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Molecular epidemiology of rodent-, shrew- and bat-borne hantaviruses in Mbeya region, Tanzania

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**MOLECULAR EPIDEMIOLOGY OF RODENT-, SHREW- AND BAT-
BORNE HANTAVIRUSES IN MBEYA REGION, TANZANIA**

Lwitiho Edwin Sudi

**A Thesis Submitted in Partial Fulfilment for the Requirements of the Master's in Life
Sciences of the Nelson Mandela African Institution of Science and Technology**

Arusha, Tanzania

March, 2019

ABSTRACT

Hantaviruses, family Bunyaviridae are emerging zoonotic RNA viruses, which originates from rodents, bats and shrews. Expanded diversity of Hantaviruses with their respective reservoir host in Africa stimulates the research on Hantavirus distribution among reservoir hosts as well as the assessment of knowledge, attitude and practices on Hantavirus infections at the community level in Mbeya region. Cross section surveys conducted between July 2017 and September 2018, involved the trapping of rodents, shrews and bats on residential areas, agricultural fields and forest areas and assessing the level of knowledge, attitude and practices on Hantavirus infections at the community level in Mbeya region. Necropsies from the internal organs were collected and screened for Hantavirus using Han-L PCR. Positive samples were purified and sequenced. Phylogenetic analysis was done to assess the evolutionary relationship among Hantavirus strains. Only (6/334) 1.8% of all bat species trapped were positive for Hantavirus while rodents and shrews were found to be negative. *Mops condylurus* was confirmed to carry Hantavirus in Mbeya region. The maximum likelihood phylogenetic analysis revealed a previous unidentified bat borne Hantavirus strain named Kiwira virus. In addition, the community has a low level of knowledge and higher practices, which may favor the transmission of the Hantavirus from the reservoir host to human population and this, endanger the community health. The discovery of new Hantavirus strain on free-tailed bats (*Mops condylurus*) in Kyela district expands the diversity of Hantavirus reservoir host in Africa. Informing the society about Hantavirus infections is more important as far as public health is concerned.

AUTHOR DECLARATION

I, Lwitiho Edwin Sudi do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this thesis titled 'Molecular Epidemiology of Rodent-, Shrew- and Bat-borne Hantaviruses in Mbeya Region, Tanzania' is my original work and has never been submitted for a degree in any other university

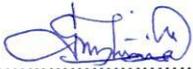
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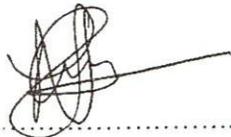
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CERTIFICATION

We Supervisors, have read this thesis and found it acceptable in terms of originality and quality for the award of Master's in Life Science.



19 March 2019

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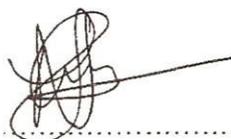
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This thesis is dedicated to my beloved mother, Rose Selaka Sanga who took moral effort and encouragement for me to reach this stage at this Year 2019. It is also dedicated to my wife, Neema Noah Kimani and My kids Daniel Lwitiho and Godvictory Lwitiho, who provide the moral support and ensure that I concentrate with research and analysis and achieve the results at this year 2019.

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LIST OF ABBREVIATION AND SYMBOLS

HPS	Hantavirus pulmonary syndrome
HFRS	Hemorrhagic fever with renal syndrome
MOHSW	Ministry of Health and social warfare
IgM	Immunoglobulin M
IgG	Immunoglobulin G
ELISA	Enzyme-Linked Immunosorbent Assay
PCR	polymerase Chain Reaction
FRNT	Focus Reduction Neutralization Assay
IFA	Immunofluorescence Assay
GPS	Geographical Positioning System
NIMR	National Institute for Medical Research
MMRC	Mbeya Medical Research Center
cDNA	Complimentary deoxyribonucleic acid
Han-L-1R/1F	Outer primers (reverse and forward) for L segment
Han-L-2R/2F	Nested primers (reverse and forward) for L segment
RNA	Riboxynucleic acid
DTT	Dithiothreitol
dNTPs	Deoxyribonucleotide triphosphates
EDTA	Ethylenediaminetetraacetic acid
CYT B	Cytochrome B

NCBI	National Center for Biotechnology Information
BIC	Bayesian information criterion
MMLV-RT	Moloney Murine Leukemia Virus Reverse Transcriptase
DFG	German Research Foundation
mRNA	Messenger RNA
%	Percentage
°C	Degree centigrade
PREDICT	Pandemic, Re-emerging and Emerging Diseases Information and Threats
RLT	Lysis Buffer
mA	Milli ampere
rpm	Revolution per minutes
bp	Base pair
kb	Kilo bytes

CHAPTER ONE

INTRODUCTION

1.1 General Background

Hantavirus is one of the most important zoonotic pathogens of humans with a global public health concern that causes either hemorrhagic fever with renal syndrome (HFRS) or Hantavirus pulmonary syndrome (HPS) (Kruger *et al.*, 2015). It is a single-stranded, enveloped, negative-sense RNA virus belong to the family *Bunyaviridae*. The genome of the virus consists of three segments, the small segment (S) 1.6 - 2 kb, medium segment (M) 3.6 kb, and the large segment (L) 6.5 kb (Plyusnin *et al.*, 1996). These segments are very important in expressing the nucleocapsid protein (N), the glycoprotein's (Gc and Gn) and the polymerase protein (L) respectively for virulence, survival and replication of the virus (Plyusnin *et al.*, 1996).

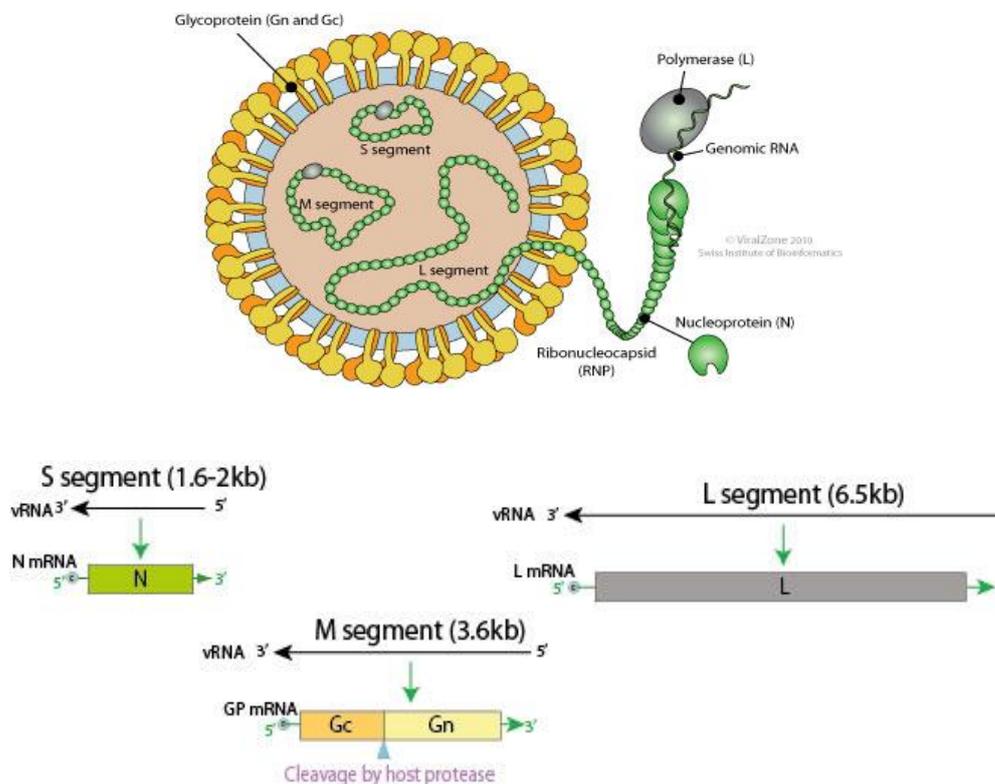


Figure 1: Hantavirus virion and the genome with S, M and L segment (source: [www.viralzone](http://www.viralzone.org))

On the Hantavirus gene expression and replication the main components are the RNA-dependent RNA polymerase (RdRp), the Nucleocapsid protein (N), and the viral genomic and antigenomic RNA templates (Jonsson and Schmaljohn, 2001). The RdRp is responsible for the synthesis of positive strand messenger RNA from the large (L), medium (M), and small (S) viral RNA segments; it is responsible for mediation of both transcription and replication (Jonsson and Schmaljohn, 2001). The RdRp binds to a promoter on each encapsidated segment and transcribes the mRNA. These segments are capped by L protein during synthesis using the cap-snatching mechanism. Transcription is terminated by a strong hairpin sequence at the end of each gene; mRNAs are capped by RdRp protein during the synthesis step. Hantavirus transcription and replication takes place in the cytoplasm of the target cell (Khaiboullina *et al.*, 2005). Virus attaches to host receptors through Gn-Gc glycoprotein dimer, and is endocytosed into vesicles in the host cell. Fusion of virus membrane with the vesicle membrane; ribonucleocapsid segments are released in the cytoplasm. Transcription, viral mRNAs are capped in the cytoplasm. Replication presumably starts when enough nucleoprotein is present to encapsidate neo-synthesized antigenomes and genomes. The ribonucleocapsid buds at the Golgi apparatus, releasing the virion by exocytosis (Khaiboullina *et al.*, 2005).

1.2 Statement of the Research Problem

Bats, rodents and shrews are arguably the most important source of emerging viruses such as Lassa-, Henipa- and Filo-viruses (Calisher *et al.*, 2006). Their ubiquity, species richness, migration patterns, large colony sizes, roosting behavior and ability to fly long distances, are among of the traits enabling the maintenance of virus lifecycles within bat populations (Allocati *et al.*, 2016). The recent discovery of Hantaviruses in shrews, rodents and bats in West Africa suggests that other genetically distinct Hantaviruses may exist in East Africa (Weiss *et al.*, 2012; Witkowski *et al.*, 2014). Evidence of Hantavirus in Uluguru and Kilimanjaro mountains revealed the circulation of the virus in shrews in Tanzania (Kang *et al.*, 2014). Moreover, the evidence of Hantavirus seropositivity in Kyela and Mbarali on the Unpublished reports (NIMR MMRC, 2012). Not only encouraged the investigations on the molecular epidemiology of Hantavirus in Kyela and Mbarali districts of Mbeya region, Tanzania but also the assessment of knowledge, attitude and practices on Hantavirus infection at the community level on study districts (Kyela, Mbeya municipal, Mbeya rural and Mbarali) of Mbeya region.

1.3 Research Justification

Hantavirus infections are zoonotic infections, which normally originate from wildlife. A human being is being infected due to the interaction that exists between human life and wildlife environment. Globally, Hantavirus infection may lead to hemorrhagic fever with renal syndrome (HFRS) and Hantavirus Cardiopulmonary syndrome (HCPS) with a case fatality rate up to 50% which rises a public health concern (Kruger *et al.*, 2015). The relationship between Hantavirus infections and febrile illness raises a concern on the community health as well as patient management. Only one article explained the circulation of Hantavirus in reservoir host such as shrews (Kang *et al.*, 2014) in Tanzania. The report on the circulation of Hantavirus in asymptomatic adult patients expanded the concern of Hantavirus infections in relationship to febrile illness (Hofinger *et al.*, 2006). A known serological prevalence of 2.4% on human plasma collected during previous cohort studies, in both Kyela and Mbarali district (Unpublished reports, NIMR MMRC) incited us for further investigation on Hantaviruses in bats, shrews and rodents as well as assessment of the level of knowledge, attitude and practices for Hantavirus infections at community level in Mbeya region, Tanzania.

1.4 Objectives

1.4.1 Main Objective

Detection and characterization of rodent-, shrew- and bat-borne Hantaviruses and their public health potential to improve management of febrile illness in Tanzania.

1.4.2 Specific Objective

- (i) To detect Hantaviruses in bats, shrews, and rodents using molecular techniques.
- (ii) To characterize the Hantaviruses detected in bats, shrews and rodents.
- (iii) To determine the level of knowledge, attitude and practices on Hantavirus infection at the community level in Mbeya region.

1.5 Research Questions and Research Hypotheses

1.5.1 Research Questions

- (i) Are the rodents, shrews and bats carries Hantavirus infections in Tanzania?
- (ii) Is the strain circulating in small wild animals already discovered elsewhere in the world?
- (iii) Is the community of Mbeya region aware of Hantavirus infections?

1.5.2 Research Hypotheses

- (i) H_0 : Hantaviruses are not circulating in small mammals in Mbeya region, Tanzania.
- (ii) H_0 : Communities living in Mbeya region are not aware of Hantavirus infections.

1.6 Significance of the Research Study

This study pointed out the circulating Hantavirus strain in small wild animals (rodents, shrews and bats), as well as provides the data on the knowledge, attitude and practice assessment for Hantavirus infections in Mbeya region. The understanding on the level of knowledge, attitudes and practices for Hantavirus infections at the community level in Mbeya region, helps on setting the priorities for awareness program within districts as well as contribute on policy change for surveillance of zoonotic pathogens in Tanzania. In addition, the data from this study provides the baseline for Hantavirus surveys in other regions within Tanzania.

CHAPTER TWO

LITERATURE REVIEW

2.1 Brief History and Distribution of Hantavirus in Africa

Hantaviruses belong to the family *Bunyaviridae* and are single-stranded, enveloped, negative-sense RNA viruses that causes two life-threatening human zoonotic diseases namely; Hemorrhagic Fever with Renal Syndrome (HFRS) and Hantavirus Cardiopulmonary Syndrome (HCPS), with case fatality rates of up to 50% (Kruger *et al.*, 2015; Simpson *et al.*, 2010). Reservoir host for Hantaviruses are rodents, shrews and bats that become a source of infections to human (Krüger *et al.*, 2011; Vaheri *et al.*, 2013). The first confirmed case of Hantavirus infection in Africa was detected in Sangassou village in Guinea (Krüger *et al.*, 2011) originated from *Hylomyscus simus*. Increased rodent population growth caused by climate, environmental changes and agricultural activities with high rates of human-rodent contacts may give rise to epidemics of Hantavirus infection elsewhere in Africa (Klempa, 2009; Sudi *et al.*, 2018).

