Sokoine University of Agriculture

PhD Thesis

Assessment of Rodent-Borne and Zoonotic Disease Pathogens in Humans, Dogs and Rodents and Community Awareness in Ngorongoro District, Tanzania

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ASSESSMENT OF RODENT-BORNE AND ZOONOTIC DISEASE PATHOGENS IN HUMANS, DOGS AND RODENTS AND COMMUNITY AWARENESS IN NGORONGORO DISTRICT, TANZANIA

Thesis Submitted to Sokoine University of Agriculture in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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EXTENDED ABSTRACT

Bacteria and viruses carried by rodents, and domestic dogs can have significant public health implications. Rodents are known reservoirs of several zoonotic diseases, such as Hantavirus hemorrhagic fever, Lassa fever virus, plague, tuberculosis and leptospirosis. Also, domestic dogs carry zoonoses like rabies, leptospirosis, salmonellosis, ehrlichiosis and borreliosis. Zoonotic diseases pose a substantial risk to communities' health, mostly in wildlife, domestic animals and human interface areas. Ngorongoro district is the home of various wildlife and domestic animals cohabiting with humans. The district is experiencing the expansion of human activities which brings rodents into close proximity with human settlements. Previous studies conducted in livestock and human patients attending the health facilities have documented the existence of antibodies of zoonoses like Rift Valley fever, Brucellosis, Anthrax, and molecular detection of Bovine tuberculosis. While previous studies have examined the transmission of pathogens in livestock and hospital-based research in humans within the district, the specific role played by rodents and domestic dogs in transmitting pathogens remains largely unknown. This research gap necessitated conducting a comprehensive metagenomics study to assess the diversity and abundance of bacteria and viruses circulating among rodents, domestic dogs and humans as well as the communities understanding of rodent-borne diseases, including RVF, in the Ngorongoro district of Tanzania. The Rift Valley fever was frequently previously reported to occur within livestock and human populations of the Ngorongoro district. The disease caused significant morbidity and mortality in both livestock including humans.

To address the objectives, the study used Illumina and Oxford Nanopore technologies to identify bacteria and viruses in 530 blood samples collected from 200 humans, 230 rodents, and 100 domestic dogs. Numerous bacteria and viruses were detected and identified by using MiSeq and MinION sequencers with a Kraken2 data analysis program. Moreover, the study used 3 focus groups, 20 key informant interviews, and the questionnaire (N=352) to collect information on communities' awareness on rodent-borne diseases and Rift Valley fever. The logistic regression model was used to examine the relationship between demographic factors and communities' knowledge, attitudes and practices toward rodent-borne diseases and RVF occurrences.

In all three hosts, various pathogenic and zoonotic bacterial species were detected. Zoonotic airborne and contagious bacteria such as *Mycobacterium* spp, *Mycoplasma* spp, *Bordetella* spp and *Legionella* spp were spotted in rodents, domestic dogs and humans. Arthropod-borne zoonotic bacteria like *Bartonella* spp, *Borrelia* spp, and *Rickettsia* spp were detected in all three hosts, while *Orientia* spp were found in rodents and dogs. *Yersinia pestis*, *Streptobacillus* spp and *Anaplasma* spp were identified in rodents only. Waterborne and foodborne zoonotic bacteria were also spotted in all three hosts, including *Leptospira* spp, *Brucella* spp, *Bacillus* spp, and *Salmonella*. Generally, rodents carried a high proportion of zoonotic bacteria compared to dogs and humans. The detection of zoonotic bacteria in all three hosts showed the possibilities of cross-species transmission of infections between animals and humans sharing the same environment.

