DETECTION OF HANTAVIRUS IN MICE AND THE ASSESSMENT OF KNOWLEDGE, ATTITUDES AND PRACTICES ON HANTAVIRUS DISEASE IN MICE CONSUMING SOCIETIES IN EASTERN PROVINCE-ZAMBIA.

By

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A thesis submitted to the University of Zambia in partial fulfilment for the award of a Degree of Master of Science Tropical Infectious Diseases and Zoonoses.

THE UNIVERSITY OF ZAMBIA

LUSAKA

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DEDICATION

This work is being dedicated to my family, lovely wife Amy and children: Sizwe and Salizwa, I thank you for your patience and endurance during my absence from home. Your mental support goes a long way to seeing this project to completion. To the almighty God who gives me knowledge and power to serve mankind I say "*kaizen*" on my part so that I serve mankind as you wish.

DECLARATION

I, **Jones Chipinga**, do hereby declare to the best of my knowledge, that the contents of this thesis are my original work and has not been previously presented for the award of a degree in this University or any other institution.

Signature: …………………………………………… Date: …………………………..

Jones V. Chipinga

CERTIFICATION OF APPROVAL

This MSc thesis of **Jones Victor Chipinga** has been approved as fulfilling the requirements for the award of the degree of Masters of Science Tropical Infectious Diseases and Zoonoses by the University of Zambia.

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ABSTRACT

Hantavirus is a zoonosis which can be rodent borne and recently the global scene has seen the expansion of its geographical distribution. Hantavirus disease is notifiable in America, Europe and Asia but it is underreported in Africa despite recent studies revealing the seroprevalence of Hantavirus both in humans and animals in Mozambique and Zambia respectively. Thus far, significant knowledge gaps remain and relatively little information is available for most rodent-borne Hantaviruses in Zambia. Therefore, we conducted a cross-section study to determine the presence of Hantaviruses in mice (n = 160) in Katete district Eastern of Province-Zambia. Using pan Hanta primers, Hantavirus was detected via RT PCR at a prevalence of 2.5% (4/160) (95% CI: 1.16-7.52) in *Mastomys natalensis* rodents collected from Katete district Eastern Province-Zambia. In parallel to laboratory analysis of samples, a cross-section survey using a semi-structured questionnaire (n = 160) was also carried out to capture information on knowledge, attitude and practices (KAP) regarding Hantavirus in mice consuming communities of Katete district Eastern Province. The average KAP score was calculated from total scores for knowledge, attitude, and practices and then assessed. The association between the level of knowledge and factors of knowledge, factors of attitude, and factors of practices was determined using Pearson chi-square. Multivariate analysis was used to determine the predictors of knowledge. Participants had an overall moderate knowledge (score = 59.57%), fair attitude (score = 51.40%), and good practices (score = 73.00%) towards Hantavirus. Only 48.72% (38/78) of participants had sufficient knowledge of Hantavirus. The study found that level of education (Secondary (OR: 0.69 (95% CI: 0.47–0.97)) (p = 0.047), not trapping mice (OR: 0.34 (95% CI: 0.16–0.82)) (p = 0.025), and consuming mice (OR: 6.05 (95% CI: 1.26–24.22)) (p = 0.034) were predictors of moderate knowledge on disease transmission from rodents. The current study reported that participants had moderate knowledge and fair attitude on top of displaying good practices towards reducing the transmission of diseases. Therefore, this study recommends community sensitization on Hantavirus and advocates for the importance of reporting suspected cases to relevant authorities for proper management.

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LIST OF ABBREVIATIONS

ANDV	Andes Virus
BCCV	Black Creek Canal Virus
CD55	Cluster of Differentiation 55
CDC	Centers for Disease Control and Prevention
cDNA	Complementary DNA
CI	Confidence Interval
cRNA	Complementary RNA
DAF/CD55	Decay Acceleration Factor
ddNTPs	Dideoxynucleotides Triphosphates
DMEM	Dulbecco's Modified Eagle Media
DNA	Deoxyribonucleic Acid
DOBV	Dobrava-Belgrade Virus
ER	Endoplasmic Reticulum
ERGIC	ER-Golgi-Intermediate Compartment
Gn	Glycoprotein
GPI	Glycosylphosphatidylinositol
HFRS	Hemorrhagic Fever with Renal Syndrome
HPS	Hanta Pulmonary Syndrome
HTNV	Hantaan Virus
IFNγ	Interferon Gamma
IL-1	Interlukin 1

KAP	Knowledge, Attitudes and Practices
KHF	Korean Hemorrhagic Fever
L	Large Segment
M	Medium Segment
mRNA	Messenger RNA
NE	Nephropathia Epidemica
NHRA	National Health Research Authority
NY-1V	New York-1 Virus
PCR	Polymerase Chain Reaction
PHV	Prospect Hill Virus
PUUV	Puumala Virus
RdRp	RNA-dependent RNA Polymerase
RER	Rough Endoplasmic Reticulum
RNA	Ribonucleic Acid
RNPs	Ribonucleoproteins
RT-PCR	Reverse Transcriptase PCR
S	Small Segment
SEOV	Seoul Virus
SNV	Sin Nombre Virus
TNF	Tumor Necrosis Factor
TULV	Tula Virus
UNZABREC	University of Zambia Biomedical Research and Ethics Committee

UUKV Uukuniemi Virus

vRNA Viral RNA

CHAPTER ONE

1.0INTRODUCTION

1.1 Background

In recent decades, repeated outbreaks of Hantavirus disease has become a public concern and has created a global public health burden(Id & Stenseth, 2019). Hantavirus spillover from natural hosts into human populations could be considered an ecological process, in which environmental forces, behavioral determinants of exposure, and dynamics at the human–animal interface affect human susceptibility and the epidemiology of the disease(Id & Stenseth, 2019). One such behavioral determinant is the consumption of mice which is a custom in some sectors of society in Zambia. Rural inhabitants of Eastern Province Zambia such as the Tumbuka, Senga, Chewa, Ngoni, and the Nsenga utilise mice as a delicacy ("Mbeba (Mice) Delicacy – Mwizenge S. Tembo," 2020).Handling and contact with rodents exposes humans to rodent urine and excreta in which pathogens like Hantavirus are shed posing a risk of disease transmission to humans. Mice have been documented to harbor pathogens of zoonotic potential such as yersiniosis, leptospirosis, salmonellosis, lymphocytic choriomeningitis virus and Hantavirus(Morand, Jittapalapong, & Kosoy, 2015). Therefore, close proximity and consumption of mice are likely predisposing factors to zoonotic infectious diseases including Hantavirus disease(Johnson, 2001; Jonsson *et.al.*, 2010;CDC, 2016).

Hantavirus disease is well documented in Europe, Asia and America, however, the discovery of Hanta viruses in Guinea in wood mice has changed the perception of the geographical distribution of these haemorrhagic fever viruses(Bryjova *et.al.*, 2022). Recently, studies done in South Africa, Namibia, Zambia and Mozambique have shown evidence of the existence of Hantavirus in these countries broadening further their geographical distribution(Chau et al., 2017; Witkowski et al., 2014). Hantaviruses are transmitted to humans from rodents and they are known to cause hemorrhagic fever with renal syndrome (HFRS) and Hantavirus pulmonary syndrome (HPS) (Schmaljohn and Hjelle, 1997; Wood *et al.*, 2006; Av *et.al.*, 2015; Mittler *et al.*, 2019; Kabwe *et al.*, 2020; Bryjova *et.al*, 2022). Although people consume mice in Zambia, the knowledge gap on Hantaviruses remain and therefore the health risks of consuming mice are not fully understood. Furthermore, there is a gap in the assessment of knowledge, attitude and practices (KAP) which contributes to the transmission of the virus from reservoir hosts to humans in Zambia. The research focused on assessing the level of community knowledge, attitudes and practices that lead to the transmission of the virus from the reservoir hosts to a human being. Furthermore, this study also aimed at determining the presence of

Hantaviruses in mice in order to provide information that will be critical to public health officials in disease surveillance and control in Zambia.

1.2 Statement of the Problem

Studies conducted in Zambia and Mozambique(Nakamura et al, 2013; Chau et al, 2017) show serological evidence of Hantaviruses both in humans and animals in these countries. In early phases of Hantavirus disease, there are characteristic universal signs and symptoms such as fever, headache, cough and muscle aches that can be confused with common endemic disease symptoms such as observed in malaria and pneumonia. Therefore, the likelihood of misdiagnosing nondescript febrile illness exhibited by Hantavirus is very high. Despite evidence of the existence of Hantavirus in Zambia and Mozambique, there is no substantive data to explain the epidemiology of Hantavirus disease in Zambia although mice consuming societies are highly exposed to rodent urine when handling mice which is a risk factor for Hantavirus transmission.

1.3 Study Justification

Recently the global scene has seen the emergence and re-emergence of infectious zoonotic diseases. One such zoonotic infection is OrthoHantavirus disease attributable to rodent mammalian hosts. Human infection is considered a spillover relative to influential environmental factors and human activities. Activities like rodent hunting, firewood collection, wild fruit gathering, agricultural practices and many more activities influence the interaction of humans and wildlife eventually putting the population at risk. A significant portion of rural population in Zambia is involved in agricultural activities and besides small holder farming activities, some sectors of society in Eastern Province Zambia utilize mice as a delicacy. In mice consuming populations hunting mice is also a prominent cultural attribute. Currently, significant knowledge gaps remain and relatively little information is available for most rodent-borne Hantaviruses. Therefore, determining the presence of Hantaviruses in mice in Zambia and assessment of Knowledge, Attitudes and Practices concerning Hantavirus disease will help in understanding the connectivity between the diverse dynamics surrounding human-animal interface. The study is important as part of infectious disease surveillance and is ideal for policy development on control measures and outbreak preparedness. Furthermore, the study will be an index to solve the puzzle on whether there is misdiagnosis owing to endemic diseases with similar universal signs and symptoms i.e. malaria and pneumonia.

1.4 Significance of the Study

Currently, there is limited data to describe the molecular epidemiology of Hantavirus disease in Zambia. Thus, findings from this study will help us understand the epidemiological status of Hantavirus and support designing effective prevention and control measures both in animals and human beings. The study will also establish the prevalence of Hantavirus by Polymerase Chain Reaction (PCR) as such the study will act as an extending surveillance programme to humans especially those at high risk in areas known to be in close contact with mice. Research findings from this study will also be a basis for other researchers that wish to further study in this area.

1.5Research Questions

The study was set to answer these questions:

i. Is Hantavirus present in mice in Zambia?
ii. What is the knowledge, attitudes and practices towards Hantavirus disease?

1.6Objectives

1.6.1 General Objective

To detect Hantavirus in mice specimens and conduct a KAP study in mice consuming communities of Eastern Province in Zambia.

1.6.2 Specific Objectives

1. To detect Hantavirus in mice specimens by PCR
2. To determine the prevalence of Hantavirus in mice based on PCR.
3. To determine knowledge, attitude, practices and possible predictors that could influence knowledge of Hantavirus disease among mice consuming societies in Katete district.

CHAPTER TWO

2.0LITERATURE REVIEW

2.1 History of Hantavirus disease

Haemorrhagic fever with renal syndrome (HFRS) dates back to 1913 in eastern Siberia, Russia but a Chinese medical account of a similar disease was documented as early as A.D. 960 (Johnson, 2001). Haemorrhagic fever with renal syndrome was connoted in view of chronic infection with urinary excretion in the murid rodent, *Apodemus agrarius,* endemic to Asia (Johnson, 2001). *Apodemus agrarius* often invade cultivated fields, gardens, haystacks, and sometimes human dwellings. Therefore, it is not surprising that 20th century human conflicts such as World War I and II, which increased human interface with rodents played a significant role in elucidating the aetiology, clinical evolution, epidemiology, and ecology of HFRS (Johnson, 2001).