Identification of new reservoir host (shrews, rodents and bats) for Hantaviruses in Africa, expands the host range and new insights into the molecular phylogeny and evolution of these zoonotic viruses (Kang *et al.*, 2014; Krüger *et al.*, 2011; Witkowski *et al.*, 2014; Zhang, 2014). Presence of reservoir hosts in residential areas and agricultural fields exposes residing human population at exposure risks (Krüger *et al.*, 2013). The existence of previously undetected Hantaviruses in Africa, following their first molecular demonstration in rodents (Klempa *et al.*, 2006), shrews (Klempa *et al.*, 2007) and bats (Klempa *et al.*, 2012; Weiss *et al.*, 2012), were subsequently confirmed and extended by independent groups in West and East Africa, leading to the discovery of additional rodent-borne Hantaviruses in Ethiopia (Meheretu *et al.*, 2012) and Madagascar (Reynes *et al.*, 2014). Similarly, shrew-borne Hantaviruses in Kilimanjaro and Uluguru mountains in Tanzania (Kang *et al.*, 2014) and Ivory Coast (Kang *et al.*, 2011; Sumibcay *et al.*, 2012), as well as a bat-borne hantavirus in Ivory coast (Sumibcay *et al.*, 2012; Witkowski *et al.*, 2014).

Implemented one-health research projects in sub-Saharan Africa revealed the circulation of Hantavirus serotypes in reservoir hosts such as bats, rodents, and shrews (Kruger *et al.*, 2015; Witkowski *et al.*, 2015). For example Makokou virus (MKV) on noacks round leaf bat (*Hipposideros ruber*) in Gabon (Witkowski *et al.*, 2016), Magboi virus (MGBV) on *Nycteris*

*hispid*a in Sierra Leone (Weiss *et al.*, 2012), Tigray virus (TGV) in *Stenocephalemys albipes* in Ethiopia, Mouyassue virus (MOUV) in *Neoromicia nanus* in Ivory coast (Sumibcay *et al.*, 2012). Not only that but also the Sangassou Virus (SANGV) on wood mice (*Hylomyscus simus*) in Guinea (Klempa *et al.*, 2006), Tanganya virus (TNGV) in *Crocidura theresae* in Guinea (Klempa *et al.*, 2007), Bowe virus (BOWV) on *Crocidura douceti* in Guinea (Gu *et al.*, 2013) and Azagny Virus (AZGV) in *Crocidura obscurior* in Ivory Coast (Kang *et al.*, 2011). Uluguru virus (ULUV) in *Myosorex geata* and Kilimanjaro virus on *Myosorex zinki* were reported in Tanzania (Kang *et al.*, 2014). The discovery of Hantavirus serotype with sister lineage as Sangassou on *Hylomyscus endorobae* in Kenya expanded the diversity of reservoir host for Hantaviruses in Africa (Těšíková *et al.*, 2017).

Screening of human sera revealed a seroprevalence ranging from 0.2 to 16.6% in the early 1980s (Witkowski *et al.*, 2014). However, after the establishment of confirmatory assays the Hantavirus prevalence ranges from 1- 4.4% in the whole of Africa region. These include 1.2- 4.4% seroprevalence in febrile patients in Guinea (Klempa *et al.*, 2010), 2.4% and 3.9% in Democratic Republic of Congo and Ivory Coast, respectively. Two percent (2%) in Mozambique (Chau *et al.*, 2017) and 1% in South Africa (Ithete *et al.*, 2014). In addition, the seropositivity for the Hantavirus detected in asymptomatic patients in Arusha highlights the circulation of the virus in Human within Tanzania (Hofinger *et al.*, 2006).

2.2 Hantavirus Transmission Routes and Survival outside the Reservoir Host

Hantaviruses originally found in small wild animals such as bats, rodents and shrews. The virus can be transmitted to human through: (a) Airborne by inhaling the contaminated aerosolized excreta, (b) direct contact with contaminated urine, feces, animal droppings or contaminated materials, (c) bitten by infected rodents or shrews, (d) eating foods contaminated with urine, droppings or saliva and (e) touching the eyes, nose, and mouth after being into contact with viral particles from contaminated urine, feces, droppings or saliva (Fulhorst *et al.*, 2007). Most of the rodent Hantaviruses have been stated to infect human being globally (Kruger *et al.*, 2015) with bat-borne and shrew-borne hantaviruses requires further investigation to establish its epidemiology. Furthermore, human to human transmission is rare with the exception of Andes virus in Argentina (Martinez *et al.*, 2005).

Once Hantaviruses are shed to the environment, they have the ability to survive outside the host in different ways for example for 12 - 15 days in contaminated beddings, 5 - 11 days at

room temperature in cell culture supernatants and 18 - 96 days at 4°C in a cell culture supernatants (Kallio *et al.*, 2006). The virus is susceptible to different disinfectants including, 1% Sodium hypochlorite, 1 - 5% clidox (Chlorine dioxide), 1 - 5% Dettol and 1 - 5% Virkon^R (Chau *et al.*, 2017; Mills *et al.*, 2002).

2.3 Hantavirus Epidemiology

Rodent-borne Hantavirus has been known to cause either Hantavirus cardiopulmonary syndrome or Hemorrhagic fever with renal syndrome in human worldwide (Jiang *et al.*, 2017). Human beings are primarily infected by aerosolized rodent feces, urine and saliva (Vaheri *et al.*, 2008), but humans have also been infected through contact with open wounds and rodent bites (Barrett and Stanberry, 2009). Further investigations on the bat-borne Hantavirus strains on the pathogenicity has to be implemented since there is no solid evidence in terms of literature or reports which shows that bat-borne Hantavirus or insectivores viruses causes diseases to a human being.

The epidemiological history of Hantavirus started from the discovery of Hantaan virus (HNTV) as the causes of Korea hemorrhagic fever (KHF) and this led to the epidemiological studies in rodents and humans (Jonsson *et al.*, 2010). Published epidemiological studies reported that most cases of both HFRS and HCPS occurs in working-age males and females and is most likely related to occupational exposure such as agricultural or forest work where individuals are most likely to come into contact with infected animals (Barrett and Stanberry, 2009; Bi *et al.*, 2008).

There is an estimation of 150 000 to 200 000 HFRS cases per year worldwide (Jiang *et al.*, 2017). Clinical cases of HFRS reported in some Asian countries such as Taiwan and South Korea which are caused by HNTV and SEOV (Bi *et al.*, 2008; Su *et al.*, 2015). While in Europe, HFRS is caused by Dobrava-Belgrade (DOBV), Puumala virus (PUUV) and Tula virus (TULV). Dobrava-Belgrade (DOBV) predominantly occurs in the Balkans and Eastern Europe, and it is the most virulent European virus with a mortality rate of up to 12% (Maes *et al.*, 2004). Puumala virus has the widest geographical range in Europe causes human infection which results in Nephropathia epidemica (NE), a milder form of HFRS with a case fatality rate of 0.1% (Clement *et al.*, 2006). The first reported outbreak of Hantavirus disease in the Americas was in 1993; disease outbreak occurred in the four corners region in the USA. The causative agent was Sin Nombre virus hosted by *Peromyscus Maniculatus*. The first

HCPS case in South America was reported in Argentina caused by the Andes virus, in 1995 with 29 cases reported (Bi *et al.*, 2008). Andes virus is the only Hantavirus for which person-to-person transmission was reported (Martinez *et al.*, 2005).

Serological evidence of Hantavirus infections (in both humans and rodents) in Africa was found in serological surveys done by Gonzalez *et al.* (1984), in Central African Republic, Gabon, Benin and Burkina Faso. Evidence of human Hantavirus infections was also found in Egypt, Nigeria, Djibouti and Senegal (Bi *et al.*, 2008). The first Hantavirus in Africa namely Sangassou virus (SANGV) was identified in Guinea, West Africa. It was named Sangassou (SANGV) after the village near which the host was trapped (Klempa *et al.*, 2006). A follow-up study done in Guinea 2009, revealed a seroprevalence of 1.2% and Sangassou specific antibodies were detected in two patients (Klempa *et al.*, 2010).

2.4 Hantavirus Pathogenesis

Hantavirus targets the endothelial cells of capillaries of various organs, primarily of the lung and kidneys, however, infection can occur in a variety of other organs and cell types such as endothelial and epithelial cells, macrophages, follicular dendritic cells, lymphocytes, neutrophils and platelets (Hjelle, 2008). The main receptor in endothelial cells for pathogenic Hantavirus is beta-3-integrin. Infection followed by impairment of the barrier function of endothelial cells, fluid extravasation and subsequent organ failure (Manigold and Vial, 2014).

Infection which occurred on endothelial cells by Hantavirus is noncytopathic in vitro system and in vivo cells, which have led to the suggestion that a strong cellular immune response, elicited by cytotoxic CD8⁺ T cells, could be responsible for Hantavirus pathogenesis in human (Hjelle, 2008). However, the absence of necrotic endothelial cells despite the presence of inflammatory T cells in lung tissue of HCPS patients (Mori *et al.*, 1999) seemed to argue against T-cell mediated organ damage. Moreover, in vitro pathogenic Hantaviruses can initiate viral entry by binding to the alpha (V) beta-3-integrin, since inactivation of virus-bound beta-3-integrins contributes to deregulation of vascular endothelial growth factor receptor-2 (VEGFR2) and diminished antagonism of vascular endothelial growth factor (VEGFA) (Ioannou *et al.*, 2017; Manigold and Vial, 2014), this may lead to impairment of vascular endothelial (VE) cadherin expression and subsequent loss of endothelial barrier function (Manigold and Vial, 2014).

2.5 Hantavirus Diseases Progression, Treatment Management, Prevention and Control Options

2.5.1 Hantavirus Disease Progression

Hantaviruses are zoonotic pathogens causing diseases in human life i.e. Hemorrhagic Fever with Renal Syndrome (HFRS) and Hantavirus Cardiopulmonary Syndrome (HCPS). Hantaan virus (HNTV), Seoul virus (SEOV), Puumala virus (PUUV) and Dobrava-Belgrade virus (DOBV) have been reported to cause HFRS (Mattar *et al.*, 2014) while other Hantavirus serotypes are responsible for HCPS.

Clinical diagnosis of the Hantavirus infections depends on the type of the disease as well as Hantavirus serotypes causing the disease. Asymptomatic stage of a patient with HFRS includes febrile, hypotensive, oliguric, polyuric and convalescence stages (Avšič-Županc *et al.*, 2015; Jiang *et al.*, 2017). On the febrile stage a patient experience fever, flu-like, backache and visual disorder sometimes. This stage takes 3 - 7 days after the incubation period of two days. After the febrile stage, a patient enters the hypotensive stage, where the level of blood platelets drops and the symptoms may lead to tachycardia and hypoxemia. This stage usually lasts for three to seven days. In oliguric stage, a patient may experience renal failure and proteinuria within three to seven days. Thereafter, follows a polyuria stage where a patient experiences an abnormal production of urine, which results in frequent urination with three to six liters of urine per day. The last stage is the convalescence stage where a patient starts to recover and the symptoms start to improve. Patients with HCPS may pass through febrile, prodromal, cardiogenic and convalescence stages during disease progression. The febrile stage may take 3 - 6 days after the incubation period and if not managed properly patient may enter the prodromal stage, where he/she may experience fever, headache, myalgia, vomiting, diarrhea and abdominal pain and may last 3 - 5 days. On the cardiogenic stage, a patient experiences dyspnea, nonproductive cough, and circulatory collapse (Avšič-Županc *et al.*, 2015). Seventy-five percent (75%) of the patients with cardiopulmonary edema may require mechanical support on ventilation and lasts for 48 hours, followed by rapid improvement from the symptoms (convalescence stage) (Avšič-Županc *et al.*, 2015; Sudi *et al.*, 2018). However, these symptoms are not pathognomonic as other febrile conditions may have similar signs and symptoms.

2.5.2 Diagnosis of Hantavirus Diseases

Laboratory diagnosis is very crucial for the detection and confirmation of Hantavirus infections. Assays available for the detection of the Hantavirus pathogens are Immunosorbent assays (IgG and IgM ELISA), immunoblotting assays (in house western blot and Microgen blot assays), immunofluorescence assay (IFA), reduction neutralization assay (FRNT) and molecular detection assays (PCRs) (Clement *et al.*, 2018; Mattar *et al.*, 2014). Molecular detection assays are very sensitive from day 2 to day 10 of infection since the viral load titer is higher in the body and decreases as the disease progresses. The sensitivity for IgM ELISA assay extends up to 13 to 15 days after that the expression of Immunoglobulin M (marker for acute infection) starts to decrease in the body, which also reflects the assay. However, confirmatory assays such as IFA and FRNT are very crucial for the laboratory confirmation of Hantavirus infection after the detection by ELISA assays (Mattar *et al.*, 2014; Witkowski *et al.*, 2014) especially in communities where febrile illnesses are common.

2.5.3 Hantavirus Diseases Prevention and Control Options

Hantavirus diseases can be prevented through the implementation of biosafety and biosecurity measures to minimize the transmission from reservoir hosts to a human being. The transmission can be blocked or minimized through avoiding touching the animal reservoir hosts such as bats, rodents, or shrews by bare hands, also avoiding cleaning places where rodents and bats reside while wearing the face protective mask (FP3) and gloves as to avoid contact or inhaling the contaminated dust with the viral particle. Building houses with higher ventilation increase the air circulation and limit the breeding sites for the rodents (Chau *et al.*, 2017; Mills *et al.*, 2002; Sudi *et al.*, 2018). In addition, disinfection of rodent breeding sites using chemicals such as hypochlorite solution (1 - 5%) ensures the health safety of the individuals as far as zoonotic diseases are concerned.

Raising the knowledge and awareness of the transmission of Hantavirus diseases in both clinical and non-clinical individuals may improve the management and prevention of diseases (Sudi *et al.*, 2018). Since prevention through vaccination is not available, it is advisable to encourage community awareness on disease transmission, risk factors and control options (Forbes *et al.*, 2018; Suzuki and Mutinelli, 2009; Walsh, 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was conducted in four out of seven districts (Mbeya rural, Mbeya municipal, Mbarali and Kyela) of Mbeya region. The region is located in the Southern Highlands zone of Tanzania. Iringa region on the East, Rukwa and Songwe regions on the West and Tabora region border Mbeya region on the North side, Malawi, and Lake Nyasa on the South. According to the 2012 national census, the region had a population of 2 707 410. Mbeya region consists of lower, middle and dense vegetation forests, which favors the life of small mammals such as bats, rodents and shrews. The study districts (Kyela, Mbarali, Mbeya Municipal and Mbeya Rural) were purposively selected based on the vegetation, agricultural activities and febrile illness report from health facilities and previous research epidemiological data from respective districts. The area has one rainy season between December and May. Majority of residents in these districts are involved in agricultural activities such as banana plantations, avocados, maize and cocoa production with other economic activities depending on their location and seasonal factors.



Figure 2: Map of Mbeya Region showing Study Districts (Source: arc GIS software)

3.2 Research Ethics and Permission to Conduct the Study

Permission to conduct the study was obtained from the relevant bodies including the regional and districts authorities. The study was approved by the National Health Research Ethical Committee (NatHREC) and Mbeya Medical Research and Ethics Committee (MMREC). Human subjects were provided with pre-informed consent and after reading and understanding the consent form, they signed and were then recruited into the study.