Several viral families and species were detected and identified in this study. A total of 20 RNA and 20 DNA viral families and unclassified RNA viruses were detected. The majority of viral families were detected in rodents when compared to domestic dogs and humans. *Peribunyaviridae*, *Hantaviridae*, *Phenuiviridae*, *Flaviviridae*, *Coronaviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Retroviridae*, *Picornaviridae*, *Arenaviridae*, *Togaviridae*, and *Tobaniviridae* are zoonotic RNA viruses identified in rodents. The zoonotic DNA viruses detected in rodents were *Adenoviridae, Poxviridae*, *Herpesviridae*, *Anelloviridae*, and *Circoviridae*. *Peribunyaviridae* and *Hantaviridae* viruses were identified in both rodents and humans, whereas *Retroviridae* was detected in rodents and domestic dogs. Unclassified +ssRNA viruses were spotted in domestic dogs and humans. Lastly, *Herpesviridae* was found in all three hosts. In comparison with domestic dogs and humans, rodents harboured potential zoonotic viruses of public health safety.

Result showed that 8.52% of respondents had good knowledge, 35.5% had a positive attitude, and 94.3% had good practices toward rodent-borne diseases. The study revealed that only 28.13% of participants were aware of rodent-borne zoonoses. The majority of them (77.27%) believe that rodents are pests that destroy crops and do not transmit pathogens. Moreover, the findings showed that the majority of respondents (82.9%) live in huts which can provide shelter for rodents. Additionally, except for education, the level of knowledge had no significant relationship with most of the participants' demographic variables. Respondents with secondary education ($p=0.017$) had good knowledge of rodent-borne diseases and management when compared to those without education. Despite showing good practices, the communities still don't have enough knowledge of rodent-borne infections.

The findings further showed that only 36.1%, 38.64%, and 16.19% of participants had good knowledge, positive attitudes and effective preventive practices about RVF occurrences, respectively. The significant demographic variables related to good knowledge and positive attitudes were gender ($P<0.05$), occupation ($P<0.05$) and education ($P<0.05$). Male respondents had good knowledge of RVF epidemiology. Individuals with formal education had good knowledge and exhibited positive attitudes towards RVF occurrences. Agropastoral members had a significantly negative attitude toward RVF occurrences compared to pastorals ($P=0.048$). The study revealed that the majority of respondents had poor knowledge, negative attitudes and ineffective preventive practices towards RVF epidemiology. Probably, these results were attributed to the lack of regular education campaigns to increase community awareness of the disease.

This study novelty is based on the fact that it is the first to provide the baseline information on the diversity of pathogenic and zoonotic bacteria and viruses in rodents, domestic dogs and humans sharing the same environment. Likewise, it established the levels of the communities' knowledge, attitudes and practices toward rodent-borne diseases including Rift Valley fever in Ngorongoro district for the first time. The study population had inadequate knowledge and negative attitudes toward rodent-borne diseases and RVF. Therefore, one health multidisciplinary approach is recommended in order to safeguard public and animal health from acquiring zoonoses. Provision of health education should be a long-term practice to prevent diseases outbreaks in Ngorongoro district and in Tanzania at large.

Keywords: Metagenomics next-generation sequencing, bacteria, viruses, rodents, domestic dogs, humans, Knowledge, Attitudes, Practice, zoonoses, Ngorongoro, Tanzania.