The period1930-1960 saw the early description of Hantavirus disease. In 1934 Japanese physicians first encountered HFRS in Manchuria but a detailed clinic pathological descriptions of the syndrome was completed six years later in 1940 (Johnson, 2001; Wang, *et al.*, 2014). A similar but less lethal syndrome was described in Scandinavia and central Russia and it was termed Nephropathia epidemica (NE) by Scandinavian authors (Johnson, 2001). These syndromes were recognized by hemoconcentration, leukemoid reaction, various hemorrhagic phenomena, a hypotensive crisis at the time of febrile resolution, a subsequent phase of renal insufficiency and a 10% mortality rate. Later, annual outbreaks of HFRS were seen in the Amur River Valley of Siberia but no laboratory host was found in which serial replication of the presumptive agent could be demonstrated (Johnson, 2001). However, soviet epidemiological studies implicated field mice as reservoir hosts due to the 1939 disease outbreak in their earth moving labor camp. The Soviet hypothesis was later backed up by the disease outbreak in Moscow Russia attributed to *Clethrionomys glareolus* and *C. rutilus* rodents in the laboratory (Johnson, 2001). It was only between 1951 and 1954 when American physicians diagnosed about 3000 cases among their troops in Korea that a more detailed description of the syndrome was done (Johnson, 2001; Jonsson *et al.*, 2010; Mittler *et al.*, 2019; Kabwe *et al.*, 2020). So the 1950s saw recognition of HFRS in other countries of Eastern Europe including Yugoslavia; Bulgaria, Czechoslovakia, and Hungary. Primary rodent hosts as well for Asian, Western European and Eastern European were also correctly identified as *Apodemus agrarius, Clethrionomys glareolus,* and *Apodemus jlavicollis,* for these regions respectively (Johnson, 2001).

The isolation of Machupo virus causing acute hemorrhagic fever in eastern Bolivia in 1963 by Investigators at the National Institute of Allergy and Infectious Diseases laboratory, led to a cascade of events that facilitated the recovery of a distinct pair of viruses from *Apodemus agrarius* (Johnson, 2001).These viruses were characterized by predominant viral antigen load in the lungs, with lesser incidence and intensity in the liver and kidney. They were known to cause Korean Hemorrhagic Fever (KHF) but proved rather difficult to isolate from blood of KHF patients (Johnson, 2001; Wang *et al.*, 2014; Ermonval *et al.*, 2016; Munir *et al.*, 2021). Since, the exact nature of this new agent was not fully understood and it was not serologically related to any of the other major taxa that caused hemorrhagic fever. Dr. Lee named it Hantaan virus after the small river that passes near the village of Songnaeri, where the prototype strain was obtained (Johnson, 2001). This is believed to be the first case of recognized Hantavirus which resulted in the race to identify and map HFRS infection and viruses. Later antibodies to Hantaan were quickly found in sera from HFRS patients in Scandinavia, Finland, Russia, China, and both eastern and western Europe. Once the viruses were cultivated in cells, neutralization tests were employed to document that four distinct agents had been discovered: Hantaan, the rat virus now named Seoul; Puumula, the virus of NE (Nephropathia epidemica) or mild HFRS; and Prospect Hill(Johnson, 2001; Wang *et al.*, 2014; Ermonval *et al*., 2016; Munir *et al.*, 2021).

2.2 Geographical distribution of Hantaviruses

Hantaviruses have a worldwide distribution although it is believed that there is underreporting in some parts of the world like in Africa (Figure 2.2). The history of Hantavirus disease dates back to ancient Asia although the first outbreak was reported between 1951 to 1954 by western physicians (Schmaljohn & Hjelle, 1997). Hantaviruses are known to occur in Europe, Asia, and in America (Escutenaire & Pastoret, 2000; Id & Stenseth, 2019; Wood et al., 2006). However, recent evidence shows the existence of Hantaviruses in African countries like Guinea, South Africa, Namibia, Zambia and Mozambique (Chau et al., 2017; Nakamura et al., 2013; Witkowski et al., 2014).

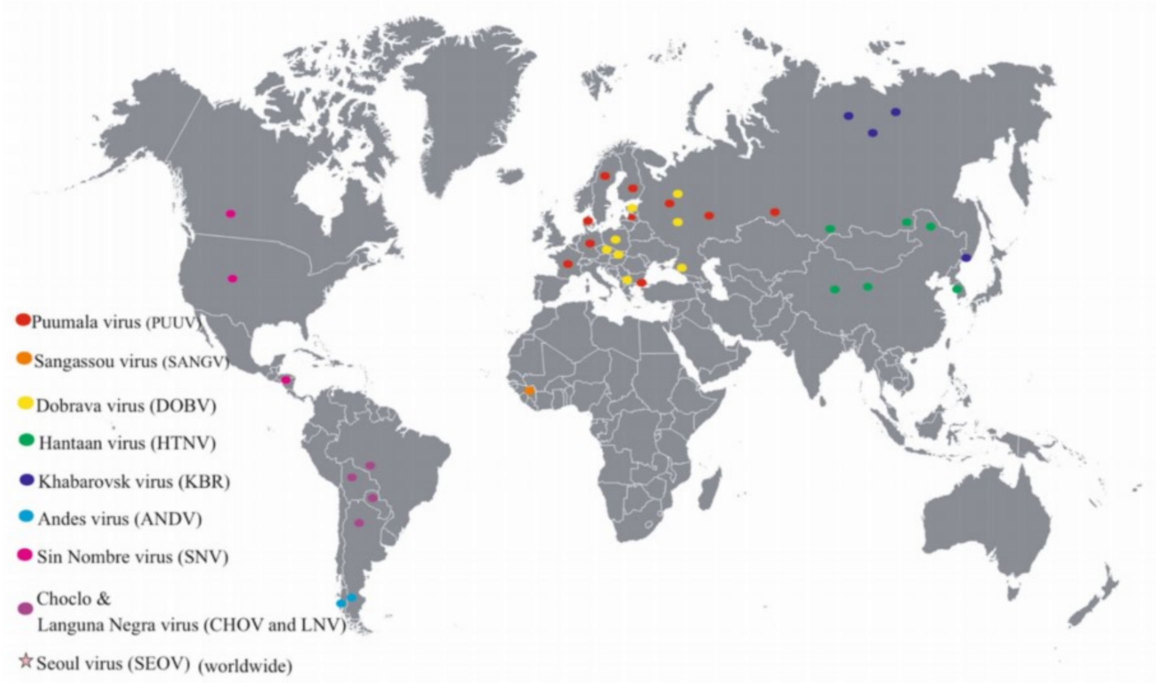

Figure 2.2: Geographical distribution of human associated pathogenic orthoHantaviruses. Adopted from Kabwe *et.al (*2020)

2.3 Etiology of Hantavirus Disease

Hantaviruses are rodent borne, zoonotic emerging viruses that cause life-threatening human diseases (Escutenaire & Pastoret, 2000; Id & Stenseth, 2019; Wood et al., 2006). Infections from Hantavirus accounts for over 50,000 cases globally each year with fatality rates of up to 12% (HFRS) and 40% Hantavirus pulmonary syndrome (HPS), depending on the infecting virus. Hantaviruses are enveloped RNA viruses belonging to the order *Bunyavirales*, family *Hantaviridae,* and genus *OrthoHantavirus.* The family *Bunyaviridae* has over 300 viruses that infect animals, plants, humans, and arthropods (Jonsson *et.al.*, 2010). Genetically there are 22 strains of Hantaviruses that are associated with disease in humans (Chau et al., 2017). Hantaviruses are known to cause hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and Hantavirus pulmonary syndrome (HPS) in America (Escutenaire & Pastoret, 2000; Id & Stenseth, 2019; Wood et al., 2006).

Hantaviruses are negative sense RNA enveloped spherical particles with a diameter of 80–120nm. Their genome comprises of three segments: a 1.8–2.1kb small segment (S), a 3.7–3.8kb medium segment (M), and a 6.5–6.6kb large segment (L). These have highly conserved 30 and 50 untranslated regions that contain complementary nucleotides. The regions recognize each other to form panhandle structures that give the RNA segments a circular appearance in some microscopic images. These panhandle structures are a hallmark of bunyaviruses and are thought to regulate viral transcription and replication (Mittler et al., 2019) (Figure2.3)

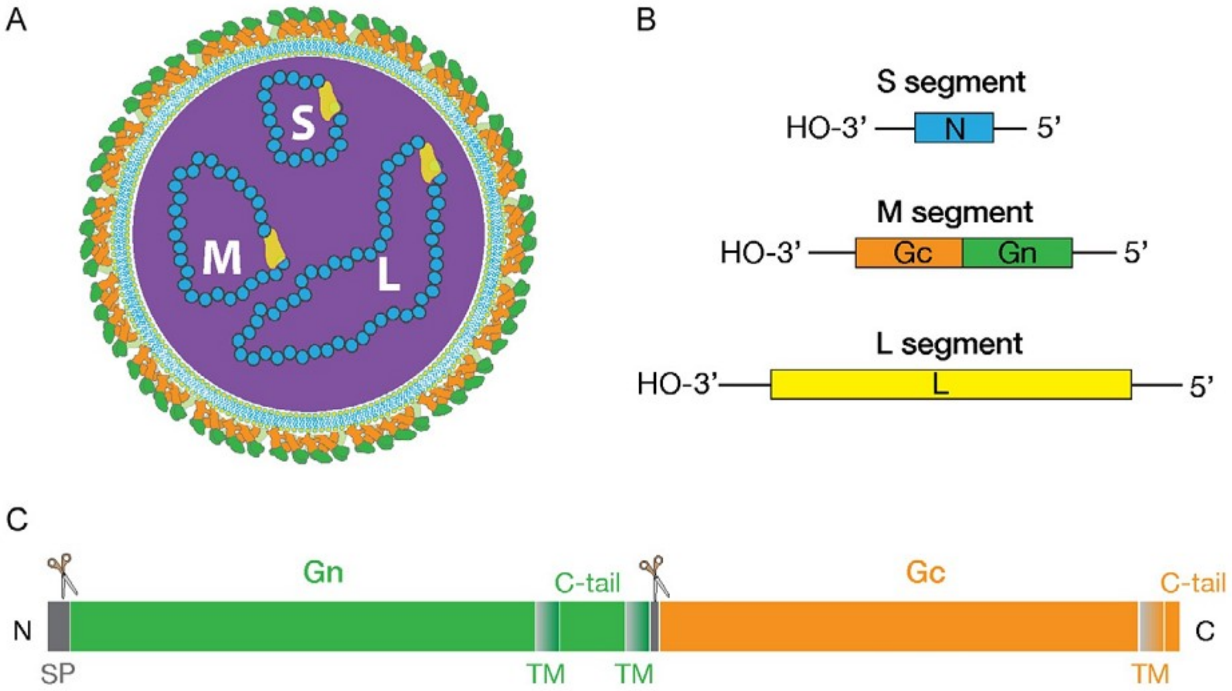

Figure2.3: Schematic representation of Hantavirus particle and genes, adopted from Mittler *et.al* (2019)

2.3.1 Host Reservoirs

Hantaviruses are maintained and transmitted through many mammalian host reservoirs including bats (order Chiroptera), shrews, moles (order Soricomorpha) and rodents (order Rodentia) via the aerosol route although human to human transmission has not been documented (Escutenaire & Pastoret, 2000; Id & Stenseth, 2019; Mittler et al., 2019; Wood et al., 2006) (Fig 3&4). Rodent hosts acquire the virus through inhalation of environmental generated aerosolized virus particles and intense contact such as biting, grooming and food resources sharing (Jonsson *et al*., 2010; Ermonval *et al.*, 2016). Infection gets well established in adult rodents as compared to juvenile rodents which are protected by maternal antibodies up to 80 days. Adult rodents are disproportionately infected and usually exhibit persistent lifelong asymptomatic infection (Ermonval *et al.*, 2016). Documented rodent hosts include *Peromyscus maniculatus* (Deer mouse), *Sigmodon hispidus* (Cotton rat), *Oryzomys palustris* (Rice rat), *Peromyscus leucopus* (White footed mouse) and *Hylomyscus simus* (African wood mouse).

Zambia boasts a wide diversity of species of mammals of which some terrestrial species are rodents (Society, 2014). However, serological evidence of Hantavirus in Zambia was seen in multimammate rat (*Mastomys sp*, subfamily Murinae) and gerbil (subfamily Gerbillinae) (Nakamura et al., 2013).