3.3 Study Design

A cross-sectional survey was conducted to determine the prevalence of Hantavirus in bats, rodents and shrews. The samples from bats, rodents and shrews were collected between April 2017 and April 2018 in selected localities within Mbeya region, Tanzania. In addition, a questionnaire-based survey as to assess the knowledge, attitude and practices associated with Hantavirus transmission in Mbeya region were conducted on between April and June 2018. The selection of participants within districts was done using random sampling selection technique.

3.4 Sample Size Calculation

3.4.1 Bat Sample Size Calculation

From the sample size (n) calculation formulae

$$n = Z^2 P(100 - P)/E^2 \quad (\text{Arya } et al., 2012)$$

Where Z is the z-value, P is the prevalence and E is the marginal error.

Then since the assumed maximum animal prevalence was 28% (Council, 1991; Spiegel *et al.*, 1980) and Z value was 1.96 for 95% confidence level then the trapped bat sample number was

$$n = 1.96^2 \times 28(100 - 28)/5^2$$

$$n = 310$$

The minimum number of bats calculated for sampling was 310.

3.4.2 Rodents/ Shrew Sample Size Calculation

From the sample size (n) calculation formulae

$$n = Z^2 P(100 - P)/E^2 \quad (\text{Arya } et al., 2012)$$

Where n is the sample size, P is the prevalence or infection rate, Z is the Z-value with a 95% confidence level, E is the marginal level of 5%. Since the confirmed maximum prevalence of Hantavirus in rodent/shrew in Sub Saharan Africa was 7% (Kang *et al.*, 2014; Witkowski *et al.*, 2014) then the minimum number of rodents/shrew required to be trapped during the study was 100.

$$n = 1.96^2 \times 7(100 - 7)/5^2$$
$$n = 100$$

The minimum number of each group of rodent/shrew to be collected is 100

3.4.3 Questionnaire Survey Sample Size Calculation

The questionnaire data were collected either to the respondent wherein the household's rodents and shrews were trapped, or close to the house surroundings where bats, rodents and shrews were trapped as to assess the level of knowledge, attitude and practices on Hantavirus infections at the community level. The individuals to participate in the survey were selected randomly in the respective areas. Since this was the first study to be done on the assessment of knowledge, attitude and practices associated with Hantavirus transmission in Tanzania, the maximum assumed level of knowledge is 50% (i.e. P = 50%).

$$n = \frac{Z^2 P(100 - P)}{d^2}$$

(Charan and Biswas, 2013; Pourhoseingholi *et al.*, 2013)

Where N is the population size, P is the level of knowledge, Z is the z-value, d is the marginal error. The level of significance held was 95% confidence interval and the marginal error of 5%

$$n = (1.96^2) \times 50(100 - 50)/5^2$$
$$n = 384$$

Since we expected some of the participants not to respond to the questionnaire then we included the non-response correction for the participant recruited. Where n is the total sample size, r is the non-response correction which is 10% and $*n$ is the sample size with non-response correction

$$*n = \frac{n}{1 - r}$$

$$*n = \frac{384}{1 - 0.1} = 427$$

The minimum number of participants required to be recruited to the questionnaire-based survey is 427.

3.5 Trapping, Handling and Collection of Bats, Rodents and Shrews

3.5.1 Bat Trapping, Collection and Handling

Both fruit and insectivorous bats were trapped by using five pockets of mist nets that were set from 0.5 to 6 meters above the ground in and around forest areas. Also around the human dwellings with higher vegetation density and along, fruit plantations. Moreover, mist nets were set close to the houses in areas with bats populations residing on the roofs of houses. All mist nets were set before the sunset and the pockets were opened at the time where the dark begins to rise. Geographical position system (GPS) coordinates were recorded in all locations where mist nets were set.



Figure 3: Investigator and field assistants setting the Mist net for bat trapping

After every one-hour, the nets were checked to remove trapped bats outside the mist nets. The direction in which the bats flew into the nets was considered before removing them from the nets. The feet were freed followed by the wings and finally the head. To ensure safety during trapping activities, all investigators wore protective equipment such as masks, leather glove on one hand and Nitrile glove on the other hand.

Trapped bats were put into cotton pockets and hanged overnight as to allow bats to have proper air circulation and remain alive until the time of sample collection.



Figure 4a and 4b: Proper handling of bats during fieldwork after removing them from the Mist net (source: Study field pictures)

3.5.2 Rodent/Shrew Trapping, Handling, and Collection

One-day prior to field activities, research team visited selected villages for familiarization and identification of sites for setting traps. In addition, bait (a mixture of peanut, flour, and dried fish) was prepared and put into the Sherman trap ready for attracting rodents and shrews.

The following day the research team set the baited Sherman traps in human dwellings (i.e. inside the houses) and on the agricultural areas (outside the houses) for trapping both rodents and shrews.

In addition, the team recorded the GPS coordinates of these locations and the areas with traps were marked with red tape. Fifty Sherman traps were set every day on selected locations and left overnight. The next morning the traps were checked if they had rodents/shrews inside. Those traps with rodents/shrews were brought to sample collection room in the field while other traps were left for a maximum of three days.

3.6 Samples Collection and Storage

3.6.1 Collection and Storage of Bat Samples

A mixture of 2% Xylazine and 10% Ketamine drugs in the ratio of 1:1 was prepared and a dosage of 0.4 mg/kg of the anesthesia was administered on the left pectoral muscle of each bat and left for 5 minutes as to ensure the animal loses sensation completely. Then the animal was then pinned on the dissecting plate. A cardiac blood draw was done for euthanasia purposes and the blood was stored in 2 ml EDTA Cryotube. After that, the animal was dissected and necropsies from spleen, liver, lung, kidney and intestine were taken at this order to avoid contamination and stored in 2 ml Cryotubes. Cryotubes were packed in the stockings and preserved in liquid nitrogen tank in the field and shipped to the center (NIMR MMRC) where are stored at -80°C .

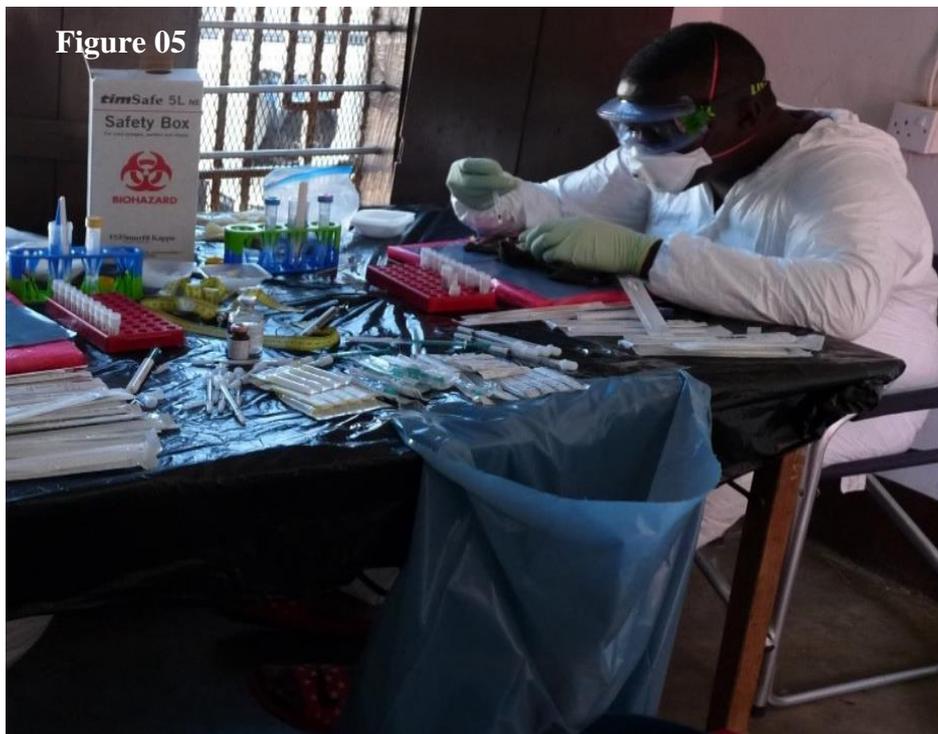


Figure 5: Scientist dissecting bats and stores the necropsies in the liquid nitrogen tank.

3.6.2 Collection and Storage of Samples from Rodents/Shrew

Cotton wool was soaked in diethyl ether and placed inside the zipped plastic bag containing the trap with rodents/shrews inside. The plastic bag was zipped to ensure the rodents inhaled for a maximum of 2 minutes until they die.

The trap was removed outside the plastic bag and the length and weight of each rodent/shrew were taken for analysis. Then the rodents/shrews were pinned on the dissecting plates and the necropsies from spleen, liver, lung, kidney and intestine were taken and stored in 2 ml Cryotubes. The Cryotubes were packed in the stockings and preserved in liquid nitrogen tank in the field and shipped to the NIMR Mbeya Center where are stored at -80°C .

3.7 Questionnaire Data Survey on Assessment of Knowledge, Attitude and Practice on Hantavirus Infections at the Community Level in Mbeya Region.

A total of 438 participants from the four study districts were interviewed using an open and closed-ended questionnaire (Appendix 4). The questionnaires were administered to both clinical and non-clinical individuals within the region. Participants with the age greater than 18 years old and mentally sounded with the ability to respond to the questions were explained the details of the study on the assessment of knowledge, attitude and practice on Hantavirus infections. Pre-informed consenting procedures was administered to each participant and those who agree to participate in the study signed the consent form, then followed by the questionnaire administration.

3.8 Laboratory Analysis of Samples

Lung samples were selected for laboratory screening due Hantavirus pathogenesis reasons (Section 2.3), the Hantavirus target the endothelia cells of the lungs and the kidney (Hepojoki *et al.*, 2014; Hjelle, 2008; Maes *et al.*, 2004). In addition, the optimized protocol for the detection of Hantavirus, on the choice of necropsies from the organ which yield better screening results was lung (Kim *et al.*, 2017). However, due to cost saving reasons, during the initial screening lung organs were only utilized. Nevertheless, depending on the positivity of the animals, other organs from positive animals were also tested as to justify the possibility of viral disseminations on the environments. However, further research on confirmation in the availability of Hantavirus on the environment has to be done as to be sure on viral transmission ability to a human being. Other necropsies from other organ were stored in the archive for further research.

A total of 334 lung tissues from bats and 251 lung tissues from both rodents and shrews were analyzed to detect the presence of Hantavirus strains. Procedures performed in the NIMR-Mbeya Medical Research laboratory includes; Riboxynucleic acid (RNA) isolation, Complimentary Deoxyribonucleic acid (cDNA) preparation, external amplification of

coding region of Polymerase gene, internal amplification of coding region of polymerase gene, gel electrophoresis, PCR product purification, sequencing of purified PCR product, sequence editing, Multiple alignment and G block analysis, phylogenetic analysis and phylogenetic tree visualization.

3.8.1 Riboxynucleic Acid (RNA) Isolation

RNA from 585 lung samples from bats, rodents, and shrews were isolated using qiagen RNA Mini easy kit protocol. Lung tissues were removed from -80°C freezer and placed in a cool container containing ice during slicing time since RNA is unstable at higher temperatures. The lung tissues were sliced using a disposable surgical blade and placed on the 2 ml Eppendorf tubes containing 400 μl RLT buffer with DTT and stainless steel beads.

The tissues were disrupted in a Qiagen tissue lyser machine in a 30 m/s for 3 minutes and the lysed tissues were centrifuged at full speed (14000 rpm). The supernatant was transferred into a 1 ml Eppendorf tube containing 350 μl of 70% ethanol and mixed through up and down pipetting. Seven hundred microliters (700 μl) of the mixture was transferred to the spin column (including the precipitate). Then the mixture was centrifuged at 8000 g for 15 seconds. The flow was discarded and the spin column was added to another collection tube and 700 μl washing buffer (RW1) was added and centrifuged at speed of 8000 g for 15 seconds. Again, the flow was discarded and the column was washed with 500 μl RPE buffer two times. Then the column was dried through centrifugation at 14000 rpm for one minute and then placed into a 1.5 ml Eppendorf tube. Fifty microliters of RNase free water was added to the column and centrifuged at the speed of 8000 g for 1 minute to elute RNA. The eluted RNA was then stored in -40°C until Polymerase chain reaction amplification (PCR) detection.

3.8.2 Complimentary Deoxyribonucleic Acid (cDNA) Preparation

For one sample a master mix containing 1.6 μl PCR water, 4 μl 5X Buffer (Invitrogen), 2 μl DTT (Invitrogen), 0.4 μl dNTPs 25 mM each (Bio line), 0.5 μl N6-primer (Roche), 1 μl Ribulock 40 U/ μl (Fermenters), 0.5 μl MMLV- RT (Invitrogen) were prepared. A mixture of 10 μl of isolated RNA and 10 μl of master mix were amplified on the Thermocycler set at 20°C for 10 minutes, 42°C for 45 minutes, 80°C for 6 minutes and then hold at 4°C until

removing the product from the Thermocycler. The cDNA samples were removed from the Thermocycler and stored in the freezer ($-20\text{ }^{\circ}\text{C}$).

3.8.3 External Amplification of the Coding Region of Polymerase Gene (Outer PCR)

For one sample, a master mix containing $12.5\text{ }\mu\text{l}$ Tempase Hot Start Mix –Blue (Ampliqon), $2.5\text{ }\mu\text{l}$ of Han-L-1F/R primers where the forward sequence was $5'$ -ATG TAY GTB AGT GCW GAT GC- $3'$ and the reverse sequence was $5'$ -AAC CAD TCW GTY CCR TCA TC- $3'$ and $7.5\text{ }\mu\text{l}$ of PCR water were prepared in Master mix preparation hood chamber then $2.5\text{ }\mu\text{l}$ of cDNA were added to $22.5\text{ }\mu\text{l}$ of master mix in a PCR tubes and amplified on thermocycler set at $95\text{ }^{\circ}\text{C}$ for 15 minutes (denaturation step), (40 cycles of ($95\text{ }^{\circ}\text{C}$ for 30 seconds, $53\text{ }^{\circ}\text{C}$ for 45 seconds, and $72\text{ }^{\circ}\text{C}$ for 45 seconds (annealing step), $72\text{ }^{\circ}\text{C}$ for 6 minutes (elongation) and hold at $4\text{ }^{\circ}\text{C}$ overnight.