IKISIRI KUU

Bakteria na virusi wanaobebwa na panya wanaweza kuwa na athari kubwa kwenye afya ya binadamu na wanyama wengine. Panya wanajulikana kuwa wanahifadhi vimelea vya magonjwa mfano virusi vinavyosababisha homa kama vile Hantavirus hemorrhagic fever, Lassa virus fever, na bacteria wanaosababisha magonjwa kama vile tauni, kifua kikuu, na leptospirosis. Pia, mbwa wa ndani wanasambaza magonjwa kama kichaa leptospirosis, salmonellosis, ehrlichiosis, na borreliosis. Magonjwa ya kuambukiza yanaleta hatari kubwa kwenye afya ya jamii, hasa katika maeneo ya mwingiliano kati ya wanyamapori, wanyama wa ndani, na binadamu. Wilaya ya Ngorongoro ni makazi ya wanyamapori na wanyama wa ndani wanaoishi pamoja na binadamu. Wilaya hiyo inakabiliwa na kuongezeka kwa shughuli za kibinadamu ambazo zinaleta panya karibu na makazi ya binadamu. Utafiti uliofanywa kwenye mifugo na binadamu waliohudhuria vituo vya afya ulithibitisha kuwepo kwa magonjwa ya kuambukiza kama vile homa ya Bonde la Ufa, homa ya kutupa mimba, kifua kikuu, na kimeta. Ingawa utafiti uliopita ulitathmini magonjwa ya kuambukiza katika mifugo na wagonjwa wilayani hapo, mchango wa panya na mbwa katika kusambaza bacteria na virusi bado ulikuwa haujulikani. Pengo hili la utafiti lilipelekea kufanya utafiti wa metagenomics ili kutambua aina za bakteria na virusi kwa panya, mbwa wa ndani, na binadamu pamoja na kutathmini uelewa wa jamii juu ya magonjwa yanayosambazwa na panya, Pamoja na ugonjwa wa Homa ya Bonde la Ufa katika wilaya ya Ngorongoro. Homa ya Bonde la Ufa iliripotiwa mara kwa mara kutokea wilayani hapo na ilisababisha vifo vingi vya mifugo pamoja na binadamu.

Utafiti huu ulitumia teknolojia za Illumina na Oxford Nanopore katika kutambua bakteria na virusi vilivyopo kwenye sampuli 530 za damu zilizokusanywa kutoka kwa binadamu 200, panya 230, na mbwa wa ndani 100. Bakteria na virusi kadhaa viligunduliwa na kutambuliwa kwa kutumia mashine za MiSeq na MinION pamoja na programu ya

uchambuzi wa data ya Kraken2. Aidha, utafiti huu ulitumia vikundi vya majadiliano 3, mahojiano ya watu wa ufahamu muhimu 20, na kujaza dodoso(N=352) ili kuweza kukusanya taarifa za ufahamu wa jamii juu ya magonjwa yanayosambazwa na panya na homa ya Bonde la ufa. Njia ya *logistic regression* ilitumika katika kuchunguza uhusiano kati ya kidemografia na ufahamu, mitazamo na mazoea ya jamii kuhusiana na magonjwa yaenezwayo na panya.

Utafiti ulitambua bakteria mbalimbali wanaosababisha magonjwa ya kuambukiza kwa binadamu na wanyama. Bakteria wanaosambazwa kwa njia ya hewa kama *Mycobacterium* spp, *Mycoplasma* spp, *Bordetella* spp na *Legionella* spp walipatikana kwa panya, mbwa na binadamu. Bacteria waenezwao kwa njia ya wadudu kama vile *Bartonella* spp, *Borrelia* spp, na *Rickettsia* spp walionekana pia kwa panya, mbwa na binadamu, *Orientia* spp ziligunduliwa kwa panya na mbwa. *Yersinia pestis*, *Streptobacillus* na *Anaplasma* walionekana kwa panya tu. Bacteria wanaoenezwa kwa njia ya vyakula, maji na mazingira walitambuliwa pia, ikiwa ni pamoja na *Leptospira* spp, *Brucella* spp, *Bacillus* spp, na *Salmonella* spp. Kwa ujumla, panya walibeba idadi kubwa ya bakteria ikilinganishwa na mbwa na binadamu. Kupatikana kwa bakteria wanaoambukiza kwenye panya, mbwa na binadamu, inaonyesha uwezekano wa kuwepo kwa maambukizo kati ya wanyama na binadamu wanaoishi Pamoja.