2.3.2 Classification of rodent-borne Hantaviruses

Overall, there are three clades of rodent-borne Hantaviruses (Mittler et al., 2019), the first clade being the New World Viruses which causes HPS. New World Viruses are carried by the *Sigmodontinae* rodent sub-family. Examples are Sin Nombre virus (SNV), New York-1 virus (NY-1V) and Andes virus (ANDV) (Ramsden *et al.*, 2008; Klempa *et al.*, 2012; Ermonval *et al.*, 2016). The second clade is that of Old World viruses which causes HFRS and they are harboured in *Murinae* rodents. Examples are Hantaan virus (HTNV), Seoul virus (SEOV), and Dobrava-Belgrade virus (DOBV) (Ramsden *et al.*, 2008; Klempa *et al.*, 2012; Ermonval *et al.*, 2016). Lastly, there are viruses associated with mild disease or are non-virulent. These are either found in the new or old world. Those associated with mild disease include Puumala virus (PUUV) while those which are non-virulent include Prospect Hill virus (PHV) and Tula virus (TULV). These are carried by *Arvicolinae* rodents (Ramsden *et al.*, 2008; Klempa *et al.*, 2012; Ermonval *et al*., 2016).

2.4 Life Cycle of Hantavirus Disease

There are 48 suggested Hantavirus species associated with asymptomatic lifelong persistent infections in Rodentia mammalian species on every continent except Australia and Antarctica (Forbes *et al.*, 2018). Besides Rodentia mammalian hosts several host switches have been described among wildlife species and this leads to genetic reassortment, hence, the co-evolution opinion (Bowen et al., 2001; Ermonval *et al.*, 2016; Forbes, Sironen and Plyusnin, 2018). Further to this environmental conditions are critical in the stability of infectiousness of Hantavirus outside the reservoir hosts with cool and damp conditions being favourable for prolonged infections (Jonsson *et al.*, 2010). Therefore, host persistent infections ensures continuous viral shedding in the environment via rodent excreta and urine. Continuous viral shedding coupled with favourable external environmental conditions are instrumental in Hantavirus maintenance in the environment. Hantavirus particles from the environment gain access to infect host cells through inhalation (Figure 2.4.a). Hantavirus infect its host through attachment of the viral glycoprotein to host's cell surface receptors on endothelial, epithelial, macrophage, follicular dendritic, and lymphocyte cells (Jonsson *et al.*, 2010) (Figure 2.4.b).

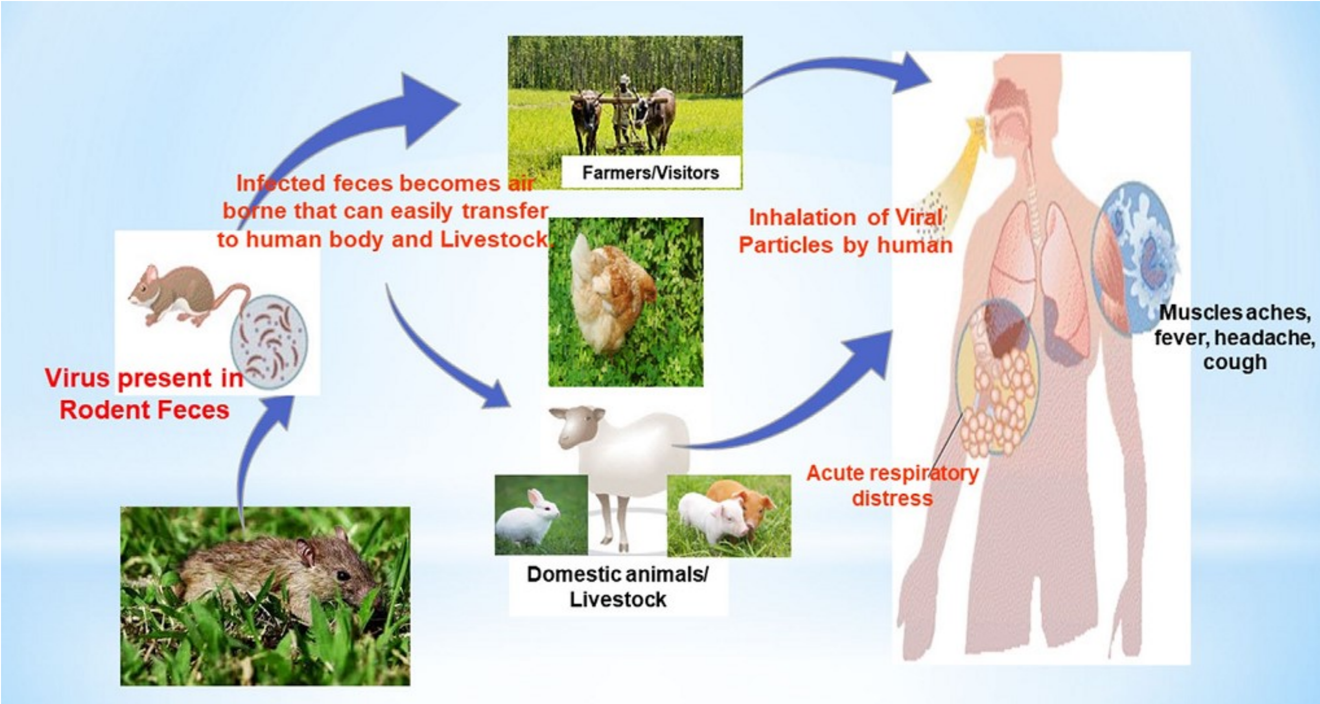

Figure 2.4a: Schematic presentation of Hantavirus infection cycle; transmission is via breath of aerosolized viral particles in the urine, faeces and saliva discharged into the environment, adopted from Munir *et.al* (2019**)**.

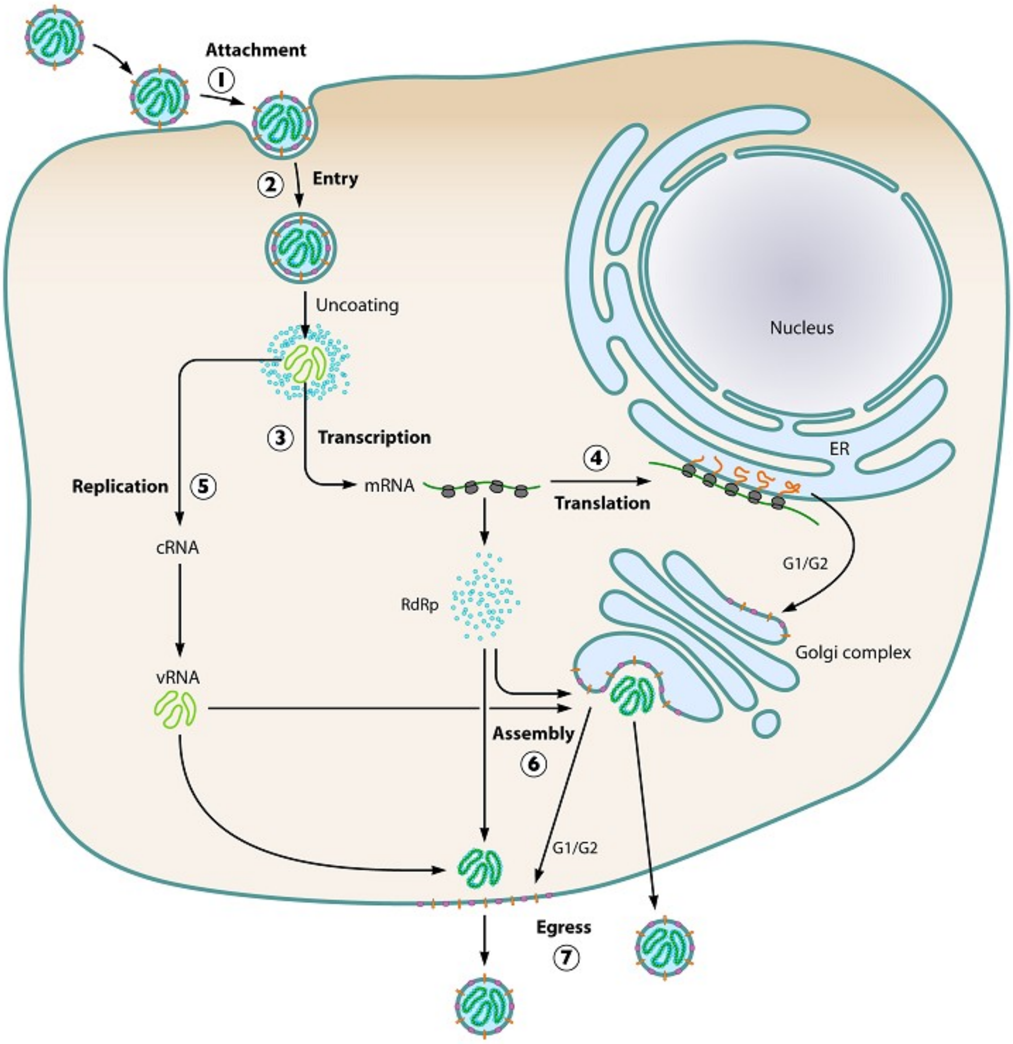

Figure 2.4b: The Hantavirus life cycle. The basic steps include the attachment of the virion particle to the cell's surface through interactions between the host's cell surface receptors and the viral glycoprotein (1); entry through the use of receptor-mediated endocytosis and the uncoating and release of the viral genomes immediately thereafter (2); transcription of complementary RNA (cRNA) from the viral RNA (vRNA) genome using host-derived primers (3); translation of L, M, and S mRNAs into viral proteins using host machinery (4); replication and amplification of vRNA, assembly with the N protein, and transport to the Golgi apparatus (5); assembly of all components at the Golgi apparatus or, possibly for New World viruses, at the plasma membrane (alternative assembly) (6); and viral egress via the fusion of the Golgi vesicle harboring the mature virion particles with the plasma membrane (7). Adopted from Jonsson *et.al* (2010).

Intergrins are suggested receptors that interact with the larger viral glycoprotein (Gn) whereby β1 integrin is for *Microtus*-borne Hantaviruses and is considered to be apathogenic whileβ3 integrin is for pathogenic Hantaviruses causing HFRS and HPS but these may not be the only receptors (Jonsson *et al.*, 2010). For Hantavirus to penetrate host cells there is need for glycosylphosphatidylinositol (GPI)-anchored protein of the complement regulatory system known as decay-accelerating factor (DAF)/CD55. When target cells are polarized Hantaviruses then enter polarized target cells from the apical and basolateral membrane surfaces. HTNV enters via clathrin-coated pits, followed by movement to early endosomes and subsequent delivery to late endosomes or lysosomes (Jonsson *et al.*, 2010). The virus gets uncoated within the endolysosomal compartments, to liberate the three ribonucleoproteins (RNPs) into the cytoplasm. The L protein or viral RNA (vRNA)-dependent RNA polymerase (RdRp) initiates primary transcription to give rise to the S, M, and L mRNAs. The translation of the S and L mRNA transcripts occurs on free ribosomes, and the M-segment transcript occurs on membrane-bound ribosomes, which is cotranslated on rough endoplasmic reticulum (ER) (RER) (Jonsson *et al.*, 2010). N protein the most abundant viral protein gets synthesized early in infection and it functions in translation, trafficking, and assembly. It is also believed that the N protein interacts with and can modulate the host immune response to infection. Viral glycoprotein precursor is proteolytically processed into Gn and Gc during import into the ER, however, a conserved amino acid motif, WAASA, located at the end of Gn is presumed to be the proteolytic cleavage site for most Hantaviruses (Jonsson *et al*., 2010).

The modification of Gn and Gc proteins happens in the ER by addition of a sugar molecule, however, the glycosylated proteins are transported to the Golgi complex where transcription is initiated. Viral polymerase can perform dual functions hence it switches from transcription to replication of the S, M, and L genomic RNAs (Figure2.4b). When vRNAs synthesis is complete, the nucleic acids are encapsidated by the N protein to form the RNPs which are ready for transportation. Transportation of RNPs is subject to the type virus, for instance, the HTNV N protein gets transported via microtubule dynein to the ER-Golgi-intermediate compartment (ERGIC), Golgi complex, or endosomes in HTNV-infected Vero E6 cells and not in ER (Jonsson *et al.*, 2010). On the other hand viral proteins and virion particles of the *Bunyaviridae* members such as Uukuniemi virus (UUKV) and Bunyamwera virus, accumulate at the Golgi complex. The UUKV N protein associates with *cis*-Golgi elements and accumulates in peripheral elements that could also include the ERGIC. This suggests that the ERGIC and the Golgi complex may be important for some aspects of virus assembly (Jonsson *et al.*, 2010). The assembly of RNPs and their trafficking is not well understood but it is suggested that RdRp is part of the RNP complex and is instrumental in how the RNPs are trafficked to the Golgi complex, and bud into and out of the Golgi complex to produce the infectious virion. Despite the stated mechanism of viral budding, New World Hantaviruses is believed to assemble and mature at the plasma membrane (Figure

2.4b) but there is no evidence for this pathway (Jonsson *et al.*, 2010). This hypothesis rests on the observation that intracellular particles were not observed in cells infected with SNV or Black Creek Canal virus (BCCV). The localization of the N protein at the Golgi complex, presumably as an RNP complex, has been observed for both New World and Old World Hantaviruses. Although, Old and New World Hantaviruses share common features of their life cycles recent studies suggest that they may have evolved differently in specific interactions with host cell machinery.