3.8.4 Internal Amplification of the Coding Region of the Polymerase Gene (Nested PCR)

For one sample, a master mix containing $12.5\text{ }\mu\text{l}$ Tempase Hot Start Mix –Blue (Ampliqon), $2.5\text{ }\mu\text{l}$ of Han-L-2F/R primers where the forward sequence was $5'$ -TGC WGA TGC HAC IAA RTG GTC- $3'$ and the reverse sequence was $5'$ -GCR TCR TCW GAR TGR TGD GCA A- $3'$ and $9.0\text{ }\mu\text{l}$ of PCR water were prepared in Master mix preparation hood chamber. A mixture of $1.0\text{ }\mu\text{l}$ of PCR product from the first round and $24\text{ }\mu\text{l}$ of master mix were amplified on thermocycler set at $95\text{ }^{\circ}\text{C}$ for 15 minutes (denaturation step), (25 cycles of ($95\text{ }^{\circ}\text{C}$ for 30 seconds, $53\text{ }^{\circ}\text{C}$ for 45 seconds, $72\text{ }^{\circ}\text{C}$ for 45 seconds) annealing step), $72\text{ }^{\circ}\text{C}$ for 6 minutes' elongation and hold at $4\text{ }^{\circ}\text{C}$ overnight.

3.8.5 Gel Electrophoresis

(i) Detection of Amplified Coding Region using Gel Electrophoresis

Two percent of Agarose gel was prepared by mixing 2 g of Agarose powder (Promega, USA) and 100 mls of 1X Tris Boris EDTA (TBE) reagent (Aniara Diagnostica) in a heat resistant flask. The mixture was swirled to obtain a uniform suspension and then boiled in a microwave oven until it is clear. The clear solution was left to cool at room temperature until it reaches a temperature of $55\text{ }^{\circ}\text{C}$, then $5\text{ }\mu\text{l}$ of Gel green stain (Gel Green®) was added and swirled gently until the stain is clearly mixed.

Casting tray was prepared by adding the appropriate combs on the tray and carefully pour the melted Agarose solution on the casting tray. The tray with a gel solution was left for 30-45 minutes for the Agarose gel to polymerize. The casting tray was placed in an electrophoretic chamber and 1X TBE was added in an electrophoretic chamber until the gel was submerged. The combs were removed slowly and carefully to avoid breaking of the solidified gel.

PCR products (5 μ l) mixed with 2 μ l of loading dye (Invitrogen) were loaded to the wells and the first and the last wells 5 μ l of 100 bp ladder marker (Invitrogen) were loaded. The cover of the electrophoretic chamber was returned, the cathode-anode directions were accurately ensured and then run at 70 volts, and 100 mA for 90 minutes (larger chamber) until the bands are clearly separated.

(ii) Gel Visualization

The gel with bands was placed on the gel documentation system for PCR band detection and the system was turned on for visualizing the band separation. The gel picture was taken for further documentation

3.8.6 PCR Product Purification using Gene Jet Purification Kit

PCR products (20 μ l) were mixed thoroughly with 20 μ l of binding buffer in 500 μ l Eppendorf tubes until the color remained yellow, which indicate optimum pH for DNA binding. The mixture was then transferred into the spin column and centrifuged at a maximum speed of 14000 rpm for 1 minute. The flow was discarded and the column was transferred in another collection tube. Seven hundred microliters (700 μ l) of wash buffer was added to the spin column and centrifuged at a maximum speed of 14000 rpm for 1 minute and the flow was discarded. The spin column in a collection tube was then centrifuged at a maximum speed of 14000 rpm for 1 minute. Then the collection tube was discarded and the column was placed in a new 500 μ l Eppendorf tube. Fifty microliters (50 μ l) of elution buffer was added to the column and centrifuged at maximum speed for 1 minute. Then pure PCR product was stored at -20 °C until sequencing.

3.8.7 Sequencing and Phylogenetic Analyses of Purified PCR Product

(i) PCR Product Preparation for Sequencing

Depending on the band size of the PCR product, the sequence master mix was prepared using Euro Fins Mix 2Seq Kit. The master mix consists of 2 µl of sequence primer (reverse primer/forward primer), 5 µl of pure PCR product and 10 µl of sequencing water. Seventeen microliters (17 µl) of the mix was transferred to the euro film sequence mix tubes, sealed by its cover, and directly imported to the euro film company for sequencing. The details of the sample sent and the choice of sequencing method was filled on the online form.

A total of 6 samples which means 12-euro fins sequence mix tubes for both reverse primer 5'-AAC CAD TCW GTY CCR TCA TC-3' and forward primer 5'-TGC WGA TGC HAC IAA RTG GTC-3' was sent for sequencing.

(ii) Sequence Editing and Blasting

The sequence obtained from the Euro film company was first edited using Geneious software to remove the primer binding part and the low-quality part and maintain the sequence quality of more than 95%. The trimmed and edited sequence was then blasted on the NCBI website for nucleotide sequence as to observe the sequence similarities with the already uploaded sequences.

(iii) Multiple Alignments

Accession number and sequence from different Hantaviruses already identified in the world were downloaded from the NCBI website (Table 1) and transferred to the Geneious software. Both new sequences and downloaded sequences were checked for proper direction and right frame and then aligned. The phylip file was saved and imported to the Sea View software.

(iv) G-Block Analysis

The Sea View software was opened and phylip file from multiple alignment procedure was imported. The proper direction of all sequences was checked and finally, the G-block analysis was commanded by selecting the G-block and allowing the gap position with the final blocks and accepting the analysis by ticking ok. The phylip file was saved with a distinct name.

(v) Phylogenetic Analysis of Hantaviruses Sequences and Phylogenetic Tree Visualization

The sequences were analyzed using online PhylM software by selecting BIC as substitution model, SPR as a type of tree improvement and with a maximum of 1000 bootstrap values. The software was commanded to start the analyses. The phylogenetic tree data obtained was visualized using Fig tree software.

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Results

4.1.1 The Diversity of Bat, Rodent and Shrew Species as far as Hantavirus is concerned in the Districts of Mbeya Region

Out of three hundred and thirty-four (334) bats samples analyzed, 86 (25.75%) were *Mops condylurus*. Other species included; *Neoromicia species* 13 (3.89%), *Rousettus species* 45 (13.47%), *Epomophorus species* 138 (41.32%). Other bat species were 52 (15.57%) (Appendix4). Majority of these species were found in Kyela district compared to Mbarali district (Table 1) due to vegetation density and altitude, which favors growth and survival of bat species. Six out of eighty-six (6/86) *Mops condylurus* were positive for Hantavirus while other species were negative.

Approximately all rodents 247/251 (98.4%) and shrews (1.59%) tested negative for Hantavirus. The *Rattus sp.* was dominant in both Mbarali and Kyela districts. The rodents and shrews were equally distributed between the districts.

Table 1: Bats/shrews/rodents distribution by districts and Hantavirus isolation

Animal trapped	Kyela	Mbarali	P value CI:95%	Animals tested positive for Hantavirus (%)
Bats			P<0.0001	
<i>Mops condylurus</i>	86	0		6 (6.97%)
<i>Neoromicia sp.</i>	10	3		0
<i>Rousettus sp.</i>	45	0		0
<i>Other species</i>	147	43		0
Rodents			P = 0.9917*	
<i>Rattus group</i>	90	96		0
<i>Mus musculus</i>	7	8		0
<i>Other species</i>	26	22		0
Shrews			P = 0.3173*	
<i>Crocidura sp.</i>	0	2		0
<i>Myosorex sp.</i>	2	0		0

4.1.2 Gel Electrophoresis showing Positives Bands for Bat-Borne Hantavirus

Six out of 86 *Mops condylurus* species were positives for Hantavirus pathogen and this mark a prevalence of 6.98% however; it contributes to a prevalence of 1.8% in all bat species trapped. A band of 395 bp was detected during the gel electrophoresis documentation (Fig. 6)

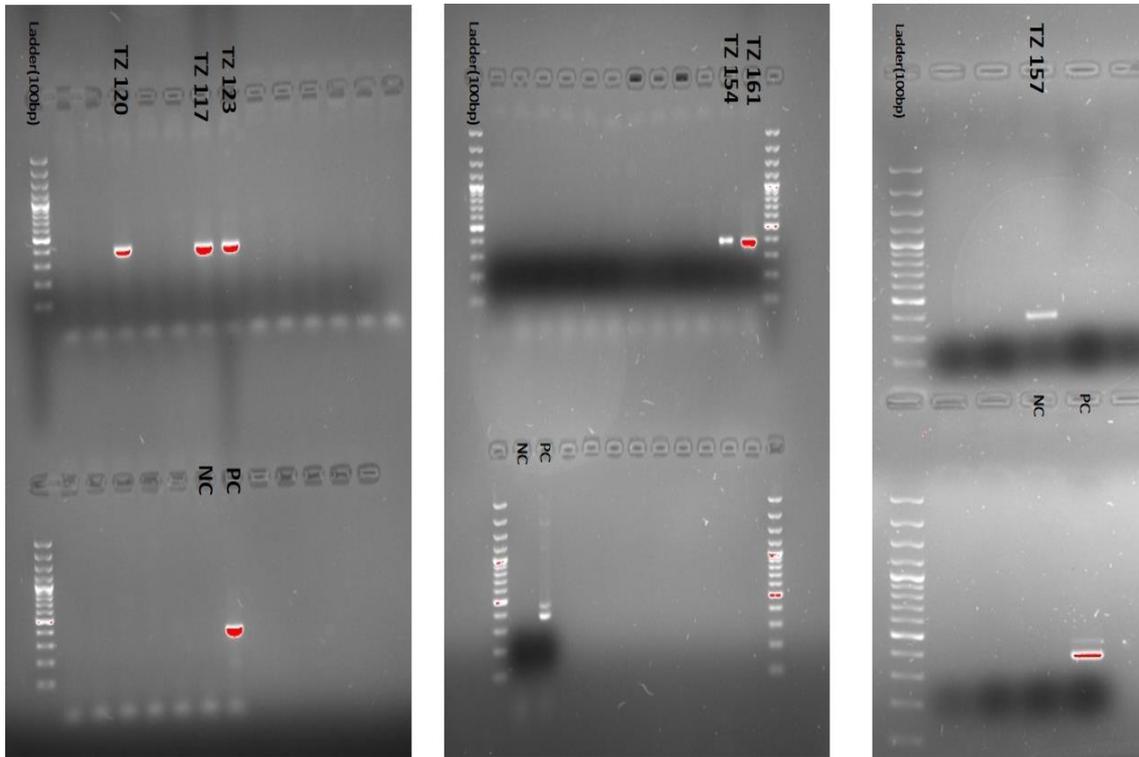


Figure 6: Positive bands (395bp) for Hantavirus, Negative controls (NC) and Positive controls (PC)

4.1.3 Rodent/Shrew Screening for Hantavirus in Mbeya Region

A total of 251 (247 rodents and 4 shrews) were trapped during the survey and the dominant group of animals trapped was *Rattus sp.*, however *Crocidura sp.* and *Myosorex sp.* were also trapped during the field trapping survey.

A total of 251 rodent's and shrew lung samples were screened for Hantavirus using Han-L PCR and the Gel electrophoresis was run but all samples were negative for Hantavirus (Fig. 7a and 7b).

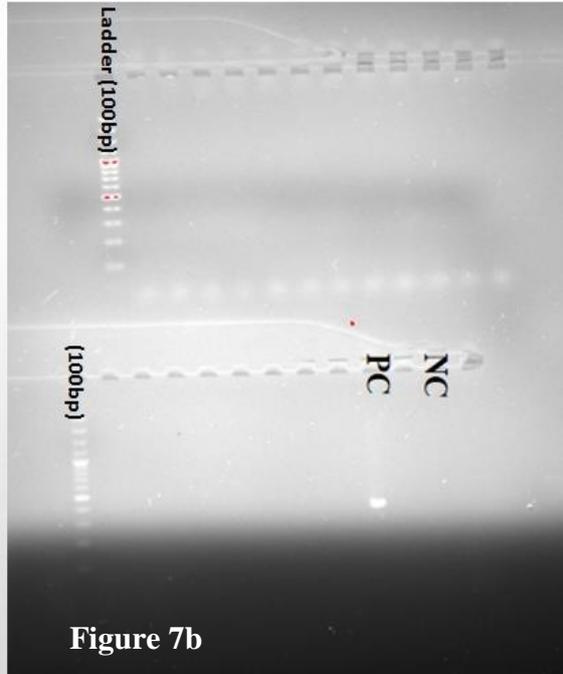
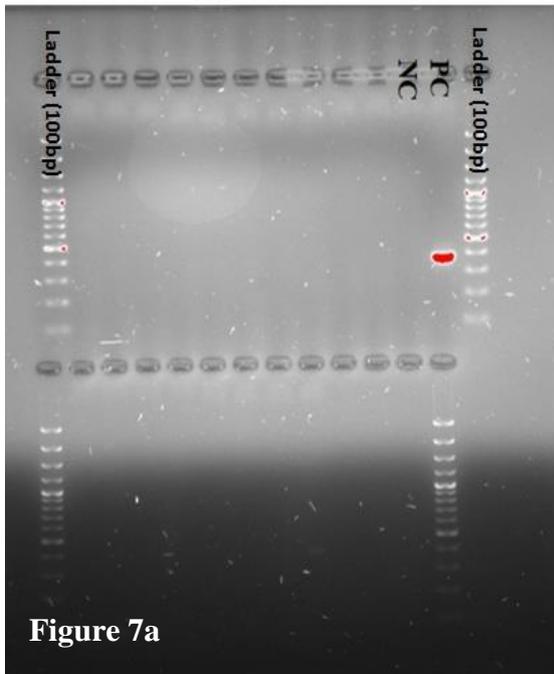


Figure 7a and 7b: Gel electrophoresis results for the Rodent and shrew lung samples screened using Han-L primers (reverse and forward)

4.1.4 Detection of Hantavirus Pathogen from Internal Organs

All organs collected i.e. Intestine, spleen, liver, kidney and spleen from all six positive *Mops condylurus* were tested for the Hantavirus and the results shows that the kidneys and intestines were also positives for Hantavirus and this indicates a probability that the viral pathogens were disseminated to the environments via urine and fecal matter (Fig. 8).

