Matokeo ya utafiti huu yatatoa takwimu kuhusu viwango vya maambukizi katika jamii za Tanzania na hivyo kuisadia serikali kupanga mipango madhubuti ya kuthibiti magonjwa ya milipuko.

Mtaalamu wa maabara atakuelezea kuhusu zoezi hili na kukuomba ruhusa kuchukua sampuli ya damu kiasi cha mililita 5-10 kutoka mkononi mwako kwa kutumia sindano maalum. Sampuli hiyo ya damu itapelekwa maabaraya Chuo Kikuu cha Sokoine cha Kilimo kwa uchunguzi wa kitaalamu. Kama kutakuwa na viashiria vyakuwepo maambukizi ya virusi, sampuli zitapelekwa kwenye maabara zenye uwezo mkubwa zaidi huko nchini Korea. Matokeo ya uchunguzi wa kimaabara yatahifadhiwa kwa usiri mkubwa.

Kitendo cha utoaji damu kinaweza kuleta usumbufu au kusababisha maumivu kidogo. Kama hilo litatokea, utapewa huduma katika vituo vya afya bila malipo.

Uko huru kushiriki katika utafiti huu. Iwapo utakubali kushiriki, hakutakuwa na malipoy oyote. Taarifa zako zote zitahifadhiwa kwa usiri mkubwa kama sheria za Jamhuri ya Muungano wa Tanzania zinavyoelekeza. Majina ya washiriki wote katika utafiti huu hayatawekwa wazi katika ripoti zote zitakazotokana na utafiti huu. Kama hutapenda kushiriki katika utafiti huu, itakuwa ni sawa tu. Bado utaendelea kupewa huduma zote za afya unazohitaji kutoka kwawa hudumu na vituo vyote vya hudumaza afya kama kawaida. Unaweza kuuliza maswali yoyote na wakati wowote kuhusu utafiti huu.

Je uko radhi kushiriki? NDYO; HAPANA (Zunguishia jibu lilotolewa)
Sahihi ya mshiriki ________________________
Kama kuna maswali yoyote kuhusu utafiti huu, unaweza kumuuliza Mtafiti Mkuu Dkt. Leonard Mboera wa wa Kituo cha Ufuatiliaji wa Magonwja Kusini mwa Afrika, S.L.B 3297, Morogoro, Tanzania; Simu: +255 754 314701; Baruapepe: lmboera@gmail.com
Kama unahitaji kuuliza maswali kuhusu haki zako kama mshiriki wa utafiti, basi wasiliana na Mwenyekiti wa Kamati ya Taifa ya Maadili ya Utafiti wa Afya, Taasisi ya Taifa ya Utafiti wa Magonjwa ya Binadamu kwa simu Na. +255 22 2121400.

Appendix 4: Child assent form seroprevalence of viral haemorragic fevers in tanzania: strengthening scientific capacity for surveillance and response

	SOKOINE UNIVERSITY OF AGRICULTURE (SUA) **SACIDS Africa Centre of Excellence for Infectious Diseases of** **Humans and Animals in Eastern and Southern Africa** **College of Veterinary Medicine and Biomedical Sciences** P.O Box 3015, Chuo Kikuu, Morogoro, Tanzania Tel: +255 23 264 0037; +255 787 011 677	

Child assent statement: *The following statement will be read to all children under the age of 18 requested participate in the survey.*

My name is ……………………………………………, and I am working for the Sokoine University of Agriculture/ National Institute for Medical Research/ Muhimbili University of Health and Allied Sciences in collaboration with the Korean National Health Institute. We are carrying out a study to establish the risk of Viral Haemorrhagic Fevers in Tanzania. It is your free choice to be part of the study. The results of the survey will lead to a better understanding of the risk of VHF in our country.