2.5 Pathogenesis of Hantavirus

The pathogenesis of Hantavirus is complex but in the lungs HPS is attributed to interaction of Gn and Gc surface glycoproteins with target endothelial cells, macrophages, and platelets that have β3 integrin receptors at the cell membrane (Jonsson *et al.*, 2010; Ermonval *et al.*, 2016; Munir *et al.*, 2021). This interaction activates a cascade of immune response events that results in changes in vascular permeability culminating into pulmonary edema followed by respiratory failure, hypotension, and cardiogenic shock. The recognition of Hantavirus by macrophages activates macrophages to secrete proinflammatory cytokines such as tumor necrosis factor alpha (TNF-), interleukin-1 (IL-1), and IL-6 (Jonsson *et al.*, 2010; Ermonval *et al.*, 2016; Munir *et al.*, 2021). CD4 T cells, after antigen recognition, differentiate into helper cells, T helper 1 (Th1) and Th2 cells. Th1 cells produce gamma interferon (IFN-γ) and TNF-β (or lymphotoxin-), responsible for cell-mediated immunity, and this differentiation is regulated by IL-12 (Jonsson *et al.*, 2010). Th2 cells produce IL-4 and IL-5 which promote humoral and allergic responses. On the other hand, inducible regulatory T cells that produce IL-10 and transforming growth factor (TGF-β), two immunosuppressive cytokines, have an important role in regulating the immune response and infection-induced pathology (Jonsson *et al.*, 2010).

The basic mechanisms behind HFRS pathogenesis also related to increased vascular permeability, but the causative agents infect endothelial cells without cytopathic effects (Jonsson *et al.*, 2010). There has not been a proper animal model for HFRS, and the Syrian hamster model for ANDV and HPS is not applicable for HFRS. However, cynomolgus monkeys infected with wild-type PUUV strains (not cell culture adapted) produce NE-like disease symptoms and clinical pathology, including elevations of nitric oxide, various cytokine (IL-10, IL-6, and TNF-), and C-reactive protein levels (Jonsson *et al.*, 2010).

The signs and symptoms of Hantavirus infection in early stages are fatigue, fever, muscle aches, headaches, dizziness, chills, nausea, vomiting, diarrhea and abdominal pain (CDC, 2016).These symptoms are universal and may coincide with other types of infections. Late symptoms of HPS include coughing and shortness of breath while common clinical features of HFRS and HPS include fever, myalgia, thrombocytopenia, leukocytosis and a capillary leak syndrome associated with shock in most severe cases (Escutenaire & Pastoret, 2000).

2.6 Diagnosis/detection of Hantavirus

Current methods of diagnosis for Hantavirus include cell culture, serological tests and molecular tests (Munir et al., 2021).In acute symptomatic HFRS and HPS serological tests that target IgM and IgG are reliable due to the presence of N protein antibodies. One such test is the indirect immunofluorescence assay (IFA) but it is less favored due to its requirements for viral infected cells for diagnosis. Besides IFA, the most common serological tests for Hantaviruses are indirect IgG and IgM enzyme-linked immunosorbent assays (ELISAs), Immunoblot assay (IBA), Focus reduction neutralization (FRNT)and IgM capture ELISAs (Ermonval *et al.*, 2016; Munir *et al.*, 2021). Furthermore, rapid IgM capture ELISA for diagnosis of HFRS and HPS is equally effective. On top of these, highly sensitive diagnostic tests have been developed based on the detection of the virus genome. The Hantavirus genome can be rapidly detected by reverse transcription-PCR (RT-PCR) with clinical samples, such as blood, serum, or organ fragments, from the first day after the onset of illness (Munir et al., 2021).Low levels of viral RNA present in human and rodent tissue samples can require nested-RT-PCR techniques using primers selected for conserved regions with high homology within the small, medium and large segments (Wood *et al.*, 2006; Munir *et al.*, 2021; Bryjova *et al.*, 2022).Hantavirus RNA has been known to continuously persist in infected rodents, therefore, nested-RT-PCR assay is a powerful tool that can be deployed to detect currently known and possible novel members of the genus *Hantavirus.* For instance the use of Pan Hanta primers to detect Hantavirus in African wood mouse in Guinea (Klempa et al., 2012).

2.7 Prevention and control of Hantavirus

Currently there is no antivirals, vaccines, or immunotherapeutics approved for any of the hemorrhagic fever viruses, including HFRS and NE but Ribavirin, Lactoferrin, ETAR, Favipiravin and Vandetanib have promising *in vitro* and *in vivo* antiviral activity against members of the *Bunyaviridae* and the *Arenaviridae* (Jonsson *et al.*, 2010; Ermonval *et al.*, 2016). Ribavirin reduces mortality and was proven effective for the treatment of lethal encephalitic suckling mice infected with HTNV. Conversely, Hantavax vaccine has been used in Korea but it does not show significant decrease in severity of HFRS disease.

The prevention of diseases caused by Hantaviruses is based on principles of rodent control (Jonsson *etal.*, 2010) such as reducing rodent shelter and food sources in and around the home, eliminating rodents inside the home and preventing them from entering the home. The use of standard precautions for preventing Hantavirus infection while rodent-contaminated areas are being cleaned up should also be adhered to. Prevention measures for persons who have occupational exposure to wild rodents, and precautions for campers and hikers should not be overlooked. In addition to minimizing the risk of Hantavirus exposure, the prevention of hantaviral disease could be augmented by effective vaccines and strategic vaccination of at-risk populations (Jonsson *et al.*, 2010).

2.8 Knowledge, attitudes, perceptions and practices regarding Hantavirus disease in Zambia

Some sectors of society in Zambia utilise mice as a delicacy (Tembo, 2020), however, consumption of mice is a risk factor for contracting zoonotic infectious diseases (Mardiana, Siska; Suminar, Jenny Ratna; Sugiana, 2019). Hantaviruses are transmitted to humans from rodents, hence, contact with rodents poses a risk of disease transmission to humans (Witkowski et al., 2014). An increasing number of outbreaks and the possibility of cases spreading over international borders has led to increased interest in these viruses and the environmental determinants that facilitate transmission. Ecological studies that evaluate the risk factors for human infection contribute significantly to understanding the dynamics of Hantavirus transmission (Guterres & de Lemos, 2018) (Figure 2.8). Outbreaks of HFRS and HPS are associated with dense rodent populations resulting from favourable climatic and environmental conditions. Human activities, such as rodent trapping, farming, cleaning rodent-infested areas, construction work, camping and hunting, are also risk factors for Hantavirus disease (Escutenaire & Pastoret, 2000).

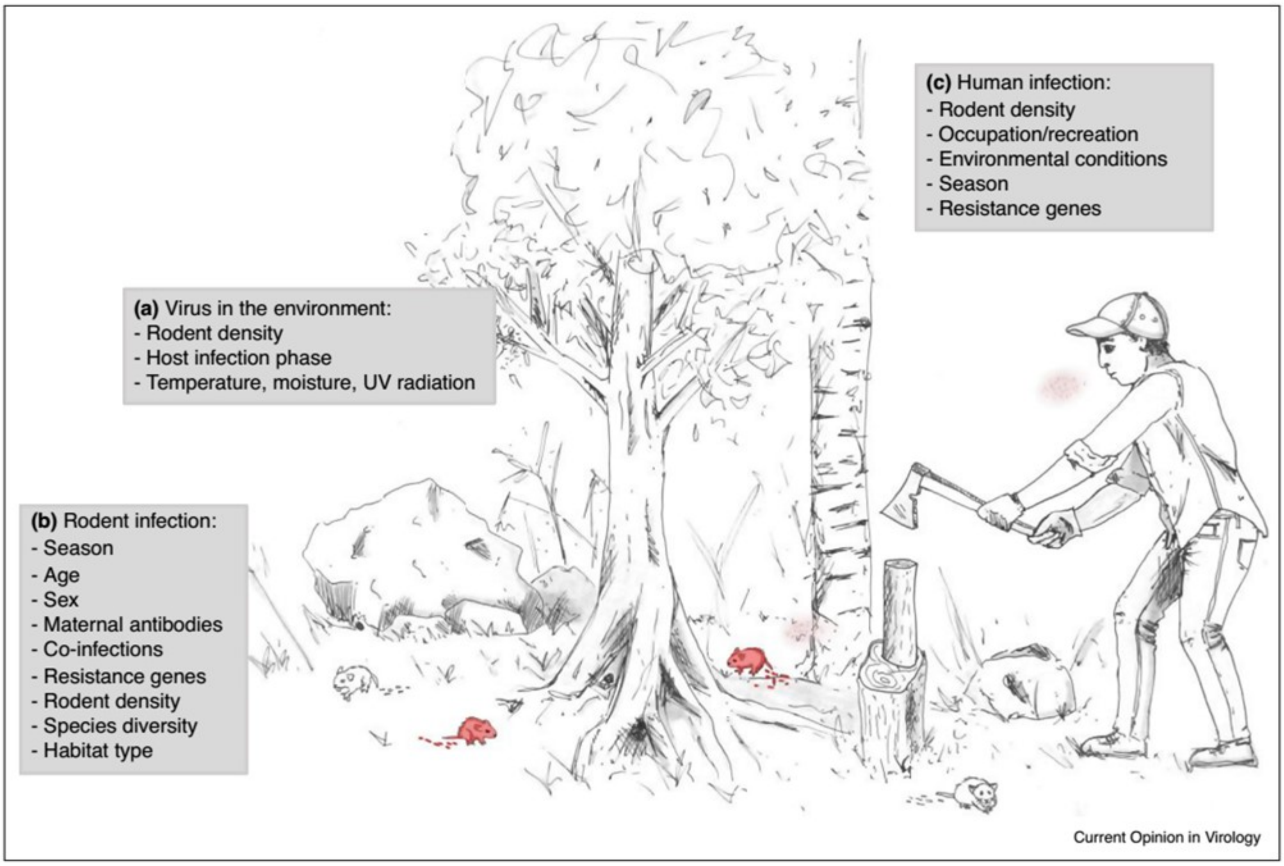

Figure 2.8: Synthetic depiction of a Hantavirus transmission setting, adopted from Forbes *et.al* (2018).

Rodents also natively known as mbeba in Zambia have a great cultural significance to many rural Zambians in the Eastern Province as they form part of the diet and make an important source of protein. Traditionally, local Zambians eat over fourteen breeds of mice (Tembo, 2020) namely Thodwe, Kabwanda, Kamzumi, Kapuku, Tondo, Mphundu, Sakachulu, Julungwere, Damba, Chivuku, Chitute, Kambinini or Kafula-fula, and Kabwira, these are described as follows: Thodwe has a rich reddish brown back and a white stomach. Kamzumi, also known Fuko, is a grayish mouse with large protruding sharp teeth. Tondo is perhaps the fastest mouse known in the area. It has a long nose and makes clear narrow paths through which it always travels to and from fetching food. Mphundu mice often burrow several of them in one hole. They are difficult to catch as they disperse through numerous escape holes once diggers close in. Sakachulu mice burrow in anthills. Julungwere mice have dark and brown stripes. It is a beautiful type Kapuku-like mouse that feeds both during day and nighttime. Chitute are fat cheeked mice that pick, collect, and eat just about anything. In its hole hunters may find stored away human hair, beads, and other odd objects from the villages. Kambinini or Kafula-fula mice exhibit behavioral characteristics like those of the Kamzumi mice. Kambinini often make several furrows on one spot and any one hole might have up to a hundred mice. Kabwira is perhaps one of the tinniest mice known. If mice hunters kill Kabwira, they Tumbuka believe it to be a sign of bad luck. Although people consume mice in Zambia, little is known about the occurrence and distribution of Hantaviruses and therefore the health risks of consuming mice are not fully understood. Furthermore, there is a gap in the assessment of knowledge, attitude and practices (KAP) which contributes to the transmission of the virus from reservoir hosts to humans in Zambia. This study therefore made an assessment of baseline understanding of Hantavirus disease, risk perception and practices regarding disease prevention in rural communities in Katete district Eastern Province using a questionnaire survey.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Design

This study was a cross-sectional study where by a structured questionnaire survey was conducted followed by laboratory analysis of collected samples.