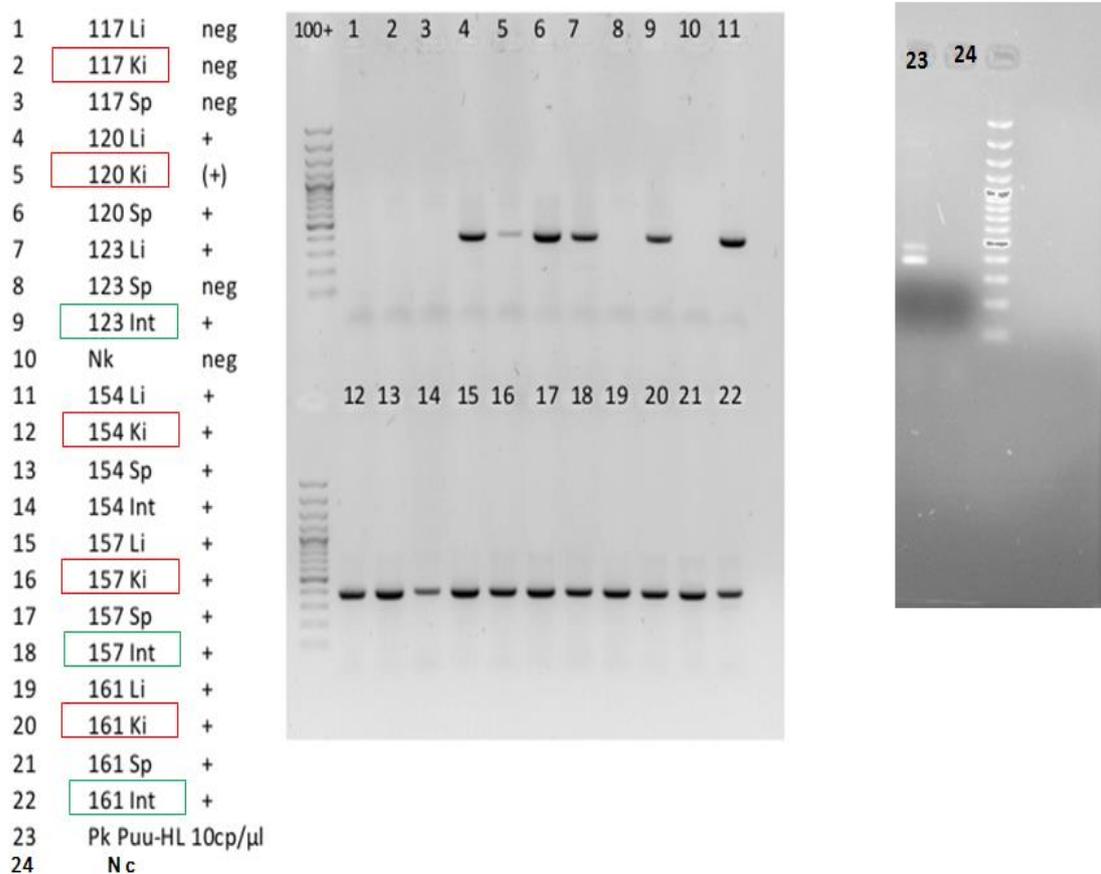


Figure 8: Gel electrophoresis results showing positivity in different organs after the Han-L-PCR results. Sp-spleen, Int-Intestine, Ki-Kidney, Li-Liver, Nk/Nc- Negative control, Pk-positive control

4.1.5 Locations where the New Hantavirus Strain has been detected

All six bats (*Mops condylurus*) infected with a new strain of Hantavirus were found alongside Kiwira river (Fig. 9 and 10) within GPS coordinates of 9° 37 ' 3.894 " S, 33° 54' 9.394 " E and 9° 36 ' 24.737 " S, 33° 55' 41.981 E. These locations are close to human population and have higher colonies of Mops.

TZ 117, TZ 120, TZ 123

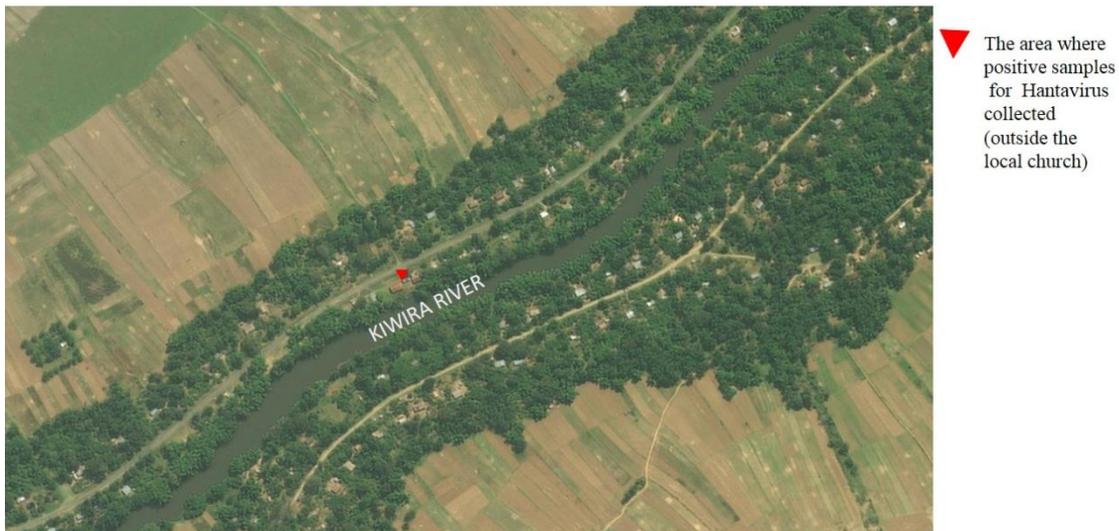


Figure 9: Location where bat animals named TZ 117, TZ 120 and TZ 123 were collected

TZ 154, TZ 157, TZ 161

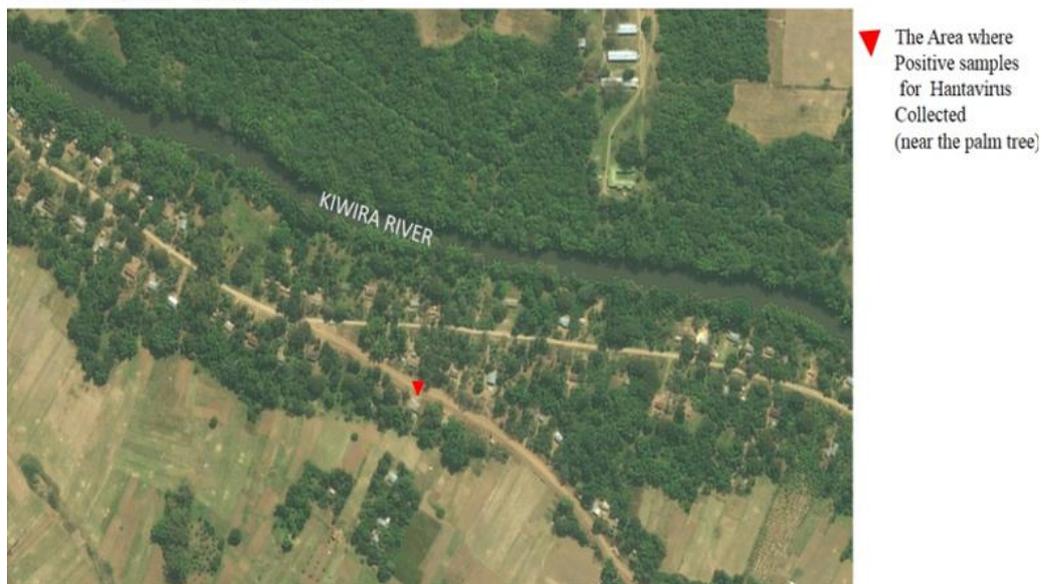


Figure 10: A palm tree where bat animals named TZ 154, TZ 157 and TZ 161 were collected

4.1.6 Phylogenetic Analysis of Bat-borne Hantaviruses detected in Mbeya Region

The phylogenetic analysis of new Hantavirus strains detected in six bats (*Mops condylurus*), close to Kiwira River and rice plantations in Kyela district. The strain was named Kiwira Virus as to locate the river where all six bats (i.e. TZ 117, TZ 120, TZ 123, TZ 154, TZ 157, and TZ 161) were collected. The sequences of Kiwira virus strain was highly related with a bootstrap value of 998, however, they have different sequence length.

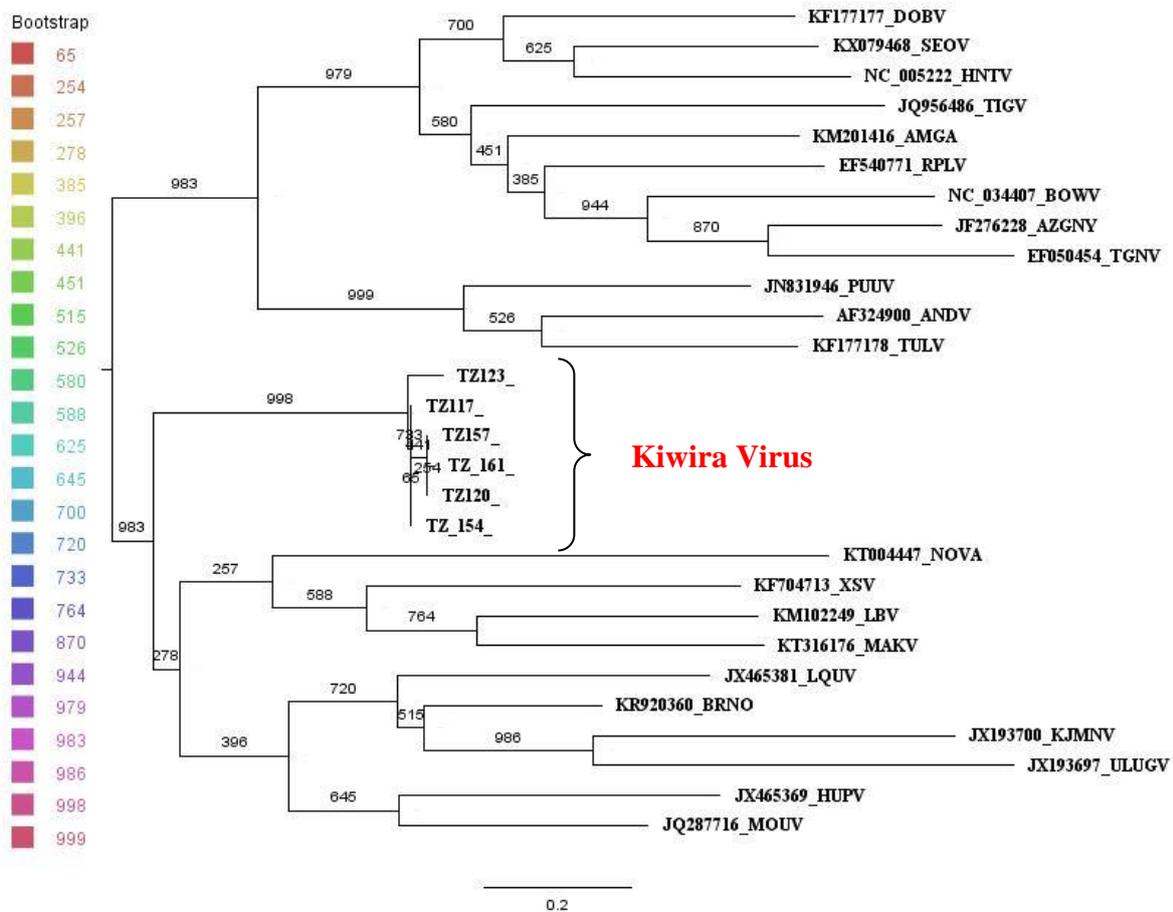


Figure 11: Maximum likelihood L-segment phylogenetic tree (Midpoint rooting) showing the evolutionary relationship between new bat-borne Hantavirus strain discovered in Tanzania and other Hantavirus strains discovered in Africa and other parts of the world

4.1.7 Assessment of Knowledge, Attitude and Practices (KAP) on Hantavirus Infections at the Community Level in Mbeya Region, Tanzania

(i) Demographic Characteristics of the Participants

Four hundred and thirty-eight (438) participants with a mean of age of 34.2 years were recruited in the study, out of which 27.17% have neither college nor university level of education. A small proportion of participants (15.07%) of all participants were health care workers (medical doctors, medical laboratory scientist, nurses etc.) and 54.8% have a net income of below 250 000 Tanzanian shillings per month. Close to one-fifth (19.41%) of the participants declined to respond to the question regarding their net income per month due to several unmentioned reasons. As shown in Appendix 1, majority of the participants (84.93%)

were members of the community with diverse occupations for example accountants, farmers, entrepreneurs, engineers and forest workers.

(ii) Assessment of the Level of Knowledge on Hantavirus Infections

A questionnaire constituting open and closed-ended questions was administered to the recruited participants as to understand the level of knowledge about the Hantavirus pathogen, its reservoir host, a disease caused by the pathogen, the relationship between Hantavirus infection and febrile illness, transmission routes of pathogens to a human being.

Almost one-third (33.3%) and 5.91% of the health care workers and other members of the community respectively know that rodents, bats and shrews are the reservoir hosts for the Hantavirus. Varying proportions i.e. 51.52% and 15.32% of health care workers and other members of the community respectively understand that Hantavirus infections can cause febrile illness. Sixty percent (60.61%) and 22.58% of both health care workers and other members of the community respectively understand the modes of transmission of Hantavirus to human i.e. through inhaling contaminated air with pathogen, being bitten by infected rodents, eating food or fruit which has been eaten by rodents or bats (Table 2).

With comparisons between districts (Kyela, Mbarali, Mbeya Rural and Mbeya Municipal), 31.79% of participants from Kyela district knows that a patient with Hantavirus infections may progress to Hantavirus cardiopulmonary syndrome (HCPS) or hemorrhagic fever with renal syndrome (HFRS). More than 90% of participants involved in the study in both Mbarali and Mbeya Rural do not know that a patient with Hantavirus infections may progress to either HCPS or HFRS depending on the Hantavirus strains infected the patients (Fig. 12).

Table 2: Proportions of participants with knowledge about Hantavirus infections.

Variables	Healthcare workers (n = 66)	Other members of the community (n = 372)	P value (CI: 95%)
Knowledge on the reservoir host and the viral pathogen causing Hantavirus infection	22 (33.33%)	22 (5.91%)	p<0.0001
Knowledge the modes of transmission for Hantavirus infection	40 (60.61%)	84 (22.58%)	p<0.0001
Knowledge on the relationship between Hantavirus and febrile illness	34 (51.52%)	57 (15.52%)	p<0.0001



Figure 12: Knowledge on whether people may get HCPS/HFRS after being infected with Hantavirus

(iii) Assessments of Attitudes on Hantavirus Infections

Twenty-two percent (22.15%) of community members believes that they could never get infections by eating food or fruits that have been eaten by reservoir hosts such as rodents or bats. This attitude endangers community health in terms of emerging infectious diseases. Five percent (5.25%) of individuals living in both districts have encouraged using organic manure collected from high bats colonies for agricultural purposes since it is more productive and this increases the risk of transmission of emerging infectious diseases since they do not use protective gears such as FP3 mask and gloves.