Jumla ya familia za virusi 20 RNA na 20 DNA zilitambuliwa katika utafiti huu. Familia nyingi za virusi zilipatikana kwenye sampli za panya ikilinganishwa na za mbwa na binadamu. *Peribunyaviridae, Hantaviridae*, *Phenuiviridae*, *Flaviviridae*, *Coronaviridae*, *Orthomyxoviridae, Paramyxoviridae, Retroviridae*, *Picornaviridae*, *Arenaviridae*, *Togaviridae*, na *Tobaniviridae* ni virusi vya RNA vyenye spishi za kuambukiza zilizopatikana kwenye sampuli za panya. Virus vya kuambukiza vya DNA vilivyotambuliwa kwenye panya ni *Adenoviridae*, *Poxviridae*, *Herpesviridae*, *Anelloviridae*, na *Circoviridae*. Virusi vya *Peribunyaviridae* na *Hantaviridae* vilipatikana kwenye panya na binadamu, wakati *Retroviridae* ilitambuliwa kutoka

kwenye panya na mbwa. Virusi visivyoainishwa +ssRNA vilipatikana kwaenye mbwa na binadamu. Familia ya *Herpesviridae* ilipatikana kwenye panya, mbwa na binadamu. Ikilinganishwa na mbwa na binadamu, panya walikuwa na virusi vingi vya kuambukiza ambavyo ni hatari kwa afya ya jamii.

Matokeo yalionyesha kuwa 8.52% ya washiriki walikuwa na ufahamu mzuri, 35.5% walikuwa na mtazamo chanya, na 94.3% walikuwa na mazoea mazuri kuhusu magonjwa yatokanayo na panya. Utafiti ulibaini kuwa washiriki wachache tu (28.13%) walikuwa na ufahamu wa magonjwa ya kuambukiza kutoka kwa panya. Wengi wao (77.27%) wanadhani kuwa panya ni mnyama mharibifu wa mazao na hawana uwezo wa kusambaza vimelea vya magonjwa. Zaidi, matokeo yalionyesha kuwa washiriki wengi (82.9%) wanaishi katika vibanda ambavyo vinaruhusu panya kuingia ndani. Isipokuwa viwango vya elimu, kiwango cha ufahamu hakikuwa na uhusiano mkubwa na sababu nyingi za kidemografia za washiriki. Washiriki wenye elimu ya sekondari ($p=0.017$) walikuwa na ufahamu mzuri juu ya magonjwa yanayoenezwao na panya ikilinganishwa na wale wasio na elimu. Licha ya kuonyesha tabia na vitendo vizuri, jamii bado haina ufahamu wa kutosha kuhusu magonjwa ya kuambukiza kutoka kwa panya.

Matokeo pia yalionyesha kuwa 36.1%, 38.64%, na 16.19% ya washiriki walikuwa na ufahamu mzuri, mtazamo chanya, na matendo madhubuti ya kuzuia matukio ya homa ya bonde la ufa. Sababu za kidemografia zinazohusiana na ufahamu mzuri na mtazamo chanya zilikuwa ni jinsi ($P<0.05$), kazi ($P<0.05$) na elimu ($P<0.05$). Washiriki wa kiume walikuwa na ufahamu mzuri wa epidemiolojia ya homa ya bonde la ufa. Watu wenye elimu rasmi walikuwa na ufahamu mzuri na walionyesha mtazamo chanya kuhusiana na kuzuia na kudhibiti matukio ya homa ya bonde la ufa. Wakulima-wafugaji walikuwa na mtazamo hasi juu ya namna homa ya bonde la ufa inavyotokea ikilinganishwa na wafugaji ($P=0.048$). Utafiti ulibaini kuwa washiriki wengi walikuwa na ufahamu duni, mtazamo hasi na tabia zisizo ridhisha za kuzuia homa ya bonde la ufa. Labda, matokeo haya yalitokana na ukosefu wa kampeni za elimu ili kuongeza ufahamu wa jamii kuhusu ugonjwa huo.

Kwa ujumla utafiti ulibaini kuwa panya walikuwa wamebeba bakteria na virusi hatari vya magonjwa ya kuambiza kuliko mbwa, na binadamu. Pia, utafiti huu ulifanya tathmini ya viwango vya ufahamu, mitazamo na tabia za jamii kuhusiana na magonjwa ya kuambukiza kutoka kwa panya hasa homa ya Bonde la Ufa katika wilaya ya Ngorongoro kwa mara ya kwanza. Washiriki wengi walikuwa na ufahamu mdogo na mitazamo hasi juu ya kuwepo kwa magonjwa yanayoenezwa na panya na pia kujikinga dhidi ya homa ya bonde la ufa. Kwahiyo, afya moja programu inashauriwa kutumika ili kulinda afya ya jamii na wanyama dhidi ya magonjwa ya kuambukiza. Utoaji wa elimu ya afya unapaswa kuwa wa muda mrefu ili kuzuia milipuko ya magonjwa katika wilaya ya Ngorongoro na Tanzania kwa ujumla.