3.2 Study Site

In this study a structured questionnaire was administered within rural households in Katete district (Azeleguza, Kachipu and Nyembe villages) followed by collection of mice samples. Collected mice samples were analysed at the University of Zambia, School of Veterinary Medicine, Molecular Biology laboratory, Department of Disease Control in Lusaka, Zambia.

3.3 Sample size

The sero prevalence of Hantavirus infection in mice in Zambia stands at 1.57% (7/446) (Nakamura et al., 2013) and sero prevalence of hantavirus in febrile patients in Mozambique is 2% (4/200) (Chau et al, 2017), however, prevalence was adjusted to 15% to attain good sample size. Considering the similar sero-prevalence of Hantavirus in rodents and humans a similar approach was used to calculate the sample size for both the questionnaire and the mice samples as follows (Wilkinson *et al.*, 1988):

$$n = \frac{Z1-\alpha/2^2\, p\,(1-p)}{d^2}$$

Where **n** is the sample size, $\mathbf{Z_{1-a/2}}$ is standard normal variate (at 5% type 1 error ($P<0.05$) it is 1.96, **p** is expected proportion in population based on previous studies or pilot studies, **d** is absolute error or precision.

The expected sample size for either the questionnaire or mice samples was calculated as:

$$n = \frac{1.96^2\, X0.15\,(1-0.15)}{0.05^2}$$

$$=196$$

Therefore 196 rodents were needed for the experiment and 196 questionnaires were to be administered.

3.4Establishing knowledge, attitude and practices regarding Hantavirus disease

3.4.1. Data collection instrument for the study

A structured questionnaire was developed through an in-depth literature search, then was translated and pretested in Nyanja. The questionnaire was pre-validated for relevance, accuracy, clarity, simplicity and understandability. The questionnaire had Cronbach`s alpha coefficient of 0.70 indicating the internal consistency and reliability of the study instrument. The questionnaire had five parts: the first part gathered information about demographics of the respondents (age, sex, area of residence, education level, employment status, monthly income, housing and housing floor); the second part obtained information about food practices (Including the type of meat they eat, the frequency on how the meat is eaten and consumption of mice). The third part of the questionnaire collected information about knowledge on Hantavirus disease (Knowledge of Hantavirus disease, causative agent, clinical signs, mode of transmission, disease prevention, and knowledge of *Mastomys* spp rodents, knowledge of zoonotic diseases contracted from mice and source of information on Hantavirus disease). The fourth part gathered information on attitude towards Hantavirus disease (Nature of severity of the disease, risk of contracting Hantavirus, class of people at risk and health service capacity to solve health problems). The last section obtained information on practices regarding reducing the risks of disease transmission from mice (including presence of rodents within the homes, trapping rodents, killing rodent predators, house cleaning practices, housing ventilation, and use of storage facilities within the house).

3.4.2. Participants identification and data collection

A structured questionnaire with mostly categorical questions to ease data processing and improve the precision of responses was administered at the household level in either Nyanja or English language. With guidance of the local veterinary office the structured questionnaire was administered face to face within the communities known to consume mice in Katete district i.e. Azeleguza, Kachipu and Nyembe villages. Simple randomization was used to select households to be included in the survey, a count of one to five was used and every 5th household was included and informed consent was sought via a written consent form.

3.4.3. Establishing KAP score

Thematic questions regarding expected knowledge, management practices and attitudes on Hantavirus disease, were answered on a “yes” or “no” basis. A correct answer was assigned 1 point, while an incorrect and “don’t know” answer was assigned 0 point. The total knowledge score ranged from 0 to 100, with a higher score denoting a better knowledge of Hantavirus disease. To determine the KAP score level, the cut-off value was based on ability of the participant to explain the rodent host, clinical symptoms and mode of transmission of Hantavirus. The overall knowledge was categorized, using Bloom's cut-off point. Those respondents who obtained KAP score above 60 were considered as high level of knowledge, attitude and practices, while the scores between 25 and 50 were considered as medium level. The score below 25 was considered as low level of knowledge, attitude and practices adapted from (Memon *et al.*, 2015).

3.5 Experimental Approach

3.5.1Sample Collection

Trapping, blood collection, dissection and harvesting of mice organs was done according to Nakamura et.al. (2013). Briefly, wire-meshed cage traps (145 × 100 × 230 mm) and Sharman-type cage traps (50 × 65 × 157 mm), baited with peanut butter-flavored bread supplement was used for animal trapping. Traps were set in the evening around 17hours and checked the following morning at 6hours. Trapped rodents were treated in accordance to the guidelines of the University of Zambia Animal Research Committee. Rodents were anesthetized using chloroform, where by rodents held by the tail were placed in a bucket containing cotton wool socked with chloroform and left for 5 minutes to be anaesthetized. Later, blood samples were collected by heart puncture using 3cc syringe fitted with 23 gauge needle and dispersed in a plain tube. Thereafter, the rodent was pinned to a cutting board and the abdomen opened using scissors. Several tissues (lung, liver and kidney) were harvested and temporarily placed on ice in a cooler box and later stored at - 20˚C.

3.5.2Nucleic Acid Isolation

Blood samples were pretreated using heat treatment for serum deactivation at 56˚C for 30 minutes using a heat block (Double Alumi Bath ALB-301, Japan) and then diluted 1:10 using phosphate buffered saline (PBS) (1part blood and 9 PBS) ready for RNA extraction. Harvested mice organs (lungs, spleen, liver and kidneys) were placed in 2ml screw cap smashing tubes containing two 2mm diameter beads and 500µl of Dulbecco's Modified Eagle Media (DMEM) (SIGMA-ALRICH, Co., 3050 Spruce St., St. Louis, MO, 63103). The tubes were then placed in an electronic micro smasher (Tomy-MeDICO LTD, Japan), set at 3500 rpm for 30 seconds and three runs for each organ were used. Total RNA extraction was performed using QIAGEN RNAeasy kit (QIAGEN, Hilden, Germany) where by 140 µl of either organ homogenate or blood (sample) were mixed with Buffer AVL-Carrier RNA mixture for cell lysis and precipitation of the freed RNA material. QIAmp spin columns were used to filter cellular particles by centrifuging at high revolution (8,000-14,000 rpm) at 4˚C. Absolute ethanol (100 percent ethanol) and provided wash solutions (AW 1 and AW 2) were used to emulsify and clean the extract. RNA was eluted in 60µl AVE buffer [RNAse free water with 0.04 percent sodium azide (NaN_3)] and was immediately stored at -80˚C until required for use. The whole protocol followed the manufacturer's instructions.

3.5.3.RT-PCR

PrimeScript™ One Step RT-PCR Kit Ver.2 (Dye Plus) kit (Takara, Bio Inc., Japan) was used for synthesis of complementary DNA (cDNA). RT-PCR was performed in a 15µL reaction mixture containing 3µl RNA template, 0.6µl PrimeScript 1 step Enzyme Mix, 7.5µl 2X 1 step Buffer (Dye Plus), 0.6µl of each primer (Reverse + Forward primers=1.2µl) and 2.7µl nuclease free water. Samples were incubated at 50˚C for 30 minutes and94˚C for 2 minutes followed by annealing at 50˚C for 30 seconds according to the primer set used (Table 2), then elongation at 72˚C for 40 seconds and final extension at 72˚C for 7 minutes. The number of cycles was 45.

Second PCR used KOD One™ PCR Master Mix –Blue-2004 (Toyobo, Co Ltd, Japan) for amplification of target cDNA. PCR was performed in a 25µL reaction mixture containing 3µl cDNA template, 12.5µl, KOD One™ PCR Master Mix, 1.25µl (0.5µM) of each primer (Reverse + Forward primers=2.5µl) and 7µl PCR grade water. The samples were incubated at 98˚C for 10 seconds followed by annealing at 55˚C for 5 seconds according to primer set used (Table 1), then elongation at 68˚C for 1 second and final extension at 68˚C for 30 seconds. The number of cycles was 40.

All reaction mixtures were prepared on ice. A master mix was prepared by mixing (after thawing) all reagents except the RNA/cDNA template. The master mix was thoroughly mixed by vortexing and spinning before dispensing appropriate volumes into the PCR tubes. PCR was run on Applied Biosystems™ Veriti™ 96-Well Fast Thermal Cycler (Applied Biosystems™, Thermo Fisher Scientific, US). PCR products were stored at 4˚C awaiting gel electrophoresis.The PCR products were visualized using 1.5% agarose gel prepared by dissolving agarose powder [(Cleaver Scientific Ltd, Rugby, UK) in TAE buffer (Tris-base 2M, Acetic acid 2M, 0.005M EDTA and distilled water) in a ratio of 1:5:1 [mass(g)/volume (ml)] and colored with a drop of ethidium bromide. Five microliters of each sample PCR products were loaded onto the gel stained with ethidium bromide(0.625mg/ml)in tandem with a 1kb ladder/marker and the PCR products subjected to gel electrophoresis (Mupid-exu submarine electrophoresis system, Japan) for 30 minutes. To check for amplification the gel was viewed under ultraviolet light in an Atto Printgraph classic WUV-20 Benchtop gel reader (Atto, Daihan Scientific, Korea).

Table 1: Primer sets for virus detection and mice identification

PRIMER NAME	TARGET GENE	PRIMER SEQUENCE(5′-3′)
HAN-L-F1(Forward)	L segment	ATGTAYGTBAGTGCWGATGC
HAN-L-R1(Reverse)	L segment	AACCADTCWGTYCCRTCATC
HAN-L-F2(Forward)	L segment	TGCWGATGCHACIAARTGGTC
HAN-L-R2(Reverse)	L segment	GCRTCRTCWGARTGRTGDGCAA
mcb398(Forward)	cytb	TACCATGAGGACAAATATCATTCTG
mcb869(Reverse)	cytb	CCTCCTAGTTTGTTAGGGATTGATCG

3.5.4. Molecular Detection of rodent species

Cytochrome *b* (cytb) gene was amplified from the extracted DNA as a standard DNA barcoding for molecular identification of mice species, a polymerase chain reaction (PCR) was performed for amplification of target cytochrome *b* gene(Cainé et al., 2006). KOD One^{TM} PCR Master Mix –Blue-2004 (Toyobo, Co Ltd, Japan) PCR was performed in a 25µL reaction mixture containing 3µl DNA template, 12.5µl, KOD One^{TM} PCR Master Mix, 1.25µl (0.5µM) of each primer (Reverse + Forward primers=2.5µl) and 7µl PCR grade water. The samples were incubated at 98˚C for 10 seconds followed by annealing at 55˚C for 5 seconds according to primer set used (Table 2 above), then elongation at 68˚C for 1 second and final extension at 68˚C for 30 seconds. The number of cycles was 40.

3.5.4.1. PCR Product purification

The amplified expected band size of PCR product were purified using Wizard® SV Gel and PCR Clean-Up System according to the manufacturer's instruction. Briefly, following electrophoresis, excise DNA band from gel was placed in a 1.5ml microcentrifuge tube. Ten (10) µl Membrane Binding Solution was added per 10mg of gel slice. The mixture was Vortex and incubated at 50–65°C to completely dissolve the gel. The SV Minicolumn was inserted into Collection Tube and dissolved gel was transferred to the Minicolumn assembly and allowed to stay for 1 minute at room temperature. It was then Centrifuge at 16,000 × g for 1 minute. The flow-through was discarded and Minicolumn was reinserted into the Collection Tube. 700µl Membrane Wash Solution (ethanol added) was added and centrifuge at 16,000 × g for 1 minute. Again the flow-through was discarded and the washing repeated with 500µl by

Centrifuge at 16,000 × g for 5 minutes. The collection tube emptied and the column assembly was recentrifuged for 1 minute to ensure complete evaporation of any residual ethanol before elution of the PCR product. The Minicolumn was carefully transferred to a clean 1.5ml microcentrifuge tube and 50µl of nuclease-free water was added to the Minicolumn. This was incubated for 1 minute at room temperature and centrifuged at 16,000 × g for 1 minute. The eluted DNA was stored at 4°C or –20°C prior to sequencing reaction.