(iv) Assessments of Practices which Contributes to the Transmission of Hantavirus Infections

About 49.5% (217/438) of the residents of Mbeya region, who participated in the interview, had rodent breeding sites in their houses, however only 6.6% (29/438) of the participants wear mask and gloves during cleaning of those sites and this increases the risk of being infected with Hantavirus whenever the infected host is around. Forty two percent (42.0%) of individuals recruited in the study uses food or fruits which had already been eaten by rodents or bats, while slightly more than half (58.2%) of the interviewees are involved in agricultural activities such as banana plantations, cocoa and palm trees which play part as the roosting sites and favor the growths of different species of bats which are potential reservoir hosts for Hantavirus. Only 8.0% (35/438) of participants are involved in the forest activities such as Timbering and charcoal making which expose them onto risk of encountering infected reservoir hosts.

4.2 Discussion

Chiropterans (bats), rodents and shrews are important reservoir hosts for emerging infectious zoonotic viral pathogens such as Hantavirus, Rubella, Corona, Arena and Paramyxoviruses (Han *et al.*, 2015). Clear understanding on the diversity of the bat, rodents and shrew species within Mbeya region produces a clear image on the possible diseases which can be disseminated by small wild animals due to inter-relationships which exist between wildlife environment and human population since viral pathogen are host specific (Guterres and de Lemos, 2018). Identification of new strain of Hantavirus pathogen on free-tailed bats *Mops condylurus* in Tanzania expands the host diversity for Hantavirus in Africa.

The discovery of a new strain of bat-borne Hantavirus in Mbeya region highlights the need for further investigations on the transmissibility of the virus to other host species such as human population.

The possibilities of the viral dissemination on the environment via urine and fecal matter have been evidenced by the detection of virus in the kidney and intestine organs. However, this needs confirmatory research on the availability of the virus in the environment which would predict the risk for the transmission of Hantavirus strain identified and other zoonotic pathogens to human.

A low number of shrews trapped during the survey in Kyela and Mbarali districts of Mbeya region is supported by publications and reports which shows the scarce of the species within the regions (PREDICT, 2016; Stanley and Esselstyn, 2010).

The results of molecular screening of rodents and shrew samples collected within districts of Mbeya region indicates that the samples were negative and this does not mean that the virus is not circulating in the region. Geographical locations for rodent/shrew trapping have to be extended as well as seasonality consideration (Guterres and de Lemos, 2018; Klempa, 2009; Vaheiri *et al.*, 2013) as it is already known that infection rate in small mammals varies with time and depends much on seasons.

Knowledge concerning Hantavirus infections is very low at the community level compared to the clinical individuals in Mbeya region as observed in this study, which endanger the community health. However, there is at least a hope for proper patient management when they arrive at health care facilities since more than 50% of health care workers are aware of Hantavirus infections. In addition, the increase in the practices that favor the transmission of Hantavirus pathogen from reservoir host to human, jeopardize the community health. Therefore, there is a need of informing the society on Hantavirus pathogen, transmission modes as well as risk factors for the disease transmission as to increases the awareness about Hantavirus infections (Hansen *et al.*, 2017; Suzuki and Mutinelli, 2009). The observed difference in the knowledge on the Hantavirus disease progression in human, following the response on the knowledge of whether Hantavirus infections may cause Hantavirus cardiopulmonary syndrome (HCPS) or hemorrhagic fever with renal syndrome between districts indicates the priority needs on the awareness programs of Hantavirus infections between the study districts.

Raising the public awareness on the Hantavirus infection is more significant as to facilitate the updates on the understanding in both clinical society and community in general. Awareness of the society on Hantavirus infections may help on the improvement of febrile patient's management since the Health workers will remain being updated and may play as the prevention and control strategy at community level within the society.

4.3 Limitations of the Study

A low number of shrews trapped during the survey, limits the inference on the detection status of the Hantavirus on shrews in Mbeya region. Short time for rodents, bats and shrews trapping and limited trapping locations due to budget limits, also provides the edge on concluding that rodents and other bat species were not carrying Hantavirus in Tanzania.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The maximum likelihood phylogenetic tree of L segment suggest the previous unidentified Hantavirus strain named Kiwira virus. Free-tailed bats (*Mops condylurus*) were confirmed through Cytochrome B (CYT B) PCR and sequencing to carry Hantavirus with a prevalence of 6.97% within *Mops condylurus* species. However this count to a prevalence of 1.80% on whole bat species trapped during the survey.

Low level of knowledge on Hantavirus infection has been observed on the community compared to the Health care workers (HCWs) which jeopardize the community health. In addition, the community have practices which increase the contact with infected Hantavirus reservoir host and this endanger the public health.

The identification of bat-borne Hantavirus in Mbeya region open further research on the ability of this new virus to infect other bat species other than *Mops condylurus*, not only that but also the possibility of intra- and interspecies transmission.

5.2 Recommendations

Following the identification of Kiwira virus in Kyela district, the Investigations on the virulence and pathogenicity of the discovered virus as well as the contribution of climatic and environmental factors on the transmissibility of the virus to infect other host species is highly recommended.

Development of pseudo recombinant protein (antigens) which may be used for screening febrile patient sample is highly needed to establish the real prevalence of Hantavirus in Mbeya region and Tanzania in general. In addition, the development of a rapid diagnostic assay using discovered Hantavirus antigens from Africa would help on capturing early Hantavirus infection on febrile patients and improve the management of febrile illness.

Further research on *Mops condylurus* and other bat species as well as rodents and shrews have to be implemented on different geographical locations in Tanzania as to detect the undiscovered Hantavirus serotypes as well as to determine the geographical range of the *Mops condylurus* as far as Hantavirus is concerned.

Preparation of awareness programs is of most importance to the community members of Mbeya region and Tanzania in general as to ensure the alertness of the society concerning Hantavirus infections.

REFERENCES

- Allocati, N., Petrucci, A., Di Giovanni, P., Masulli, M., Di Ilio, C. and De Laurenzi, V. (2016). Bat–man disease transmission: zoonotic pathogens from wildlife reservoirs to human populations. *Cell Death Discovery*, 2, 16048.
- Arya, R., Antonisamy, B. and Kumar, S. (2012). Sample size estimation in prevalence studies. *Indian Journal of Pediatrics*, 79(11), 1482 - 1488.
- Avšič-Županc, T., Saksida, A. and Korva, M. (2015). Hantavirus infections. *Clinical Microbiology and Infection*, doi:10.1111/1469-0691.12291
- Barrett, A. D. and Stanberry, L. R. (2009). Vaccines for Biodefense and Emerging and Neglected diseases: *Academic Press*.
- Bi, Z., Formenty, P. B. and Roth, C. E. (2008). Hantavirus infection: a review and global update. *The Journal of Infection in Developing Countries*, 2(01), 003-023.
- Calisher, C. H., Childs, J. E., Field, H. E., Holmes, K. V. and Schountz, T. (2006). Bats: important reservoir hosts of emerging viruses. *Clinical Microbiology Reviews*, 19(3), 531 - 545.
- Charan, J. and Biswas, T. (2013). How to calculate sample size for different study designs in medical research? *Indian Journal of Psychological Medicine*, 35(2), 121.
- Chau, R., Bhatt, N., Manhiça, I., Cândido, S., de Deus, N., Guiliche, O..., Monteiro, V. (2017). First serological evidence of Hantavirus among febrile patients in Mozambique. *International Journal of Infectious Diseases*, 61, 51 - 55.
- Clement, J., Lee, A., Verpooten, G., Laenen, L., Vergote, V., De Samblanx, H..., Maes, P. (2018). Acute Hantavirus infection presenting as haemolytic-uraemic syndrome (HUS): the importance of early clinical diagnosis. *European Journal of Clinical Microbiology and Infectious Diseases*, 37(1), 135 - 140.
- Clement, J., Maes, P. and Van Ranst, M. (2006). Hantaviruses in the old and new world. *Perspectives in Medical Virology*, 16, 161 - 177.
- Council, N. R. (1991). Infectious diseases of mice and rats: *National Academies Press*.

- Forbes, K. M., Sironen, T. and Plyusnin, A. (2018). Hantavirus maintenance and transmission in reservoir host populations. *Current Opinion in Virology*, 28, 1 - 6.
- Fulhorst, C. F., Milazzo, M. L., Armstrong, L. R., Childs, J. E., Rollin, P. E., Khabbaz, R. . . . , Ksiazek, T. G. (2007). Hantavirus and arenavirus antibodies in persons with occupational rodent exposure, North America. *Emerging Infectious Diseases*, 13(4), 532.
- Gu, S. H., Nicolas, V., Lalis, A., Sathirapongsasuti, N. and Yanagihara, R. (2013). Complete genome sequence and molecular phylogeny of a newfound Hantavirus harbored by the Doucet's musk shrew (*Crocidura douceti*) in Guinea. *Infection, Genetics and Evolution*, 20, 118 - 123.
- Guterres, A. and de Lemos, E. R. S. (2018). Hantaviruses and a neglected environmental determinant. *One Health*.
- Han, H. J., Wen, H. I., Zhou, C. M., Chen, F. F., Luo, L. M., Liu, J. W. and Yu, X. J. (2015). Bats as reservoirs of severe emerging infectious diseases. *Virus Research*, 205, 1 - 6.
- Hansen, A., Xiang, J., Liu, Q., Tong, M., Sun, Y., Liu, X. . . . , Han, G. S. (2017). Experts' Perceptions on China's Capacity to Manage Emerging and Re-emerging Zoonotic Diseases in an Era of Climate Change. *Zoonoses and Public Health*, 64(7), 527 - 536.
- Hepojoki, J., Vaheri, A. and Strandin, T. (2014). The fundamental role of endothelial cells in Hantavirus pathogenesis. *Frontiers in Microbiology*, 5, 727.
- Hjelle, B. (2008). Pathogenesis of Hantavirus infections. In: UpToDate: Wellesley, MA, USA.
- Hofinger, D., Nofchissey, R., Jacobson, M. and Goade, D. (2006). Serological survey of Hantavirus in adult humans living in Tanzania. In: *BMJ Publishing Group Limited*.
- Ioannou, A., Andreou, M., Watson, D., Chra, P. and Panos, G. (2017). Hantavirus Infections in the European Region: A Mini-Review of the Literature. *Journal of Expanded Microbiology*, 5(1), 00139.

- Ithete, N., Matthee, S., Auste, B., Witkowski, P., Klempa, B., Kruger, D. and Preiser, W. (2014). Evidence of Hantavirus infection in South Africa. *International Journal of Infectious Diseases*, 21, 182 - 183.
- Jiang, H., Zheng, X., Wang, L., Du, H., Wang, P. and Bai, X. (2017). Hantavirus infection: a global zoonotic challenge. *Virologica Sinica*, 32(1), 32 - 43.
- Jonsson, C. B., Figueiredo, L. T. M. and Vapalahti, O. (2010). A global perspective on Hantavirus ecology, epidemiology, and disease. *Clinical Microbiology Reviews*, 23(2), 412 - 441.
- Jonsson, G. and Schmaljohn, S. (2001). Replication of Hantaviruses. Eds.: Scmaljohn SC, Nichol ST: Hantaviruses. In: Berlin. *Springer-Verlag Press*.
- Kallio, E. R., Klingström, J., Gustafsson, E., Manni, T., Vaheri, A., Henttonen, H..., Lundkvist, Å. (2006). Prolonged survival of Puumala Hantavirus outside the host: evidence for indirect transmission via the environment. *Journal of General Virology*, 87(8), 2127 - 2134.
- Kang, H. J., Kadjo, B., Dubey, S., Jacquet, F. and Yanagihara, R. (2011). Molecular evolution of Azagny virus, a newfound Hantavirus harbored by the West African pygmy shrew (*Crocidura obscurior*) in Côte d'Ivoire. *Virology Journal*, 8(1), 373.
- Kang, H. J., Stanley, W. T., Esselstyn, J. A., Gu, S. H. and Yanagihara, R. (2014). Expanded host diversity and geographic distribution of Hantaviruses in sub-Saharan Africa. *Journal of Virology*, 88(13), 7663 - 7667.
- Khaiboullina, S. F., Morzunov, S., St, J. and Stephen, C. (2005). Hantaviruses: molecular biology, evolution and pathogenesis. *Current Molecular Medicine*, 5(8), 773 - 790.
- Kim, H. C., Kim, W. K., Klein, T. A., Chong, S. T., Nunn, P. V., Kim, J. A..., Song, J. W. (2017). Hantavirus surveillance and genetic diversity targeting small mammals at Camp Humphreys, a US military installation and new expansion site, Republic of Korea. *PloS One*, 12(4), e0176514.
- Klempa, B. (2009). Hantaviruses and climate change. *Clinical Microbiology and Infection*, 15(6), 518-523.

- Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H..., Krüger, D. H. (2007). Novel Hantavirus sequences in shrew, Guinea. *Emerging Infectious Diseases*, 13(3), 520.
- Klempa, B., Fichet-Calvet, E., Lecompte, E., Auste, B., Aniskin, V., Meisel, H..., Krüger, D. H. (2006). Hantavirus in African wood mouse, Guinea. *Emerging Infectious Diseases*, 12(5), 838.
- Klempa, B., Koivogui, L., Sylla, O., Koulemou, K., Auste, B., Krüger, D. H. and ter Meulen, J. (2010). Serological evidence of human Hantavirus infections in Guinea, West Africa. *The Journal of Infectious Diseases*, 201(7), 1031 - 1034.
- Klempa, B., Witkowski, P. T., Popugaeva, E., Auste, B., Koivogui, L., Fichet-Calvet, E..., Krüger, D. H. (2012). Sangassou virus, the first Hantavirus isolate from Africa, displays genetic and functional properties distinct from those of other murinae-associated Hantaviruses. *Journal of Virology*, 86(7), 3819 - 3827.
- Kruger, D. H., Figueiredo, L. T. M., Song, J. W. and Klempa, B. (2015). Hantaviruses—globally emerging pathogens. *Journal of Clinical Virology*, 64, 128 - 136.
- Krüger, D. H., Schönrich, G. and Klempa, B. (2011). Human pathogenic Hantaviruses and prevention of infection. *Human Vaccines*, 7(6), 685 - 693.
- Krüger, D. H., Ulrich, R. G. and Hofmann, J. (2013). Hantaviruses as zoonotic pathogens in Germany. *Deutsches Ärzteblatt International*, 110(27-28), 461.
- Maes, P., Clement, J., Gavrilovskaya, I. and Van Ranst, M. (2004). Hantaviruses: immunology, treatment, and prevention. *Viral Immunology*, 17(4), 481 - 497.
- Manigold, T. and Vial, P. (2014). Human Hantavirus infections: epidemiology, clinical features, pathogenesis and immunology. *The European Journal of Medical Sciences*, 144.
- Martinez, V. P., Bellomo, C., San Juan, J., Pinna, D., Forlenza, R., Elder, M. and Padula, P. J. (2005). Person-to-person transmission of Andes virus. *Emerging Infectious Diseases*, 11(12), 1848.