Maneno muhimu: Metagenomics next-generation sequencing, bakteria, virusi, panya, mbwa, binadamu, ufahamu, Mitazamo, magonjwa ya kuambukiza, Ngorongoro, Tanzania.

DECLARATION

I, Amina Radhamani Issae, hereby declare to the Senate of Sokoine University of Agriculture that this thesis is entirely my original work accomplished within the period of registration. This work has neither been submitted nor concurrently submitted for degree award in any other institution.

………………………………… ……………………………
Amina Ramadhani Issae Date
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The above declaration is confirmed by;

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Prof. Christopher J. Kasanga Date
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DEDICATION

In profound honour and everlasting love, I dedicate this work to my beloved parents (Mr. Ramadhani Issae (late) and Mrs.Fatuma Selemani) and my siblings (Maimuna R. Issae, Husseni R. Issae, Zena R. Issae, Yahaya R. Issae, Nuhu R. Issae, Twalibu R. Issae and Bashiri R. Issae). Their unwavering love, prayers and support have been the guiding light throughout my academic journey. They instilled in me the values of perseverance, determination, and resilience, which have shaped my character and enabled me to overcome challenges. Though my daddy is no longer with me physically, his spirit and unwavering love continue to inspire me every day. This work stands as a tribute to their boundless sacrifices and serves as a testament to their enduring legacy in my life.

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LIST OF ABBREVIATIONS AND ACRONYMS

&	And
+ssRNA	Positive Single Stranded Ribonucleic Acids
µl	Microliter
ACEII-IRPM	African Centre of Excellence for Innovative Rodent Pest Management
BCL	Binary Base call
BTD	Biosensor Technology Development
cDNA	Complementary Deoxyribonucleic Acids
CI	Confidence interval
COSTECH	Tanzania Commission of Science and Technology
DNA	Deoxyribonucleic Acids
dsDNA	Double Stranded Deoxyribonucleic Acids
et al	and others
F	Female
FGD	Focus Group Discussion
HFRS	Hemorrhagic Fever with Renal Syndrome
KAP	Knowledge, Attitudes and Practices
KI	Key Informant
LFA	Large Folding Aluminum
M	Male
ml	Milliliter
n	Sample size
N	Total sample size
NA	Not Applicable
NCBI	National Center for Biotechnology Information
NIMR	National Institute for Medical Research
ONT	Oxford Nanopore Technologies
OR	Odd Ratio
P-value	Probability Value
QC	Quality control
r	Correlation coefficient
RNA	Ribonucleic Acids
RVF	Rift Valley fever
SD	Standard Deviation

ssDNA	Single Stranded Deoxyribonucleic Acids
-ssRNA	Negative Single Stranded Ribonucleic Acids
SUA	Sokoine University of Agriculture
TAWIRI	Tanzania Wildlife Research Institute
USA	United States of America

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Introduction and Literature Review