A trained laboratory technologist will describe to you about the collection of blood and urine for testing of VHF viruses. He/she will seek your permission to collect the specimen from you. She/he will ask for 5-10mls of blood which he/she will take from your hand using a syringe and needle. The blood specimen will be taken to a laboratory at Sokoine University of Agriculture in Morogoro and tested for VHF viruses. If it is found to be positive then the specimen will be shipped to a laboratory in Korea for further testing and approval. The results of the test will be kept confidential. There is the possibility of mild discomfort, bruising and very rarely infection at the site where the blood is taken. But, should you be injured as a direct result of participating in this survey, you will be provided with medical care at a local public health facility at no cost.

You are free to choose to be part of this survey. However, if you accept to take part in this study, there will be no payment to you. The facts about you from this survey will be kept confidential as directed by the Laws of the United Republic of Tanzania. No names will be used on any of the survey reports, publications or presentations. Only we, the researchers, will ever see the surveys with people's names.

If you sign this paper, it means that you have read this and that you want to be in the study. If you don't want to be in the study, don't sign this paper. Being in the study is up to you, and no one will be upset if you don't sign this paper or if you change your mind later.

Your signature (Child): __________________________________ Date _____________
Your name: ____ Date _____________
Signature of person obtaining consent: _____________________ Date _____________
Name of person obtaining consent: _______________ ________ Date _____________

If you have any questions regarding this research, you may ask the research staff or contact Dr. Leonard Mboera, Leader, Emerging and Vector-borne Diseases, Southern African Centre for Infectious Disease Surveillance, P.O. Box 3297, Morogoro, Tanzania; Telephone: +255 754 314701; E-mail: lmboera@gmail.com

If you have questions about your rights as participant in this research, please contact the chairperson of the National Health Research Ethics Review Committee of the National Institute of Medical Research, at 255 22 2121400.

Appendix 5: Kiambatanisho 2b: Tamko la kukubali kushiriki utafiti kuhusu magonjwa yasababishayo uvujaji wa damu mwilini nchini tanzania: kujenga uwezo wa ufuatiliaji na udhibiti

<table><tr><td></td><td>SOKOINE UNIVERSITY OF AGRICULTURE (SUA)
SACIDS Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Eastern and Southern Africa
College of Veterinary Medicine and Biomedical Sciences
P.O Box 3015, Chuo Kikuu, Morogoro, Tanzania
Tel: +255 23 264 0037; +255 787 011 677</td><td></td></tr></table>

Taarifa ifuatayo itasomwa kwa kila mtoto mshiriki (umri miaka <18) katika utafiti huu.

Mini ni ………………………………………, kutoka Chuo Kikuu cha Sokoine cha Kilimo/ Taasisi ya Taifa ya Utafiti wa Magonjwaya Binadamu/ Chuo Kikuu cha Tiba na Sayansi Shirikishi cha Muhimbili. Tunafanya utafiti kuhusu uwepo na kiwango cha maambukizi ya magonjwa ya virusi yanayosababisha uvujaji wa damu katika binadamu. Magonjwa hayo ni pamoja na Homa ya Manjano, Ebola na Homa ya Bonde la Ufa. Matokeo ya utafiti huu yatatoa takwimu kuhusu viwango vya maambukizi katika jamii za Tanzania na hivyo kuisadia serikali kupanga mipango madhubuti ya kuthibiti magonjwa ya milipuko.

Mtaalamu wa maabara atakuelezea kuhusu zoezi hili na kukuomba ruhusa kuchukua sampuli ya damu kiasi cha mililita 5-10 kutoka mkononi mwako kwa kutumia sindano maalum. Sampuli hiyo ya damu itapelekwa maabaraya Chuo Kikuu cha Sokoine cha Kilimo kwa uchunguzi wa kitaalamu. Kama kutakuwa na viashiria vyakuwepo maambukizi ya virusi, sampuli zitapelekwa kwenye maabara zenye uwezo mkubwa zaidi huko nchini Korea. Matokeo ya uchunguzi wa kimaabara yatahifadhiwa kwa usiri mkubwa.

Kitendo cha utoaji damu kinaweza kuleta usumbufu au kusababisha maumivu kidogo. Kama hilo litatokea, utapewa huduma katika vituo vya afya bila malipo.