3.5.4.2. Cycle sequencing reaction

Cycle sequencing reaction was done in a10µl reaction volume mix comprising; 1 µl of purified DNA, 0.5µlBigDye™ Terminator v3.1 Ready Reaction Mix, 2.0 µl of 5X Sequencing Buffer, 4.0 µl of Deionized water (RNase/DNase-free) and 0.5µlof primer set. The Cycling Program was as follows: incubation at 96 °C for 1 minute; denaturation at 96 °C for 10 seconds; annealing at 50 °C for 5 seconds; extension at 60°C for 2 minutes for 30 cycles and hold at 4 ˚C till infinity.

3.5.4.3. Purification of sequencing reaction

The sequencing reaction was cleaned to remove the Bigdye terminator and it was removed using Agencourt CleanSEQ Dye-Terminator Removal (Beckman Coulter, Inc.) according to the manufacturing instruction. Briefly, The Agencourt CleanSEQ was mixed thoroughly to ensure no visible bead pellet remains in the bottle. 10µl of the Agencourt CleanSEQ was added to each sample, 85% ethanol was then added to the mixture and mix by vortexing 7 times. The tubes containing the sample was then placed on the Agencourt plate for 3-5 minutes to allow beads to attach to the magnet and the clear supernatant was withdrawn carefully. Another 100 µl of 85% ethanol was added and allowed for 30 seconds and washing the step was repeated and ensuring that all ethanol was removed. The tubes were allowed to air dry for 10 minutes on/off the Agencourt plate. 40 µl Deionized water (RNase/DNase-free) was added to the air dry tubes and 35µl of the cleaned product was collected for sequencing after 5 minutes and stored at 5°C until sequencing by capillary sequencer. This was performed using SeqStudio Genetic Analyzer (Applied Biosystems, Foster City, CA).

3.5.4.4. Sequence analysis

The nucleotide sequences were assembled and edited in GENETYX, version 13 (GENETYX, Tokyo, Japan) using the ATGC software to obtained a consensus sequence for each pair of sequences. The consensus sequences were compared with available sequences using the Basic Local Alignment Search Tool (BLAST. http://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the organisms.

3.6Data Analysis

Data was collected through administration of a structured questionnaire obtaining individual information of knowledge, attitudes, perception and practices regarding Hantavirus disease. This was followed by laboratory analysis of blood and harvested organ samples. All data was entered, cleaned and validated in Microsoft™Office Excel®2019. The data was grouped according to intended information as socio-demographic, awareness, attitude and management practices. All descriptive and inferential analysis were performed in IBM SPSS version 21 (SPSS Inc., Chicago IL) statistical software and Microsoft™ excel spread sheet. Data was run through three steps of analysis; univariate, a bivariate and multivariate analysis. At multivariate analysis, independent variables with *p* value less than 0.05 were considered significant determinants of Hantavirus knowledge. Analysis involved largely calculating counts and frequencies. Chi-square (X2) test was used to explain the response of the participants while observed different laboratory experimental parameters were presented in terms of tables.

3.7Ethical considerations

The University of Zambia Biomedical Research and Ethics Committee (UNZABREC), Lusaka, Zambia (REF: 2432-2021) and National Health Research Authority (REF: NHREB00004/01/04/2022) cleared the study for academic and laboratory work. Community participants consented in writing but those who could not write consented orally before interviews. Animal welfare during sample collection was ensured by using appropriate restraining methods (Sharman traps) and administration of anaesthesia (Chloroform) before heart puncture for blood collection and harvesting of organs.

CHAPTER FOUR

4.0 RESULTS

4.1Knowledge, attitude, practices (KAP) of participants towards Hantavirus disease and possible predictors that could influence knowledge of Hantavirus disease.

4.1.1 Socio-demographic characteristics of study participants

We enrolled 78participants from rural Katete as follows; 36 from Azeleguza, 31 from Kachipu and 11 from Nyembe. Demographic characteristics are shown in Table 2. A higher proportion of women were enrolled than men. Consistent with low socioeconomic characteristics of the commune, the educational level was lower and inadequate housing higher in all the sites.

Table 2: Socio-demographic characteristics of study participants

Demographic characteristics	Levels	Frequency	Proportion (%) n = 78	95% CI
Sex	Male	32	41.03	30.00-52.70
	Female	46	58.97	47.30-70.00
Age group (years)	18-35	29	37.18	27.70-50.20
	≥36	49	62.82	49.80-72.30
Residential area	Urban	1	1.28	00.00-06.90
	Rural	77	98.72	85.70-97.90
Education level	≤Primary	68	87.20	77.70-93.70
	Secondary	10	12.82	06.30-22.30
Employment status	Employed	10	12.82	06.30-22.30
	Unemployed	68	87.18	77.70-93.70
Monthly income	≤5,000.00	74	94.87	87.40-98.60
	6,000.00 – 9,000.00	4	5.13	01.40-12.60
House	Cement	31	39.74	18.60-40.50
	Mud/dirt	47	60.26	58.50-81.40

n = number of respondents; % = Percentage; CI = Confidence interval

4.1.2 Knowledge about Hantavirus disease

A good proportion 51.28% (40/78) of the participants did not know about Hantavirus disease and about their rodent reservoir hosts but when shown a picture of a Mastomys rodent, 79.49%(62/78) had seen the rodent. With regard to the disease, 64.10% (50/78) reported knowing its symptoms. When asked to describe the symptoms, fever was correctly mentioned by 3.80% (6/78) followed by flu like symptoms at 5.13% (4/78) and 35.90% (28/78) of the participants had no knowledge of any symptoms of

Hantavirus disease. With respect to the mode of transmission, 38.46% (30/78) declared that they did not know the mode of transmission, however, a small proportion of those who knew only 1.30% (2/78) mentioned that it is transmitted by rodents or their droppings. Over sixty percent (54/78) of participants reported that they knew how to prevent the disease but correct measures, like keeping the home and surroundings clean and rodent control, were rarely mentioned. The main information source regarding Hantavirus disease was relatives and friends. The average KAP score of Hantavirus disease knowledge of the participants was found to be 66.12% (462.82/7) which indicated moderate knowledge with regard to the understanding of Hantavirus disease (Table 3.)

Table 3: Participants knowledge on Hantavirus disease

Factors under knowledge	Levels	Frequency (n = 78)	Proportion (%)	95% CI	KAP Score (%)
Are you aware that there are diseases that can be transmitted from animals to humans and mice specifically?	Yes	64	82.05*	71.70-89.80	82.05
	No	14	17.95	10.20-28.30	
Are you aware of any illnesses in this village that have been associated with contact, handling or consumption of animals?	Yes	53	67.95*	56.40-78.10	67.95
	No	25	32.05	21.90-43.60	
Do you know hantavirus disease?	Yes	38	48.72*	37.20-60.30	48.72
	No	40	51.28	39.70-62.80	
Have you seen the rodent (picture of mastomys sp)	yes	62	79.49*	68.80-87.80	79.49
	No	16	20.51	12.20-31.20	
Do you know the symptoms of hanta disease?	yes	50	64.10*	52.40-74.70	64.10
	No	28	35.90	25.30-47.60	
Do you know how hantavirus is transmitted?	Yes	48	61.54*	49.80-72.30	61.54
	No	30	38.46	27.70-50.20	
Do you know someone who has had Hanta disease?	Yes	54	69.23*	57.80-79.20	69.23
	No	24	30.77	20.80-42.20	
Do you know how to prevent hanta disease?	Yes	54	69.23*	57.80-79.20	69.23
	No	24	30.77	20.80-42.20	
Average KAP score on knowledge of Hantavirus disease (462.82/7)					66.12

n = number of respondents; % = Percentage; *= Proportion considered as KAP score; CI = Confidence interval

4.1.3 Attitudes and risk perception regards to Hantavirus.

Seventy-six percent (60/78) of the participants classified Hantavirus as a severe/very severe disease that can cause death. Sixty-nine percent (54/78) reported that they knew someone who had Hantavirus disease. Rural inhabitants were perceived as the group with highest risk of disease, followed by farm workers and forestry workers. 8.97% (7/78) of participants considered themselves at high/very high risk of acquiring Hantavirus disease, compared with a perceived risk of 11.54% (9/78) for their families and 16.70% (13/78) for members of their communities. The reasons mentioned for being at risk were living or working in a high risk place, presence of rodents in the environment and farming activities. Fifty-three percent (42/78) of the people reported that they had thought at some point that they had Hantavirus disease, and 41.03% (32/78) indicated that they sought medical attention, hence, they reported that they would fear if they were diagnosed with Hantavirus disease. The average KAP score of Hantavirus disease attitude and risk perception of the participants was found to be 55.90% (279.49/5) which indicated moderate risk perception with regard to Hantavirus disease (Table 4.)

Table 4: Participants attitude towards Hantavirus disease

Factors under Attitude	**Levels**	**Frequency (*n*=78)**	**Proportion (%)**	**95% CI**	**KAP Score (%)**
Are cases of hanta reported to health authorities?	Yes	33	42.31*	31.20-54.00	42.31
	No	45	57.69	46.00-68.80	
Do you believe that someone can die from hantavirus?	Yes	51	65.38*	53.80-75.80	65.38
	No	27	34.62	24.20-46.20	
Do you think the severity of Hanta disease in humans is high and life threatening?	Yes	60	76.92*	66.00-85.70	76.92
	No	18	23.08	14.30-34.00	
Have you ever thought you have hantavirus disease?	Yes	42	53.85*	42.20-65.20	53.85
	No	36	46.15	34.80-57.80	
Did you seek health attention at that time?	Yes	32	41.03*	30.00-52.70	41.03
	No	46	58.97	47.30-70.00	
Average KAP score on Attitude towards Hantavirus disease (279.49/5)					55.90

n = number of respondents; % = Percentage; *= Proportion considered as KAP score; CI = Confidence interval

4.1.4 Practices regarding reducing risks of disease transmission from mice

Almost all participants reported presence of rodents inside their homes during the past year. Nearly forty percent (34/78) reported holes within the walls and spaces beneath the roof and doors through which rodents can enter. Fifty-six percent (44/78) reported to have rodents in their storerooms. With respect to cleaning practices of places with rodent droppings, 79.49% (62/78) answered spontaneously that they cleaned with chlorine or disinfectant. Regarding food practices seventy-three percent (57/78) of the participants consume mice and ninety percent (70/78) of the participants reported a contagious cough attributed to seasonal abundance of mice. Despite lack of knowledge by the majority of the participants on Hantavirus disease, 82.05% (64/78) were aware of zoonotic infections. Faint understanding of correct preventive practices like ventilating closed dwellings, using bleach or detergent and not raising dust when cleaning places with rodent droppings was observed. Average KAP score on participant's practices regarding reducing the risk of Hantavirus disease transmission from mice was 60.42% (483.34/8) which indicated good practice with regard to the reducing risk of Hantavirus disease transmission from mice (Table 5.)