Rodents play a crucial role in the transmission of zoonotic pathogens to humans and other animals globally (Mazigo *et al*., 2010). Rodents such as rats and mice, have been recognized as important reservoirs and carriers of bacteria and viruses, which pose significant public health risks (Rabiee *et al*., 2018). These rodents have been implicated in the transmission of diseases like Leptospirosis (Biggs *et al*., 2011), Salmonellosis and Plague, Rift Valley fever (Sumaye *et al*., 2004), and Hantavirus hemorrhagic fever (Mazigo *et al*., 2010). They potentially spread infectious agents to humans and animals through various routes such as i) direct contact with infected rodents by consumption or exposure to their urine, faeces, saliva and blood (Mazigo *et al*., 2010); ii) ectoparasites, such as fleas, ticks, and mites are found on rodents and can transmit diseases to other animals and humans via bites (Sumaye *et al*., 2004). For instance, fleas transmit *Yersinia pestis* a causative agent of Plague, and *Bartonella* species (Theonest *et al*., 2019), and Murine typhus (Sumaye *et al*., 2004), and ticks can transmit Lyme disease and Rocky Mountain spotted fever (Theonest *et al*., 2019); iii) contaminated water and food: it has been reported that rodents can contaminate water sources and food with their droppings, urine and saliva (Mgode *et al*., 2014). If animals and humans drink or eat contaminated water or food can get infections. Iv) Contaminated environments: rodents can introduce zoonoses indirectly by contaminating the environment with their pathogens, and then animals and humans acquires the infections from the environment (Mgode *et al*., 2014).

Additionally, it has been found that the expansion of human activities in the Ngorongoro district brings rodents and their ectoparasites into close contact with humans and domestic animals (Shilereyo *et al*., 2021; Shilereyo *et al*., 2022). This can contribute to the transmission of rodent-borne pathogens to humans and domestic animals (Shilereyo *et al*., 2022). Crops cultivation is a significant human activity in the district, and cereal crops and improper storage of food attract rodents to crop fields and inside houses. Livestock keeping is another essential economic activity in the district which contributes to attracting rodents close to human settlements (Shilereyo *et al*., 2022). It has been found that rodents are attracted to livestock feed and some insects from decomposed manures (Shilereyo *et al*., 2021). Improper waste management practices, such as leaving garbage uncovered or improperly disposing of organic waste attract rodents to human residents (Shilereyo *et al*., 2022). Rodents that feed on wastes can pick up pathogens and then transmit them to humans or other animals through contact or contaminated food or water sources. Poorly constructed houses with inadequate sanitation in some communities can provide a favourable environment for rodents' inhabitation and hence increase the risk of spreading zoonoses. Another activity includes the increased human population which leads to the expansion of settlement: As human settlements expand, they encroach on natural rodent habitat, bringing rodents into closer contact with humans (Shilereyo *et al*., 2021). The expansion of human settlements is usually accompanied by deforestation which disrupts the natural habitats of rodents, causing them to seek shelter and food in human houses (Shilereyo *et al*., 2021).

Furthermore, husbandry practices of domestic dogs in Tanzania, including Ngorongoro district pose significant risks for zoonotic disease transmission to humans and other animals (Swai *et al*., 2016; Issae, 2018). While dogs can be wonderful companions, their interactions with humans, and the environment create opportunities for disease transmission if proper husbandry practices are not followed (Ghasemzadeh *et al*., 2015; Swai *et al*., 2016; Issae, 2018). Some ways in which these practices can lead to disease transmission include: 1) lack of vaccination and veterinary care: In many rural areas of Tanzania, access to veterinary service is limited

(Swai *et al*., 2016; Issae, 2018). As a result, domestic dogs do not receive the necessary vaccination and routine care to prevent diseases, such as rabies, parvovirus, leptospirosis, ehrlichiosis, and borreliosis (Cleaveland *et al*., 2000; Ghasemzadeh *et al*., 2015). This can lead to occurrences of these diseases in humans and other animals through dog bites, direct contact, contaminated water or environment, and bites from ectoparasites (Cleaveland *et al*., 2000). 2) Stray and free-roaming dogs: Uncontrolled breeding and a lack of responsible animal ownership may result in large population of stray and free roaming dogs (Ghasemzadeh *et al*., 2015). It has been found that stray and free-roaming dogs scavenge for food in garbage dumps and hunt wild animals like small mammals which increase the risk of acquiring infectious agents (Cleaveland *et al*., 2000; Swai *et al*., 2016). 3) Close proximity to humans and other animals: Domestic dogs often lives in close proximity to humans and their livestock in rural settings (Issae, 2018). This closeness can facilitate the transmission of diseases from dogs to humans including livestock and vice versa. 4) Inadequate waste management practices often lead to environmental contamination and spread of pathogens. Dog faeces can carry parasites and pathogens that may persist in the environment and infect humans and other animals when proper hygiene practices are not observed (Ghasemzadeh *et al*., 2015).