Uko huru kushiriki katika utafiti huu. Iwapo utakubali kushiriki, hakutakuwa na malipo yoyote. Taarifa zako zote zitahifadhiwa kwa usiri mkubwa kama sheria za Jamhuri ya Muungano wa Tanzania zinavyoelekeza. Majina ya washiriki wote katika utafiti huu hayatawekwa wazi katika ripoti zote zitakazotokana na utafiti huu. Kama hutapenda kushiriki katika utafiti huu, itakuwa ni sawa tu. Bado utaendelea kupewa huduma zote za afya unazohitaji kutoka kwa wa hudumu na vituo vyote vya huduma za afya kama kawaida.

Sahihi ya mtoto: ________________________________ Tarehe ___________

Jina la mtoto: __________________________________ Tarehe ___________

Sahihi ya Mtoa Ridhaa (Mzazi/Mlezi): _________________ Tarehe ___________

Jina la Mtoa Ridhaa: ______________________________ Tarehe ___________

Kama kuna maswali yoyote kuhusu utafiti huu, unaweza kumuuliza Mtafiti Mkuu Dkt. Leonard Mboera wa Kituo cha Ufuatiliaji wa Magonwja Kusini mwa Afrika, S.L.B 3297, Morogoro, Tanzania; Simu: +255 754 314701; Baruapepe: lmboera@gmail.com

Kama unahitaji kuuliza maswali kuhusu haki zako kama mshiriki wa utafiti, basi wasiliana na Mwenyekiti wa Kamati ya Taifa ya Maadili ya Utafiti wa Afya, Taasisi ya Taifa ya Utafiti wa Magonjwa ya Binadamu kwa simu Na. +255 22 2121400.

UTAFITI KUHUSU MAGONJWA YASABABISHAYO UVUJAJI WA DAMU MWILINI NCHINI TANZANIA: KUJENGA UWEZO WA UFUATILIAJI NA UDHIBITI

TAMKO LA KUKUBALI KUSHIRIKI

SOKOINE UNIVERSITY OF AGRICULTURE (SUA)

SACIDS Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Eastern and Southern Africa

College of Veterinary Medicine and Biomedical Sciences

P.O Box 3015, Chuo Kikuu, Morogoro, Tanzania

Tel: +255 23 264 0037; +255 787 011 677

TAMKO LA KUKUBALI KUSHIRIKI

DODOSO LA NYUMA

UTAMBULISHO WA KAYA

Jina la Wilaya: ______________________________

Jina la kijiji: ______________________________

Jina la kitongoji: ______________________________

Namba ya kaya: ______________________________

Jina la mtafiti: ______________________________

Tarehe ya mahojiano: _________________

Muda wa kuanza mahojiano: _________________

I: DEMOGRAFIA

1. Jaza taarifa kwa kila mwana kaya katika kaya hii:

SN	1a. Jina	1b. Jinsi [1] Me [2] Ke	1c. Umri ***(kama ana umri chini ya miezi 12, anza na "0." Ama sivyo Kama umri ni chini ya mwezi mmoja andika "0"; Kama hujui weka "-9"***	1d. Uhusiano na mkuu wa kaya: [1] Mkuu wa kaya [2] Mke au Mume [3] Mtoto wa Kiume/Kike [4] Shemeji/Wifi [5] Mjukuu [6] Mzazi [7] Mkwe [8] Kaka au dada [9] Ndugu wengine [10] Hatuna uhusiano [-9] Sijui	1e. Ndoa [1]Ameoa/ameolewa [2] Mtalaka [3]Wametengana [4] Mjane/ Mgane [5] Hajaoa/ Hajaolewa [6]Wanaishi pamoja	1f. ***(Kwa Mwanamke wa umri wa miaka 15-49).*** Je ni mjamzito? [1] Ndiyo [2] Hapana [-9] Sijui	1g. (Kama ana umri wa ≤2) Je, bado ananyonya? [1] Ndiyo [2] Hapana [-9] Sijui	1h. Je, (jina) amepata elimu? [1] Ndiyo [2]Hapana	1i. Kama 1h jibu ni Ndiyo, nini kiwango cha juu cha elimu (jina) [1] Chekechea [2] Msingi [3] Sekondari [4] Elimu ya chuo baada ya Sekondari [-9] Sijui	1j. Katika miezi 12 iliyopita nini shughuli kuu ya kila mwanakaya? [1] Kilimo [2] Ufugaji [3] Kilimo na ufugaji [4] Mwajiriwa [5] Ajira binafsi/kujiajiri [6] Mganga wa jadi [7] Mwanafunzi [8] Mama wa nyumbani [9] Hana shughuli maalum [10] Haimhusu
1*		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
2		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
3		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
4		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
5		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
6		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
7		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
8		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
9		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
10		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
11		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10
12		1 2	____ -9	1 2 3 4 5 6 7 8 9 10 -9	1 2 3 4 5 6	1 2 -9	1 2 -9	1 2	1 2 3 4 -9	1 2 3 4 5 6 7 8 9 10