Table 5: Participant's practices regarding reducing the risks of disease transmission from mice

Factors under practices	Levels	Frequency (*n*=78)	Proportion (%)	95% CI	KAP score (%)
What type of meat do you eat?	Beef and goat meat	69	88.46	79.20-94.60	
	others	9	11.54	5.40-20.80	
Do you consume mice?	Yes	57	73.08	61.80-82.50	
	No	21	26.92*	17.50-38.20	26.92
Have you had rodents inside your home this year?	Yes	39	50.00	38.50-61.50	
	No	39	50.00*	38.50-61.50	50.00
Does your home have holes where mice can enter?	Yes	34	43.59	32.40-55.30	
	No	44	56.41*	44.70-67.60	56.41
Have you had rodents in your storerooms this year?	Yes	44	56.41	44.70-67.60	
	No	34	43.59*	32.40-55.30	43.59
Do you trap rodents?	Yes	17	21.79	13.20-32.60	
	No	61	78.21*	67.40-86.80	78.21
When you clean closed dwellings, do you avoid raising dust?	Yes	59	75.64*	64.60-84.70	66.25
	No	19	24.36	15.30-35.40	
Do you clean places with rodent droppings with chlorine/detergent?	Yes	62	79.49*	68.80-87.80	79.49
	No	16	20.51	12.20-31.20	
Do you clean with detergent or bleach places with signs of rodents?	Yes	57	73.08*	61.80-82.50	73.08
	No	21	26.92	17.50-38.20	
Average KAP score on practices regarding reducing transmission of Hantavirus disease (483.34/8)					60.42

n = number of respondents; % = Percentage; *= Proportion considered as KAP score; CI = Confidence interval

4.1.5. Mean Knowledge, Attitude and Practices across the Socio-Demographic Characteristics

The study found that women had higher mean scores of 57.10± 20.76and 60.81± 11.46 than males for attitudes, and practices, at $p = 0.024$, and $p = 0.041$, respectively. Additionally, mean scores statistically differed among education, employment status, monthly income and housing (Table 6.). The age group 18-35 years had a higher mean score of 40.17± 9.41 for practices, followed by the ≥ 36 age group at the same level, with a mean score of 59.83± 15.78 (Table6).

Table 6: Mean knowledge, attitude and practice scores across Socio-demographic characteristics

Variable	Mean knowledge score		Mean attitude score		Mean practice score	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
Sex						
Male	44.43	15.032	42.90	17.097	39.19	4.234
Female	55.57	19.752	57.10	20.761	60.81	11.463
p-value	**0.066**		**0.024**		**0.041**	
Age (years)						
18-35	39.57	12.799	34.14	9.965	40.17	9.411
≥ 36	60.43	25.310	65.86	13.446	59.83	15.784
p-value	**0.028**		**0.033**		**0.019**	
Residential Area						
Urban	1.13	1.11	0.33	0.05	0.92	0.075
Rural	92.28	15.367	93.39	26.406	91.29	16.066
p-value	**0.087**		**0.011**		**0.037**	
Education						
Primary	88.87	12.694	87.22	19.731	88.10	14.653
Secondary	10.60	3.80	12.79	13.664	11.90	4.375
p-value	**0.041**		**0.045**		**0.044**	
Employment						
Employed	13.11	8.712	11.87	5.12	12.52	8.105

Unemployed	86.89	16.466	88.13	14.687	87.16	8.668
p-value	**0.017**		**0.026**		**0.036**	
Monthly Income						
≤5,000.00	95.29	17.677	96.51	13.390	94.26	11.414
6,000.00 – 9,000.00	4.71	1.31	3.49	2.280	5.84	2.19
P-value	**0.028**		**0.024**		**0.022**	
House						
Cement	28.38	9.032	27.78	26.329	29.68	11.148
Mud/Dirty	71.62	14.736	72.22	26.126	70.32	14.233
p-value	**0.042**		**0.031**		**0.051**	
Overall	59.57	4.79	51.40	6.35	73.00	11.58
Range	28-110		10-91		43-128	

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

4.1.6 Analysis of the Association between Hantavirus very high score Participants and the Potential Predictors

The study that found only 30.77% (24/78) of participants had very high score about Hantavirus at a cutoff point of 60%. The Pearson chi-square showed an association between the potential predictors and the Hantavirus knowledge. There was an association between very high score and the potential predictors such as education (X^2 = 481.4011, p = 0.045); knowledge of transmission (X^2 = 321.931, p = 0.001), feelings that Hantavirus disease is severe in humans (X^2 = 81.805, p = 0.001) and the practice of trapping mice (X^2 = 213.993, p = 0.001) and practice of consuming mice (X^2 = 7.993, p = 0.031). Thereafter, the variables were screened for multicollinearity using univariate linear regression (Table 7.)

Table 7: Summary of univariate analysis between potential determiners of knowledge, attitude and practices towards Hantavirus

	Number tested (*n*=78)	Very high (*n*=24)	Positivity (%)	OR	95% CI	*p* =Value
Potential risk factors for demographic						
Sex(*n*= 78)						
Male	32	8	25.00	ref		
Female	46	16	34.78	1.063	1.00-189.42	0.146*
Education (*n*= 78)						
Primary	68	22	32.35	ref		
Secondary	10	2	20.00	0.331	0.02-0.58	0.044***
Age (*n*= 78)						
18-35	29	2	06.89	ref		
>35	49	22	44.89	6.049	4.55-38.12	0.034***

Potential risk factors for knowledge						
Do you know ways of prevention (*n*=78)						
Yes	54	15	27.78	ref		
No	24	9	37.50	2.390	1.21-18.99	0.246*
Do you know ways of its transmission (*n*=78)						
Yes	48	16	33.33	ref		
No	30	8	26.67	0.301	0.18-0.64	0.211*
Have you ever seen the rodent (*n*=78)						
Yes	63	17	26.98	ref		
No	15	7	46.67	4.577	1.13-18.42	0.051*
Potential risk factors for attitude						
Is it severe in humans (*n*=78)						
Yes	60	18	30.00	ref		
No	18	6	33.33	2.016	1.06-44.25	0.041***

Do you believe that someone can die (*n*=78)						
Yes	51	15	29.42	ref		
No	27	9	33.33	2.016	1.06-44.25	0.041***
Is it a risk to the family members (*n*= 78)						
Yes	44	13	29.56	ref		
No	34	11	32.35	2.016	1.06-44.25	0.041***
Potential risk factors for practice						
Do you consume mice (*n*=78)						
Yes	57	15	26.32	ref		
No	21	9	42.86	2.103	1.74-19.59	0.003***
Do you trap rodents (*n*=78)						
Yes	53	12	22.64	ref		
No	25	12	48.00	5.526	2.29-34.81	0.048***

Do you clean with detergents (*n*=78)							
	Yes	51	12	23.53	ref		
	No	27	12	44.44	2.866	1.11-23.64	0.036***

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

4.1.7 Predictors of Hantavirus disease Knowledge for the Participants

After the adjustment for other variables in the stepwise binary logistic regression model, significant predictors of Hantavirus knowledge for participants were with a *p*-value < 0.05. Variables with a *p*-value < 0.250 in the bivariate analysis were included in the model. The test had an insignificant Hosmer–Lemeshow goodness-of-fit statistic (*p* = 0.922), and the Omnibus Test of Model Coefficients values of *p*< 0.000 were obtained, indicating the goodness of fit of the generated model. The significant predictors were the level of education which contributed to the participants understanding of how Hantavirus is transmitted, and the respective adjusted odds ratios (aORs) are presented in Table 8. Participants with secondary level of education were (aOR: 0.693 (95% CI: 0.47-0.97) times more likely to be less knowledgeable about Hantavirus than participants with primary level of education (*p* = 0.047). Participants who do not trap mice were (aOR: 0.34 (95% CI: 0.16–0.82) times more likely to be less knowledgeable about Hantavirus than participants who trapped mice (*p* = 0.025). Participants who consume mice were (aOR: 6.049 (95% CI: 0.03–0.62) times more likely to be less knowledgeable about Hantavirus than participants who do not consume mice (*p* = 0.034).

Table 8: Summary of maximum-likelihood estimates for predictors associated with Hantavirus knowledge.

Variable	Level	aOR	95% CI	*p*-Value
Education (*n* =78)	Primary	Ref		
	Secondary	0.693	0.47–0.97	0.047***
Trapping mice (*n* =78)	Yes	Ref		
	No	0.342	0.16–0.82	0.025 ***
Consuming mice (*n* =78)	No	Ref		
	Yes	6.049	1.26–24.22	0.034 ***

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

4.2 To determine the prevalence of Hantavirus in Zambia based on polymerase chain reaction (PCR)

A total of 400 RNA extracts were obtained from different mice organs. The extracted RNA was pooled (n=76) by organ and sampling area (Table 9.). All pools had 5 organs per pool except pool number 76 which had 6 organs. About 3µl of RNA from individual organ was used to contribute towards making each pool. Samples were stored overnight at -80˚C and then used the following day for downstream application.

Table 9: Pools of RNA showing the number of organs per individual pool and sampling area.

Number of pools	Number of organs/Tissues per pool	Organ/Tissue types	Sites
76	5	Blood Spleen Liver Lung Kidney	Kachipu Nyembe Azeleguza

4.2.1 Mice Identification using Cytochrome b gene

Hantavirus PCR positive rodent samples were identified using Cytochrome *b* gene. Positive pools of PCR product (Cytochrome b subunit) size of approximately 450base pairs were obtained on 2, 3,4 and 5 (Figure 4.2a). NC and lane numbers 1-6 correspond to ladder, negative control (containing the PCR reaction mix with sample) and field samples respectively.

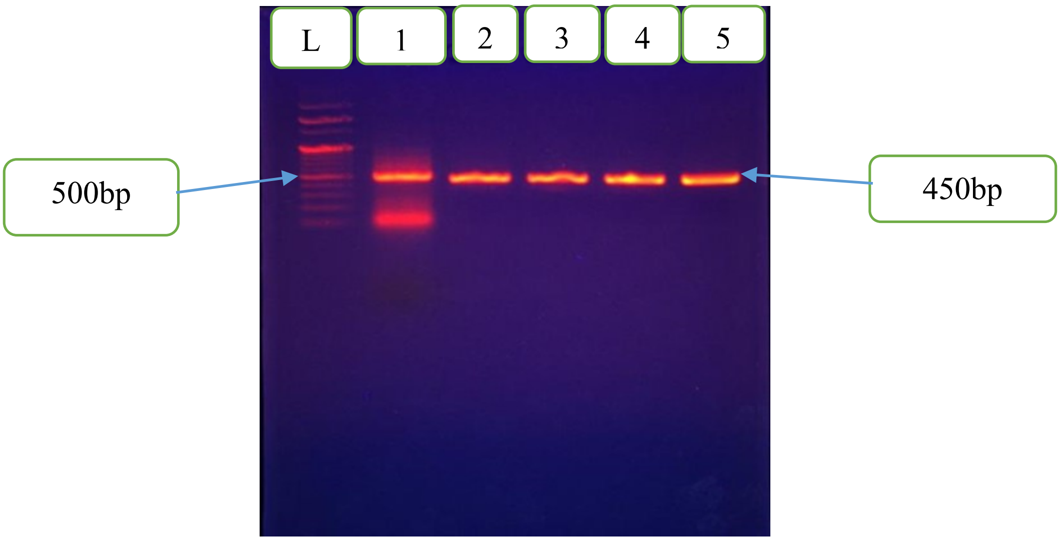

Figure 4.2.a: Gel image of fragments amplified by PCR. Samples 2, 3, 4 and 5 are positive and L is the 1kb DNA ladder.

4.2.2 DNA Barcoding for Mice Identification

The amplified product for molecular characterization of the identified Mice genera confirmed the species in samples 2, 3, 4 and 5 as *Mastomys natalensis* as shown in Figure 4.2.a. The *Mastomys natalensis* were all collected from Azeleguza village in Katete district Eastern Province. The blast result from NCBI websites for these samples showed 98.86% identity with 2, 3, 4, and 5 as shown in Table 10.