Despite the potential threats associated with rodent-borne pathogens globally, there was limited knowledge regarding the diversity of zoonotic bacteria and viruses circulating in rodents, domestic dogs, and humans in Tanzania. The inclusion of rodents, domestic dogs and humans in diseases surveillance aligns with the one health approach, which recognizes the interconnections between human health, animal health, and the environment (Chauhan *et al*., 2020). The majority of zoonoses have complex ecological dynamics involving various hosts, and understanding the pathogens existing in interface areas is crucial for disease prevention and control (Chauhan *et al*., 2020). Monitoring infections in domestic dogs, rodents, and people at the same time leads to a thorough understanding of disease patterns, dynamics of transmission, and risks of cross-species transmission of pathogens. Therefore, the main goal of this study was to conduct a comprehensive metagenomics screening of pathogenic bacteria and viruses in rodents, domestic dogs and humans as well as assessing knowledge, attitudes and practices (KAP) towards rodent-borne diseases in the Ngorongoro district, Tanzania. The study established the diversity, proportions and abundance of pathogenic and zoonotic bacteria and viruses, and levels of KAP of communities towards rodent-borne diseases, with a specific focus on Rift Valley fever (RVF).

Rift Valley Fever (RVF) is a zoonotic disease caused by the RVF virus, first reported in 1913 in Kenya’s Rift Valley region. It is endemic in Sub-Saharan Africa, the Arabian Peninsula, and other African countries (Sindato *et al*., 2014). The virus infects various mammals, transmitted primarily by Aedes and Culex mosquitoes during periods of heavy rainfall (Mweya *et al*., 2018). RVF affects a wide range of animals, with sheep and goats being particularly susceptible, while humans primarily contract the disease through contact with infected animals or mosquito bites (Sindato *et al*., 2014). Prevention in animals relies on vaccination and vector control. The disease’s socio-economic impact includes animal and human deaths, disruption of livestock markets, and financial losses, making it a significant concern for both veterinary and public health authorities. RVF episodes have been repeatedly reported in various parts of Tanzania including the Ngorongoro district (Mweya *et al*., 2018). The disease caused substantial morbidity and mortality in both animals and humans (Sindato *et al*., 2014; Mweya *et al*., 2018). Even though we haven’t found RVF virus in this study, its viral family, *Phenuiviridae* was found in rodents. Through an in-depth exploration facilitated by the KAP study, elucidating the intricacies of Rift Valley Fever (RVF) transmission dynamics and risk factors becomes attainable. Armed with this knowledge, targeted educational initiatives can be implemented to disseminate crucial information regarding the etiology, transmission pathways, and preventive measures pertaining to RVF. By fostering a comprehensive understanding of RVF within the community, individuals can adopt proactive measures to mitigate the risk of infection, safeguarding both human and animal populations. This concerted effort promotes a collaborative approach towards disease containment, thereby curtailing the adverse impact of RVF on public health and agricultural sustainability.

Studies documented that prevention and control of zoonotic diseases require various approaches, including improved surveillance and monitoring, effective diseases control measures, and public health education and awareness campaigns (Mweya *et al*., 2018; Ndumu *et al*., 2020). Knowledge, attitudes and practices (KAP) towards diseases are essential factors which have influence on transmission of infections (Ndume *et al*., 2020; Mohamed *et al*., 2021). Understanding the KAP of local communities help to identify gaps in knowledge, attitudes, and practices related to zoonoses, and guide the development of targeted education campaigns and interventions. This thesis has provided the baseline information for understanding knowledge, attitudes and practices of the communities in the Ngorongoro district on rodent-borne diseases as well as RVF.