- Mattar, S., Garzon, D., Tadeu, L., Faccini-Martínez, A. A. and Mills, J. N. (2014). Serological diagnosis of Hantavirus pulmonary syndrome in a febrile patient in Colombia. *International Journal of Infectious Diseases*, 25, 201 - 203.
- Meheretu, Y., Čížková, D., Těšíková, J., Welegerima, K., Tomas, Z., Kidane, D..., Günther, S. (2012). High diversity of RNA viruses in rodents, Ethiopia. *Emerging Infectious Diseases*, 18(12), 2047.
- Mills, J. N., Corneli, A., Young, J. C., Garrison, L. E., Khan, A. S., Ksiazek, T. G., Control, C. f. D. and Prevention. (2002). Hantavirus pulmonary syndrome-United States: Updated recommendations for risk reduction. *Morbidity and Mortality Weekly Report, Recommendation Reports*, 51(9).
- Mori, M., Rothman, A. L., Kurane, I., Montoya, J. M., Nolte, K. B., Norman, J. E..., Ennis, F. A. (1999). High levels of cytokine-producing cells in the lung tissues of patients with fatal Hantavirus pulmonary syndrome. *The Journal of Infectious Diseases*, 179(2), 295 - 302.
- Plyusnin, A., Vapalahti, O. and Vaheri, A. (1996). Hantaviruses: genome structure, expression and evolution. *Journal of General Virology*, 77(11), 2677 - 2687.
- Pourhoseingholi, M. A., Vahedi, M. and Rahimzadeh, M. (2013). Sample size calculation in medical studies. *Gastroenterology and Hepatology from Bed to Bench*, 6(1), 14.
- PREDICT (2016). Reducing Pandemic Risk, Promoting Global Health, Supporting the Global Health Security Agenda. Retrieved from *University of California, Davis*: <http://www.vetmed.ucdavis.edu/ohi/predict/index.cfm> on November 12, 2018.
- Reynes, J. M., Razafindralambo, N. K., Lacoste, V., Olive, M. M., Barivelo, T. A., Soarimalala, V..., Lavergne, A. (2014). Anjozorobe Hantavirus, a new genetic variant of Thailand virus detected in rodents from Madagascar. *Vector-Borne and Zoonotic Diseases*, 14(3), 212 - 219.
- Simpson, S. Q., Spikes, L., Patel, S. and Faruqi, I. (2010). Hantavirus pulmonary syndrome. *Infectious Disease Clinics*, 24(1), 159 - 173.

- Spiegel, A., Erichsen, S. and Solleveld, H. (1980). Animal quality and models in biomedical research. Paper presented at the *Symposium of the International Council for Laboratory Animal Science 1979*: Utrecht.
- Stanley, W. T. and Esselstyn, J. A. (2010). Biogeography and diversity among montane populations of mouse shrew (Soricidae: Myosorex) in Tanzania. *Biological Journal of the Linnean Society*, 100(3), 669 - 680.
- Su, S. W., Wu, Y. C., Chang, S. F., Ho, L. L. and Su, C. F. (2015). Epidemiological Investigation of Hantavirus in Rodents at International Ports in Taiwan, 2010-2013. *Epidemiology Bulletin*, 31(14), 121 - 121.
- Sudi, L., Mangu, C., Tarimo, T., Ntinginya, N. and Shirima, G. (2018). Hantaviruses in East and Central Africa. *American Journal of Research Communication*, 6(6), 1 - 14.
- Sumibcay, L., Kadjo, B., Gu, S. H., Kang, H. J., Lim, B. K., Cook, J. A..., Yanagihara, R. (2012). Divergent lineage of a novel Hantavirus in the banana pipistrelle (*Neoromicia nanus*) in Cote d'Ivoire. *Virology Journal*, 9(1), 34.
- Suzuki, K. and Mutinelli, L. E. (2009). Knowledge and practices about Hantavirus pulmonary syndrome in a cluster of Japanese communities in Argentina. *Revista Panamericana de Salud Pública*, 25, 128 - 133.
- Vaheri, A., Henttonen, H., Voutilainen, L., Mustonen, J., Sironen, T. and Vapalahti, O. (2013). Hantavirus infections in Europe and their impact on public health. *Reviews in Medical Virology*, 23(1), 35 - 49.
- Vaheri, A., Vapalahti, O. and Plyusnin, A. (2008). How to diagnose Hantavirus infections and detect them in rodents and insectivores. *Reviews in Medical Virology*, 18(4), 277-288.
- Walsh, K. (2018). E-learning modules in new and emerging infectious diseases improve the applied knowledge and problem-solving skills of healthcare professional learners. *BMJ Simulation and Technology Enhanced Learning*, doi:10.1136/bmjstel-2017-000291.

- Weiss, S., Witkowski, P. T., Auste, B., Nowak, K., Weber, N., Fahr, J..., Drosten, C. (2012). Hantavirus in bat, Sierra Leone. *Emerging Infectious Diseases*, 18(1), 159.
- Witkowski, P. T., Drexler, J. F., Kallies, R., Ličková, M., Bokorová, S., Mananga, G. D..., Drosten, C. (2016). Phylogenetic analysis of a newfound bat-borne Hantavirus supports a laurasiatherian host association for ancestral mammalian Hantaviruses. *Infection, Genetics and Evolution*, 41, 113 - 119.
- Witkowski, P. T., Klempa, B., Ithete, N. L., Auste, B., Mfuné, J. K., Hoveka, J..., Krüger, D. H. (2014). Hantaviruses in Africa. *Virus Research*, 187, 34 - 42.
- Witkowski, P. T., Leendertz, S. A., Auste, B., Akoua-Koffi, C., Schubert, G., Klempa, B..., Krüger, D. H. (2015). Human seroprevalence indicating Hantavirus infections in tropical rainforests of Côte d'Ivoire and Democratic Republic of Congo. *Frontiers in Microbiology*, 6, 518.
- Zhang, Y. Z. (2014). Discovery of Hantaviruses in bats and insectivores and the evolution of the genus Hantavirus. *Virus Research*, 187, 15 - 21.

LIST OF APPENDIXES

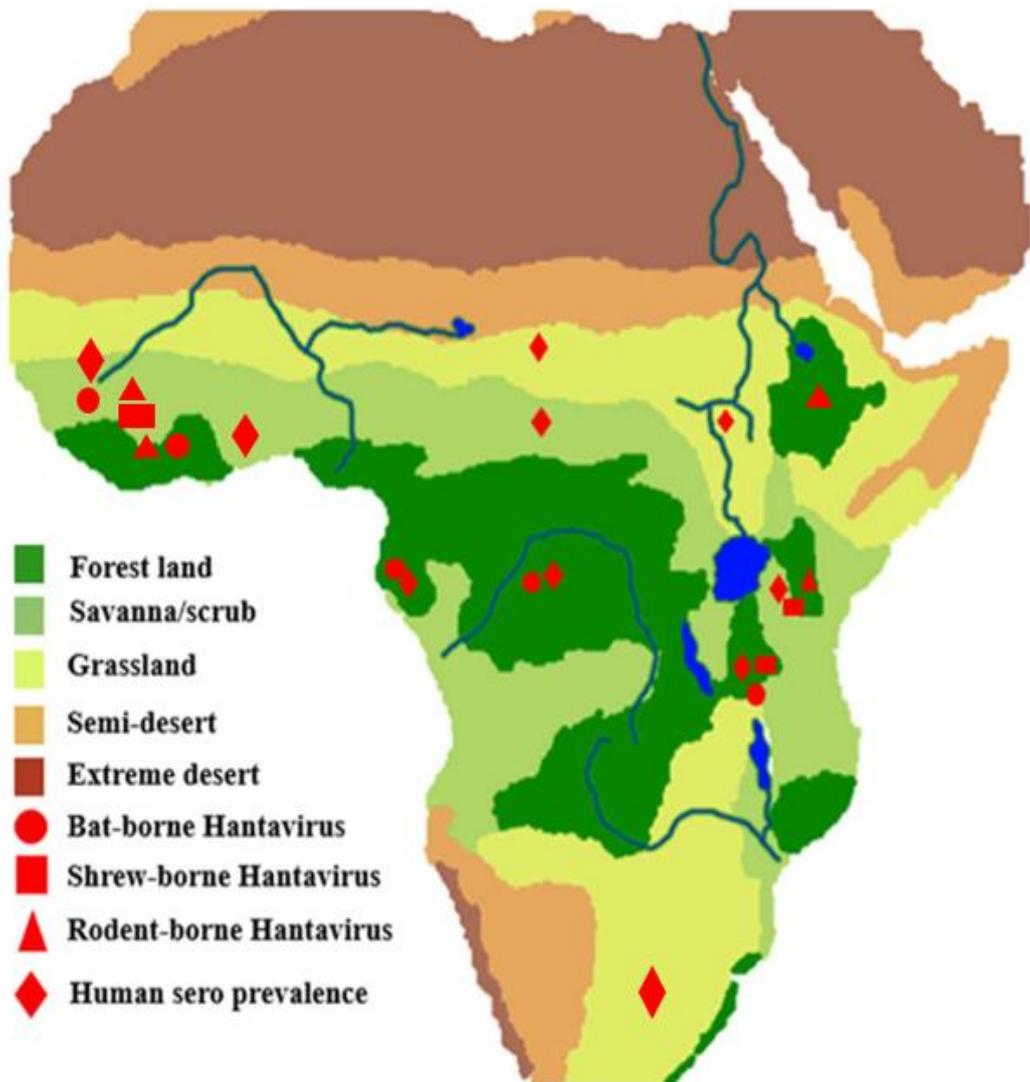
Appendix 1: Table showing Characteristics of Participants involved in the Survey

Characteristics (n=438)	Percentages
Age group	
18-25 years	98 (22.37%)
26-45 years	277 (63.24%)
>45 years	63 (14.38%)
Sex	
Female	262 (59.82%)
Male	176 (40.18%)
Education	
No formal education	26 (5.94%)
Primary level	207 (47.26%)
Secondary level	86 (19.83%)
College/University	119 (27.17%)
Occupation	
Entrepreneur's	149 (34%)
Farmers	165 (37.67%)
Other occupations	58 (13.24%)
Medical doctors	23 (5.25%)
Nurses	26 (5.94%)
Medical Laboratory personnel	17 (3.88%)
Income generation per months (Tshs)	
<100 000	127 (29.00%)
100 000 – 250 000	113 (25.8%)
250 000 – 1 000 000	92 (21.00%)
>1 000 000	21 (4.79%)
Districts involved in the interview	
Kyela	151 (34.47%)
Mbarali	96 (21.96%)
Mbeya municipal	98 (22.37%)
Mbeya rural	93 (21.23%)

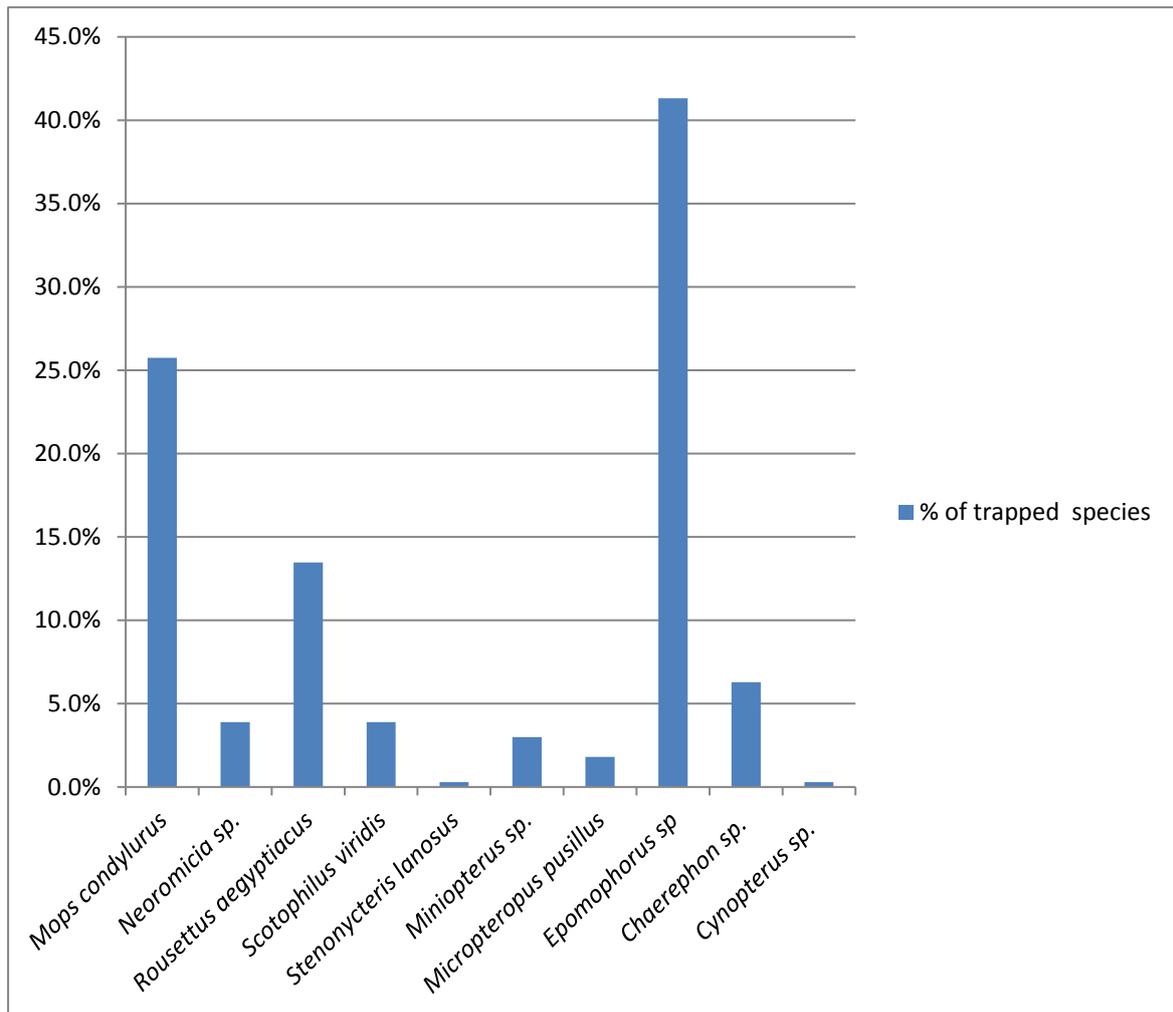
Appendix 2: Hantavirus Strains used in the Phylogenetic Tree Preparation