***** Mhojiwa***

Appendix 6: Permission and ethical clearance certificates

UNITED REPUBLIC OF TANZANIA
MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

SOKOINE UNIVERSITY OF AGRICULTURE
SACIDS FOUNDATION FOR ONE HEALTH
P.O. Box 3297, Morogoro, Tanzania
Tel: +255 23 2640 037, Fax: +255 232 604 647
E-mail: secretariat@sacids.org Website: http://www.sacids.org

Please refer to:

Our ref: SACIDS/ADM/21/01 **Date:** 04 January 2021

The Regional Administrative Secretary,
P.O. Box 5429,
DAR ES SALAAM

RE: MAPPING PANDEMIC POTENTIAL AND IMPROVING ON-SITE SCREENING AND DIFFERENTIATION OF VIRAL HEMORRHAGIC FEVERS DURING OUTBREAKS

Sokoine University of Agriculture through the SACIDS Foundation for One Health is conducting a research aimed at generating data to guide strengthening of surveillance of viral haemorrhagic fevers (VHF) in human and animal populations in Tanzania. The research aims at establishing the risk of introduction and spread of VHF in the country, targeting different cross-border ecosystems.

This letter is to request for your permission for the research to be carried out in Dar es Salaam from 2-20 January 2021. We kindly request to conduct individual interviews with officials from airports, ground-crossing borders, ports, citizens and refugees in the cross-border ecosystem.

I would like to introduce Dr. Sima Rugarabamu and Ms. Janeth George who are PhD Students and leading the research implementation team for this purpose. This study has received the attached ethical approval from the Medical Research Coordination Committee (Ref. NIMR/HQ/R.8c/Vol.1/1612). Please accept the assurance of my highest consideration and esteem.

Misinzo

Associate Professor Gerald Misinzo
COORDINATOR

JAMHURI YA MUUNGANO WA TANZANIA
WIZARA YA AFYA, MAENDELEO YA JAMII, JINSIA, WAZEE NA WATOTO

Telegram '**AFYA' DODOMA**
Simu NA. : **255-26-2323267**
(Barua zote zipitie kwa
Katibu Mkuu)
Unapojibu tafadhali taja

Mji wa Serikali –Mtumba,
Barabara ya Afya,
S.L.P. 743
40478 DODOMA.

Kumb. RA.81/249/01/173

27 Februari, 2020

Sima Rugarabamu
Chuo cha Kilimo (SUA)
S.L.P 3297,
MOROGORO.

YAH: MAOMBI YA KIBALI CHA KUAGIZA VIFAA NA DAWA ZA UTAFITI

Tafadhali husika na somo tajwa hapo juu.

Wizara ya Afya, Maendeleo ya Jamii, Jinsia Wazee na Watoto inakiri kupokea barua yako isiyo na kumbu kumbu namba ya tarehe 28 Januari, 2020 inayohusu ombi la kibali cha kuagiza vifaa na dawa za utafiti.