Table 10: Molecular Identification of Mice

Mice Genus	Species Name	Closely Related (Accession No)	Blastn		
			Identity	Query Cover	E-Value
Mastomys	*natalensis*	MK454508.1	98.86 %	99%	0.00
Mastomys	*natalensis*	MK454500.1	98.86 %	99%	0.00
Mastomys	*natalensis*	MK454496.1	98.86 %	99%	0.00
Mastomys	*natalensis*	MK454424.1	98.86 %	99%	0.00

4.2.3 Prevalence of Hantavirus in Mice

A total of 160 mice, representing 81.6% of the total number (196) of required mice by sample size calculation were collected in October 2021, in Katete district Eastern Province, Zambia owing to the sampling period (out of season for mice) and shorter sampling duration (1 week).76 pools were generated from aliquots of 5samples except pool number 76 which contained 6 samples. Mice were positive for orthohantavirus with a pooled prevalence of 5.26% (4/76). *Mastomys natalensis* rodents, all from Azeleguza village were identified and found to be positive for Hantavirus. Of these 160 individual mice tested, 4were positive for Hantavirus and this gave a prevalence of 2.5%. (Figure 4.2.b) (Table 11)

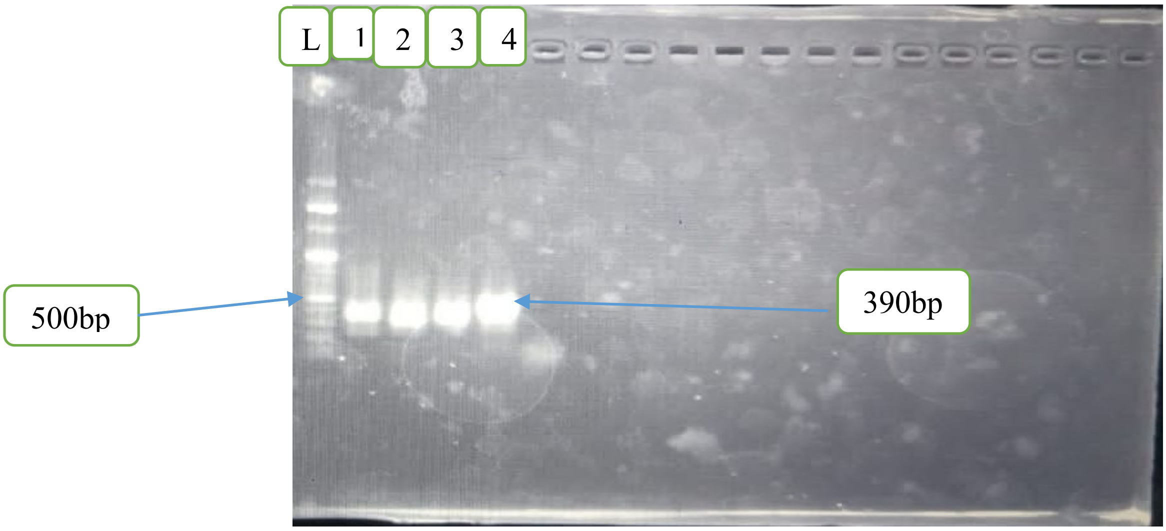

Figure 4.2.b: Gel image of fragments amplified by PCR. Samples 1, 2, 3 and 4 are positive and L is the 1kb DNA ladder.

Table 11: Prevalence of Hantavirus in mice

Factor	**Category**	***n***	**PCR Positive**	**Prevalence (%)**	**95% C I**
Overall	Mouse	160	4	2.5	1.16-7.52
Areas	Azeleguza	148	4	2.7	1.25-8.11
	Kachipu	8	0	0.00	0.00-40.23
	Nyembe	4	0	0.00	0.00-60.42
Species	*Mastomys natalensis*	159	4		
	Rattus rattus	1	0		
	Total	160	4	2.5	1.16-7.52

CHAPTER FIVE

5.0 DISCUSSION

Hantavirus disease is a notifiable disease in America, Europe and Asia. The present study detected Hantavirus in rodent samples and has further enabled the assessment of baseline understanding of Hantavirus disease, risk perception and practices regarding disease prevention in three rural communities in Katete district eastern Province Zambia. The study revealed moderate knowledge, fair attitude and good practices towards Hantavirus disease amongst the majority of participants although only a handful had heard about its rodent reservoir. One percent of the participants were aware that the disease is transmitted by rodents and their droppings besides participants reporting to have seen the specific reservoir, *Mastomys* rodent. Lack of knowledge on the rodent reservoir could likely explain why mice consumption is still significant besides culture, tradition and custom. Significant consumption of mice could possibly compromise prevention and good practice efforts towards reducing the risk of Hantavirus transmission from the rodent reservoir host.

Good knowledge, attitudes and practices regarding Hantavirus amongst mice consuming societies form a great foundation for infection prevention and control amongst people at high risk of Hantavirus infection. The assessment of participant's knowledge, attitudes and practices in sectors deemed to be at high risk is critical because knowledge is essential in curbing the spread of viral hemorrhagic infectious diseases including Hantavirus (Shoemaker, 2020). Furthermore, deciphering the influence of management practices and attitudes toward infectious diseases is crucial to improving prevention and control efforts.

Gender and age are among the important demographic factors that influenced knowledge level, attitude, and practices of study participants (Sarker *et al.*, 2005; Sadati *et al.*, 2010). By integrating gender and age in a sampling of the respondents, the response bias could be reduced, and the findings could be easily generalized. Education opens the way for awareness and fosters a better understanding of the interlinkage between disease dynamics and traditional customs. The impact of positive education influence was noted based on the mean score of secondary and tertiary education as seen in other studies (Chitaukali and Kock, 1989; Tebug *et al.*, 2012; Chingala *et al.*, 2017).

This study established that a good number of participants knew about Hantavirus with friends and relatives depicted as the highest mode of communication regarding the disease. People in these communities were not aware that mice can transmit Hantavirus despite having seen the reservoir rodent host but they perceived that Hantavirus disease could be severe and can cause death. However, they never perceived themselves to have had the disease. Overall, participants demonstrated fear for endemic diseases likely those with similar characteristic attributes as Hantavirus disease. This suggests that many diseases with similar clinical signs as malaria could be perceived in like manner. The fear for endemic diseases may be attributed to living in communities where cases and deaths have occurred from such diseases. Katete district was one such area in Zambia with high prevalence of malaria as documented by National Malaria Control Centre (2010), hence, this explains the health seeking behavior in participants who ever thought they were diseased. This revelation concurs with a study conducted by Bwalya (2019) who unearthed that people of Eastern Zambia demonstrate urgency in health care practices and health seeking behavior to enhance their wellness. Therefore, observed attitudes of participants could be considered a mediator between knowledge and practices and have a significant role in directing the choice of a disease's prevention and control measures.

Over fifty percent of the participants consume mice, except during the rainy season when rodents are worm infested. 90% of the participants reported a contagious cough attributed to seasonal abundance of mice soon after harvesting the fields and farms. The reporting of a contagious cough attributed to seasonal abundance of mice could suggest that a lot of rodent borne diseases exist but have not been confirmed or described. The assertion that a lot of rodent borne diseases exist and are yet to be described is affirmed by the detection of Hantavirus in this study. The detection of Hantavirus in Zambia (prevalence 2.5% (4/160)) appeals for molecular characterisation and viral ecological mapping of Hantavirus. Furthermore, Hantavirus is a notifiable disease in America, Europe and Asia, similarly, Zambia could adopt the same policy considering that Hantavirus has a potential to cause diseases outbreaks. In this study the majority of participants were aware of zoonotic infections such as anthrax and plague. Participants acknowledged that mice were capable of transmitting diseases to humans. About 50% reported presence of rodents inside their homes, which concurs with a study by Munõz-Zanzi *et al.*(2014) that found higher presence of rodents in rural and slum households. However, there are no studies conducted in Zambia to ascertain the proportion of rodents in both urban and rural settings. Therefore, control of rodent infestation of homes will probably require an improvement in housing standards to avoid indoor contact with rodents.

In Zambia public authorities, local governments, health and educational services and media have widely disseminated information about other endemic zoonotic diseases and their mode of transmission unlike Hantavirus disease. Although there is moderate knowledge on Hantavirus disease and its prevention, the detection of Hantavirus in mice using RT PCR would call for intensifying disease surveillance and sound control measures. Therefore, civic education is needed in mice consuming societies because frequent contact with rodents due to hunting mice and close contact inside homes constitute an important risk for acquiring Hantavirus and other zoonotic diseases.

All viral hemorrhagic infectious diseases, including Hantavirus, require special attention in prevention and control(WHO, 2011). This study reported associations between factors under knowledge and some demographic characteristics that positively influenced the understanding of Hantavirus disease (Table 7) (Monje, Erume, Mwiine, Kazoora, & Okech, 2020). This association indicated the dependence between the level of education and the practice of trapping and consuming mice. Participants with tertiary level of education presumed to have more understanding compared to primary and secondary school participants had less likelihood to be less knowledgeable of how Hantavirus is transmitted, manifested and prevented (Table 8). Similarly, participants who trap and consume mice were more likely to be less knowledgeable regarding disease transmission, symptoms and prevention and this depended on their level of education.

In conclusion, this study has highlighted moderate knowledge and practices regarding Hantavirus disease in Zambia. Despite high confidence in health services and health personnel, knowledge of zoonotic diseases, risk perception, confidence in health system, better preventive practices and culture may influence epidemiological disease dynamics and health seeking behaviours. Therefore, adequate research and education to ensure sufficient understanding of the risks and benefits of proper implementation of feasible preventive practices in management of zoonotic infections is needed.

5.1 LIMITATIONS OF THE STUDY

In this study we could not manage to conduct molecular characterization and phylogenetic analysis of the detected Hantavirus due to time factor and lack of resources. Secondly, the required sample size of 196 rodents could not be attained owing to the sampling period (out of season for mice) and shorter sampling duration (1 week). Thirdly, during this study time factor was a gross challenge considering that 160 questionnaires were required to be administered face to face within the different households in mice consumption catchment area in concurrent with mice trapping.

5.2 RECOMMENDATIONS AND FUTURE WORK

Molecular characterization and phylogenetic analysis of Hantavirus is needed in Zambia. Further to this mapping the Hantavirus distribution in Zambia would help to pinpoint viral hotspots making it easier for disease outbreak and epidemic management. Secondly, a KAP study covering a significant geographical portion and population of Zambia should be conducted to get a country representative understanding. Thirdly, this study recommends community sensitization on Hantavirus disease and the possibility of transmission of other zoonotic pathogens and advocates for the importance of reporting suspected cases to relevant authorities for proper management. Lastly, we suggest the investigation of Hantavirus sero-prevalence in humans particularly those societies deemed highly predisposed due to frequent contact with mice.

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APPENDICES

1. UNZABREC ETHICAL CLEARANCE

UNIVERSITY OF ZAMBIA
BIOMEDICAL RESEARCH ETHICS COMMITTEE

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

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

1st February, 2022.

Your REF. No. 2432-2021

Mr. Jones Victor Chipinga,
University of Zambia,
School of Veterinary Medicine,
P.O Box 32379,
Lusaka.

Dear Mr. Chipinga,

RE: MOLECULAR EPIDEMIOLOGY OF HANTAVIRUSES IN MICE IN ZAMBIA (REF. NO. 2432-2021)

The above-mentioned research proposal was presented to the Biomedical Research Ethics Committee meeting on 26th January, 2022 and the following concerns were raised:

CORRECTIONS:

1. Dissemination of study results is missing in the proposal.

Approval will only be granted after the raised concerns have been addressed. Please Resubmit An ELECTRONIC copy of the revised proposal to unzarec@unza.zm, with highlighted changes. This should be done within **TWO (2)** weeks period.

Yours sincerely,

Munsaka.

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

2. NHREB CLEARANCE

NATIONAL HEALTH RESEARCH AUTHORITY

Paediatric Centre of Excellence, University Teaching Hospital, P.O. Box 30075, LUSAKA
Chalala Office Lot No. 18961/M, Off Kasama Road, P.O. Box 30075, LUSAKA
Tell: +260211 250309 | Email: znhrasec@nhra.org.zm | www.nhra.org.zm

REF:NHREB00004/01/04/2022 **Date: 1st April, 2022**

The Principal Investigator
Jones Chipinga,
University of Zambia,
Lusaka, Zambia.

Dear Dr Chipinga,

Re: Request for Ethical Clearance and Authority to Conduct Research

The National Health Research Ethics Board (NHREB) is in receipt of your request for ethical clearance and authority to conduct research titled **"Molecular epidemiology of Hantaviruses in mice in Zambia".**

I wish to inform you that following submission of your request to the Board, its review of the same and in view of the ethical clearance, this study has been **approved** on condition that:

1. **A Material Transfer Agreement is obtained and cleared by the National Health Research Ethics Board should there be any need for samples to be sent outside the country for analysis.**
2. The relevant Provincial and District Medical Officers where the study is being conducted are fully appraised;
3. Progress updates are provided to NHRA quarterly from the date of commencement of the study;
4. The final study report is cleared by the NHRA before any publication or dissemination within or outside the country;
5. After clearance for publication or dissemination by the NHRA, the final study report is shared with all relevant Provincial and District Directors of Health where the study was being conducted, and all key respondents.

Yours sincerely,

Dr Fusya Goma
Vice Chairperson
National Health Research Ethics Board