Virus	Abbreviation	Sources of Virus	Origin	GeneBank Accession Number
Mouyassue Virus	MOUV	<i>Neoromicia nanus</i>	Ivory Coast	JQ2877162
Huangpi Virus	HUPV	<i>Pipisterus abramus</i>	China	JX465369
Uluguru Virus	ULUV	<i>Myosorex zinki</i>	Tanzania	JX193697
Kilimanjaro Virus	KJMNV	<i>Myosorex geata</i>	Tanzania	JX193700
Brno Virus	BRNO	<i>Nyctalus noctula</i>	Czech Republic	KR920360
Longquan Virus	LQUV	<i>Rhinolophus afinis</i> , <i>Rhinolophus sinicus</i> , <i>Rhinilophus monoceros</i>	China	JX465381
Makokou Virus	MKV	<i>Hipposideros rubber</i>	Gabon	KT316176
Laibin Virus	LBV	<i>Taphozous melapogon</i>	China	KM102249
Xuan son Virus	XSV	<i>Hipposideros pomona</i> , <i>Neoromicia nanus</i>	Vietnam, Ivory Coast	KF704713
Nova Virus	NOVA	<i>Talpa europaea</i>	Belgium	KT004447
Tula Virus	TULV	<i>Microtus subterraneus</i> , <i>Microtus arvalis</i>	Serbia	KF177178
Andes Virus	ANDV	<i>Oligoryzomys longicaudatus</i>	Argentina, Chile	AF324900
Puumala Virus	PUUV	<i>Myodes glareolus</i>	Europe	JN831946
Tanganya Virus	TGNV	<i>Crocidura theresae</i>	Guinea	EF050454
Azagny Virus	AZGNY	<i>Crocidura obscurior</i>	Ivory Coast	JF276228
Bowe Virus	BOWV	<i>Crocidura douceti</i>	Guinea	NC034407
Amga Virus	AMGA	<i>Sorex caecutiens</i>	Russia	KM201416
Tigray Virus	TIGV	<i>Stenocephalemys albipes</i>	Ethiopia	JQ 956486

Appendix 3: Map of Africa Showing Hantavirus Distribution in Relation to Vegetation Density in Africa



Appendix 4: Bat Species Identified using Cytochrome B PCR and Sequencing



Appendix 5: Questionnaire to Assess the Knowledge, Attitude and Practices on Hantavirus Infections at the Community Level in Mbeya Region, Tanzania

Please provide your assistance by giving your answers to the following questions.

(Please circle the correct answer)

Registration number: 2018MB -----

Bio data:

1.1 Surname and given name of the participant:

1.2 Date of Birth: _____ age: _____

1.3 Gender: (a) Male (b) Female

1.4 Phone number: _____

1.5 Education level of a participant

(a) No education (b) primary (c) secondary (d) college (e) university

2 Location and general information

2.2 a) Village..... b) Ward.....

2.2 District 2.3 GPS: S.....E

3 Date questionnaire was taken:

4 Name of Investigator taking questionnaire:.....

5 Signature of the investigator taking the questionnaire:.....

General Questions

6 Occupation of participant:

(a) Doctor (b) teacher (c) engineer (d) Agriculturalist

(e) Lawyer (f) nurses (g) accountant (h) entrepreneur

(i) Others; specify _____

7 The number of family members: _____

8 Type of house

- (a) Made of cement block (b) made of normal block
(c) Made of wood (d) others; specify_____

9 What is your monthly income in terms of cash?

- (a) <100 000 Tanzanian shillings (b) 100 000 -250 000 Tanzanian shillings
(c) 250 001- 500 000 Tanzanian shillings (d) 500,001-1,000,000 Tanzanian shillings
(e) >1 000 000 Tanzanian shillings (f) I don't know or decline to answer

10 The roof of your house is made of

- (a) Grasses (b) Aluminum sheet

11 Have you noticed a bat in your roof.

- (a) Yes (b) No

Assessment of Knowledge, Attitude and Practices Associated with Hantavirus Transmission

12 Did you ever hear anything about Hantavirus infections in your life?

- (a) Yes (go to Qn.13) (b) No

13 What cause Hantavirus infection?

- (a) Bacteria (b) parasite like malaria (c) Virus (d) I do not know

14 What have you heard about Hantavirus infections?

- (a) Pathogen causing the diseases
(b) The reservoir host of the diseases
(c) The risk factors of the Hantavirus exposure
(d) The disease itself
(e) Others (please write)_____
- (f) I do not know

15 From which sources did you get information about the Disease?

- (a) From health workers (b) TV and radio
(c) Newspapers, publications and journals (d) Taught at the school/university/college

(e) Others (Please write) _____

16 Do you know that human being can progress to Hantavirus cardiopulmonary syndrome/renal complication when he/she is exposed to the infections?

- (a) Yes (go to Qn.17-18) (b) No

17 What do you think; how people can get infected with Hantavirus?

- (a) Inhaling viral contaminated dust
(b) By Sexual contact
(c) Dirty hands
(d) Being bitten by rodents/shrew or bat
(e) Cleaning a house where bat or rodents resides
(f) Others _____

18 Have you heard anything about febrile illness?

- (a) Yes (go to question 19-21) (b) No

19 Mention the pathogen, which causes febrile illness?

- (a) Bacteria (b) Virus (c) Fungus (d) Others; _____

20 Do you know that Hantavirus can cause febrile illness?

- (a) Yes (b) No

21 Febrile illness is manifested by:

- (a) Headache (b) Fever (c) Loss of appetite (d) Others specify.....

22 Did have febrile illness for the past 12 months?

- (a) Yes (b) No

23 If Yes how many times

- (a) Once. (b) Twice (c) Thrice (d) More than 3 times.

24 How many times did you take drugs before going to hospital or to dispensary?

- (a) None (b) Once (c) Twice (d) All times

25 What kind of drugs did you take?

(a) Paracetamol (b) antibiotics (c) Herbs (d) Others _____

26 In your house did you ever seen rodents.

(a) Yes (b) No

27 Is there a breeding site of rodents in your house?

(a) Yes (b) No

If **Yes** go to question number 28-29 and if **No** go to question number 30

28 Do you clean places where rodents stay/breed?

(a) Yes (b) No

29 When you are cleaning a breeding site in your house, do you wear a mask?

(a) Yes (b) No

30 Do you eat food or fruits, which is eaten by a rodent/bat or contaminated with feces?

(a) Yes (b) No

31 Do you consume rodent/shrew or bat meat?

(a) Yes (b) No

32 Do you have trees (i.e. cocoa or palm etc.) close to your house?

(a) Yes (b) No

33 Have you ever seen a bat roosted in a tree close to your house?

(a) Yes (b) No

34 Do you involve yourself in any agricultural activities such as cocoa, banana or any fruit plantation?

(a) Yes (b) No

35 Have you ever see a fruit eaten before picking it?

(a) Yes (go to Qn.36) (b) No

36 Did you eat those fruits, which found eaten after picking?

(a) Yes (b) No

37 Are you involved in Charcoal making or timbering in the forest as an economic activity?

(a) Yes (b) No

38 What means do you use for cooking every day meal?

(a) Wood (b) Charcoal (c) Others_____

39 If you are using woods as source of energy during cooking, where do you collect them?

(a) In the forest (b) from the trees around the house (c) buy them from the market

40 Do you use organic manure from bat as a source of fertilizer?

(a) Yes (b) No

41 When collecting organic manure do you wear a mask and gloves?

(a) Yes (b) No

Thank you very much for your time

Appendix 6: Informed Consent



INFORMED CONSENT AND CONFIDENTIALITY FORM

This Informed Consent Form is for men and women who are normal citizens of this region, and who I am inviting them to participate in Hantavirus research on the knowledge, attitude and practices associated with risk factors for transmission of Hantavirus infections in Mbeya region, Tanzania

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the research with you)**
- **Certificate of Consent (for signatures if you agree to take part)**

You will be given a copy of the full Informed Consent Form

My name is _____, a student from Nelson Mandela African Institution of Science and Technology, Arusha. Taking Master of life science (health and biomedical science specialization), I am conducting a research on the assessment of knowledge on and practices which associates with Hantavirus transmission in Mbeya region, Tanzania. After understanding the information about the research, I invite you to be the part of this research.

Hantaviruses are enveloped, negative sense RNA viruses found in the family *Bunyaviridae* that cause two life-threatening human zoonoses namely, hemorrhagic fever with renal syndrome (HFRS) and Hantavirus cardiopulmonary syndrome (HCPS), with case fatality rates of up to 50%. Hantaviruses can infect wild life animals and cause persistent apparent infection while infected humans develop non-persistent infection and acute illness

Hantaviruses are a prime example for pathogens naturally occurring in wildlife, whose emergence in humans is due to factors largely attributable to human activities and favored by certain weather patterns, which may become more common as climate change progresses.

This study aims at assessing the level of knowledge about the viral infections within the community and the practice associated with the risk factors for the transmission of the diseases

The results from this study will contribute on health policy making as to plan and to put forward the dissemination strategy for the knowledge for the knowledge of Hantavirus infections in Tanzania.

Participation and Confidentiality

The interview process will take about 15 - 20 minutes, and our conversation will be used for research purposes only; your participation in this research is voluntary. It is your choice whether to participate or not but the benefit to participate in this research is that you will be part of people who will tell the community about the Hantavirus and who will give the reasons of this study to push through. Your information will not be shared with anyone, including your neighbors or community health workers. The records of this study will be kept private. No one will have access to the information except the Investigators. In addition, you are not obliged to answer any question you do not want to, and there are no right or wrong answers in our discussions. So please do not feel pressured to give a specific response and do not feel shy if you do not know the answer to a question instead you are allowed to ask for more clarification. I would like you to answer questions honestly.

Certificate of consent

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research.

Print Name of Participant _____

Signature of Participant _____

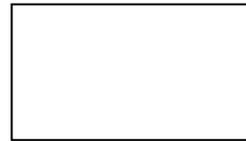
If the participant is illiterate (the witness part)

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness _____ and

Thumb print of participant

Signature of witness _____



Date _____

Day/Month/Year

Researcher/Interviewee

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent _____

Signature of Researcher /person taking the consent _____

Date _____

Day/month/year

Appendix 7: NIMR Ethical Clearance



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: headquarters@nimr.or.tz

Ministry of Health, Community
Development, Gender, Elderly & Children
6 Samora Machel Avenue
P.O. Box 9083
11478 Dar es Salaam
Tel: 255 22 2120262-7
Fax: 255 22 2110986

NIMR/HQ/R.8a/Vol. IX/2468

25th April 2017

Prof. Detlev Kruger
University of Charite, Berlin, Germany
C/o Dr. Chacha Mangu
NIMR-MMRC
Mbeya Medical Research Center
P.O. Box 2410
MBEYA

CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: Emerging viruses in Africa: Molecular identification and characterization of rodent-, shrew-, and bat-borne hantaviruses and assessment of their public health potential (Kruger D. *et al*) whose local PI is Dr. Chacha Mangu 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. A progress report is submitted to the Ministry of Health, Community Development, Gender, Elderly & Children, the National Institute for Medical Research, Regional and District Medical Officers after every six months.
2. Permission to publish the results is obtained from the 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. Site: Mbeya region.

Approval is for one year: 25th April 2017 to 24th April 2018.

Name: Prof. Yunus Daud Mgaya

Name: Prof. Muhammad Bakari Kambi

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

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

CC: RMO of Mbeya
DMOs/DEDS of selected districts of Mbeya.

Appendix 8: Ethical Clearance for Knowledge, Attitude and Practice Assessment for Hantavirus Infection in Mbeya Region

UNITED REPUBLIC OF TANZANIA MINISTRY OF HEALTH COMMUNITY DEVELOPMENT GENDER ELDER AND CHILD

Cable Referral Hospital
Telephone: 2503456/2503351
Fax 2503577
Email: info@mzrh.go.tz



MBEYA ZONAL REFERRAL HOSPITAL
P. O. BOX 419
MBEYA

28 May 2018.

Ref No. GB.152/377/01/200

Mr Lwitiho E. Sudi,
NIMR-MMRC,
P.O.Box 2410,
Mbeya, Tanzania.

RE: **Request for Ethical Clearance for Protocol sub-study: The knowledge, attitude and practices associated with risk factors for transmission of Hantavirus infections in Mbeya region (South-Western Tanzania).**

Reference is made for the aforementioned subject.

The Mbeya Medical Research and Ethics Review Committee received and discussed your response with regard to protocol queries raised by its members from the meeting held on 15th May 2018.

I would like to inform you that, the Committee has granted Ethical Clearance of the above mentioned study protocol for the period of one year, from **28th May 2018 to 27th May 2019**.

This Ethical Clearance bears the following specifications;

1. As the PI, if you completed the work earlier than you had planned you must submit a final report to the Mbeya Medical Research and Ethics Committee as soon as the work is completed;
2. You must notify the Mbeya Medical Research and Ethics Committee in writing regarding any alteration or deviation to the protocol;
3. To submit progress reports to the Committee every six months;
4. If the research has been completed, abandoned, discontinued or not completed for any reason you are required to submit a final report on the research to the Committee;
5. This approval is given for twelve months from **28th May 2018 to 27th May 2019**;
6. If unable to complete your research within the one year validation period you will be required to write to Mbeya Medical Research and Ethics Committee to request for an extension;
7. You must notify immediately the Mbeya Medical Research and Ethics Committee of any adverse event and/or unforeseen events that might affect continued ethical acceptability of the research;
8. To comply with approved study proposal, and at all times you are responsible for ethical conducts of your research.

Sincerely,

Executive Director
Mbeya Zonal Consultant Hospital
P. O. Box 419
Mbeya, Tanzania

.....
Dr. Godlove F. Mbwanji, MD, MMED
Chairman
Mbeya Medical Research and Ethics Review Committee

.....
Dr. Ruby D. Mcharo, MD, MPH-IH
Secretary

cc: The Chairman; National Health Research Ethics Review Committee;
P.O.Box 9653, Dar es Salaam.

cc: RMO - Mbeya.

All Communication should be addressed to the Chairman, Mbeya Medical Research and Ethics Committee

RESEARCH OUTPUTS

Accepted Manuscript in the Peer Review Journal (2019)

Phylogenetic analysis of previous unidentified Hantavirus strain on free-tailed bats (*Mops condylurus*) in Mbeya Region, Tanzania (*Journal of Virology*).

Published Review Paper (2018)

Hantavirus in East and Central Africa (*American Journal of Research communication*).

Published Research Paper (2019)

Assessment of Knowledge, Attitude and Practices (KAP) on Hantavirus infections at community level in Mbeya region, Tanzania (*Journal of Tropical Diseases*)

Poster presentation

Molecular epidemiology of rodent-,shrew-, and bat-borne Hantaviruses in Mbeya Region, Tanzania