Wizara ya Afya inakushauri kuwasiliana na Mamlaka ya Chakula na Dawa (TMDA) ili kupata utaratibu na kibali cha kuagiza vifaa na dawa husika. Kwa mawasilino tumia barua pepe '*info@tfda.go.tz*' au sanduku la Posta 1253 '*Mwanza Avenue*', Block T, Plot No. 6, Dodoma.

Asante kwa ushirikiano wako.

Daudi Msasi
KNY: KATIBU MKUU -AFYA

TANZANIA MEDICINES AND MEDICAL DEVICES AUTHORITY

TMDA

TMDA/DMC/MDC/P/003

SPECIAL PERMISSION TO IMPORT MEDICAL DEVICES

Section 75(1) of Tanzania Food Drugs and Cosmetics Act 2003

Permit No: [illegible]

PART A: PARTICULARS OF IMPORTER

Name of importer: **Sokoine University of Agriculture**, Postal Address **P.o Box 3000 Morogoro Tanzania** Tel. No. 0232640006
Exporting Country **CHINA** Invoice No. **1217B** Date **17-12-2019**

Exporter/Sender: **SUNLONG BIOTECH Co LTD** Postal address **0,CHINA**
Arrival expected by ship, air or motor vehicle Via **Julius Nyerere International Airport** port of entry

	Name of Product		Product	Quantity to be	Value of
S/n	Brand Name	Common Name	Registration No.	Imported	Product(s)
1	HUMAN DENGUE VIRUS IGG(DV-IGG) ELISA KIT	KITS		3 Pieces	450.00 USD
2	HUMAN DENGUE VIRUS IGM	KITS		1 Pieces	150.00 USD
3	HUMAN CHIKUNGUNYA VIRUS	KITS		3 Pieces	450.00 USD
4	HUMAN CHIKUNGUNYA VIRUS IGM	KITS		1 Pieces	150.00 USD
5	HUMAN YELLOW FEVER VIRUS (YFV-IGG) ELISA KIT	LABORATORY REAGENT		3 Pieces	450.00 USD
6	HUMAN YELLOW FEVER VIRUS	KITS		1 Pieces	150.00 USD
7	HUMAN RIFT VALLEY FEVER IGG	KITS		3 Pieces	450.00 USD
8	HUMAN RIFT VALLEY IGM	KITS		1 Pieces	150.00 USD
9	HUMAN CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IGG	KITS		3 Pieces	450.00 USD
10	HUMAN CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS	KITS		1 Pieces	150.00 USD
	TOTAL (FOB Value)				3,000.00 USD

Fees: TSH 138,000.00 Receipt No.: [illegible] Dated: 04-Mar-2020

PART B: GRANTING PERMISSION

Permission is hereby **granted** to import the above mentioned product(s). The importer has to contact the Port TMDA Inspector to examine the approved product(s) before entry into Tanzania Mainland.

09/03/2020
Date

AKIDA MBALLAH KHEA
FOR: DIRECTOR GENERAL

Prepared By: Sunday Kisoma

PART C: DECLARATION BY TMDA INSPECTOR

I ____________________ being TMDA Inspector at **Julius Nyerere International Airport** port Office has examined the above listed product(s) and I therefore **grant/not grant** entry into Tanzania Mainland.

Date **SIGNATURE OF TMDA PORT OFFICER AND STAMP**

This permit is valid from: 29-02-2020 to 29-08-2020

Note:

- The Inspector has to return immediately a completed copy of this permit bearing import stamp to TMDA Zone Manager.

MISSION:
To protect and promote public health by ensuring quality, safety and effectiveness of medicines, medical devices and diagnostics

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: nimrethics@gmail.com

Ministry of Health, Community Development, Gender, Elderly & Children
University of Dodoma, College of Business Studies and Law
Building No. 11
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8a/Vol. IX/3110

23rd May, 2019

Dr. Sima Rugaranamu
Sokoine University of Agriculture
C/o Prof. Gerald Misinzo
SUA-SACIDS
P.O. Box 3297
Morogoro

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Mapping pandemic potential and improving on-site screening and differentiation of viral hemorrhagic fevers during outbreaks (Rugaranamu S. et al), whose supervisor is Prof. Gerald Misinzo of SUA-SACIDS has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from National Institute for Medical Research.
3. Copies of final publications are made available to the Ministry of Health, Community Development, Gender, Elderly & Children and the National Institute for Medical Research.
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10(2).
5. Sites: Buhigwe District in Kigoma region, Kalambo District in Rukwa region, Kyela District in Mbeya region, Kinondoni District in Dar es Salaam, Kilindi District in Tanga region, Mvomero District in Morogoro region, Kondoa District in Dodoma region and Ukerewe District in Mwanza region.

Approval is valid for one year: 23rd May 2019 to 22nd May 2020.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Muhammad Bakari Kambi

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY & CHILDREN

UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY AND CHILDREN

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

In reply please quote:

Ref. No: NIMR/HQ/P.12 VOL XXXIII/126 Date: 10th December 2021

Dr. Sima Ernest Rugarabamu
Sokoine University of Agriculture
College Of Veterinary Medicine and Biomedical Sciences
Department of Veterinary Microbiology, Parasitology & Biotechnology
P.O. Box 3019 Chuo Kikuu
MOROGORO.

Dear Dr. Rugarabamu,

RE: PERMISSION TO PUBLISH

Reference is made to your request to publish data from a study with ethical clearance number NIMR/HQ/R.8a/Vol. IX/3110.

2. Permission has been granted to publish a manuscript titled: "**Estimating risk of introduction of Ebola Virus Disease from the Democratic Republic of Congo to Tanzania: a qualitative assessment**" by authors: Sima Rugarabamu, Kennedy M. Mbanzulu, Gaspary Mwanyika and Gerald Misinzo and Leonard E.G. Mboera.

3. Please submit an electronic copy of the published manuscript to the National Institute for Medical Research through email publications@nimr.or.tz.

Dr. Ndekya Maria Oriyo

DIRECTOR OF RESEARCH INFORMATION, TECHNOLOGY AND COMMUNICATION

Headquarters: 3 Barack Obama Drive, P.O. Box 9653, 11101 Dar es Salaam, Tanzania,
Email: info@nimr.or.tz, Website: www.nimr.or.tz

UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH, COMMUNITY DEVELOPMENT, GENDER, ELDERLY AND CHILDREN

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

In reply please quote:

Ref. No: NIMR/HQ/P.12 VOL XXXIII/125 Date: 10th December 2021

Dr Leonard Mboera
Sokoine University of Agriculture (SUA)
SACIDS Foundation for One Health
P.O. Box 3297, Chuo Kikuu,
MOROGORO.

Dear Dr Mboera,

RE: PERMISSION TO PUBLISH

Reference is made to your request to publish data dated 7th December 2021 with reference number MB/P2P/CEVBD/02/2021/04 from a study with ethical clearance number NIMR/HQ/R.8a/Vol IX/2724 and NIMR/HQ/R.8a/Vol. IX/2688.

2. Permission has been granted to publish the following manuscripts:

i. Title: "**Viral haemorrhagic fevers and malaria co-infections among febrile patients seeking health care in Tanzania**" by authors: Sima Rugarabamu, Susan F. Rumisha, Gaspary O. Mwanyika, Calvin Sindato, Hee Young Lim, Gerald Misinzo and Leonard E.G. Mboera.

ii. Title: "**Comparative assessment of the human and animal health surveillance systems in Tanzania: opportunities for an integrated one health surveillance platform**" by authors: Irene R. Mremi, Susan F. Rumisha, Calvin Sindato, Sharadhuli I. Kimera and Leonard E.G. Mboera.

3. Please submit electronic copies of the published manuscripts to the National Institute for Medical Research through email publications@nimr.or.tz.

Dr. Ndekya Maria Oriyo

DIRECTOR OF RESEARCH INFORMATION, TECHNOLOGY AND COMMUNICATION

Headquarters: 3 Barack Obama Drive, P.O. Box 9653, 11101 Dar es Salaam, Tanzania,
Email: info@nimr.or.tz, Website: www.nimr.or.tz