In recent years, metagenomics, coupled with Next-generation Sequencing (NGS) technologies, have improved the field of infectious diseases studies (Handelsman, 2004; Kilianski *et al*., 2015). Metagenomics allows the study of complex microbial communities by directly sequencing the collective genetic material present in a sample, without the need for culturing the microbes (Handelsman, 2004). It has been found that metagenomics NGS provides a comprehensive understanding of the diversity, and abundance of microorganisms in various samples, including the identification of novel pathogens (Kilianski *et al*., 2015). Next-generation Sequencing technologies such as Illumina short reads and Nanopore long reads have been used in identification of microbes in samples through partial or whole genome isolation (Overholt *et al*., 2020). Combining the two technologies allows researchers to get an entire view of the genome, capturing both subtle differences within genome and more obvious structural variations throughout the genome, greatly improving microbial identification sensitivity and accuracy (Overholt *et al*., 2020). This study established the first metagenomics next-generation sequencing surveillance of infectious agents present in rodents, dogs and humans from the Ngorongoro district.

1.2 Problem Statement and Justification for the Study

1.2.1 Problem statement

Zoonotic diseases pose significant threats to public health, especially in regions where humans, wildlife, and domestic animals frequently interact (Niboye, 2010). Previous research in the Ngorongoro district has identified various bacterial and viral infections in livestock and humans, including Anthrax, Bovine tuberculosis, Leptospirosis, Brucellosis, and Rift Valley fever (Mellau *et al*., 2009; Katale *et al*., 2013; Mbugi *et al*., 2015; Mwakapeje *et al*., 2018; Ahmed et al., 2018; Makala *et al*., 2020; Motto *et al*., 2021;). However, while studies have explored pathogen transmission among livestock and humans, the role of rodents and domestic dogs in spreading these pathogens remains largely unexplored. Given their close association with humans, there is a potential for these animals to transmit zoonotic pathogens. Addressing this research gap is crucial for understanding disease dynamics and assessing public health risks in the Ngorongoro district. Therefore, this study aimed to comprehensively investigate the contribution of rodents and domestic dogs to bacterial and viral infection transmission in Ngorongoro district, enhancing our understanding of disease dynamics and potential risks to public health.

1.2.2 Justification for the study

The significancy for conducting the study in the Ngorongoro district of Tanzania is multifaceted and deeply rooted in the need to address potential public health threats posed by zoonotic pathogens. Firstly, the interactions among humans, wildlife, and domestic animals in the district create suitable environment for the transmission of infectious diseases, particularly from rodents and domestic dogs to humans and other animals (Onyuok *et al*., 2019; Xu *et al*., 2020; Wang *et al*., 2021). Given the limited knowledge regarding the diversity and transmission dynamics of these pathogens in the area, there exists a critical research gap that necessitates comprehensive surveillance and investigation. Furthermore, the presence of specific zoonotic diseases caused substantial morbidity and mortality in both livestock and humans like Rift Valley Fever (RVF) underscores the importance of understanding disease patterns and risk factors to implement targeted interventions. By employing cutting-edge technologies such as metagenomics coupled with Next-generation Sequencing (NGS), the study aimed at providing a holistic view of bacteria and viruses landscape, identifying both known zoonotic pathogens without the need for culturing. Additionally, the assessment of community Knowledge, Attitudes, and Practices (KAP) towards rodent-borne diseases and RVF is crucial for tailoring educational campaigns and interventions effectively. Ultimately, this research endeavor aligns with the One Health approach, recognizing the interconnectedness of human health, animal health, and the environment, and seeks to empower local communities to proactively mitigate the risks posed by zoonotic diseases and safeguard the well-being of both humans and animals in the Ngorongoro district.

1.3 Objectives

1.3.1 General objective

To perform a metagenomic surveillance of pathogenic bacteria and viruses found in rodents, domestic dogs, and humans, while also evaluating the community's awareness of rodent-borne diseases, notably Rift Valley Fever (RVF) in Ngorongoro district, Tanzania.

1.3.2 Specific objectives

1. To explore the pathogenic and zoonotic bacterial species found in rodents, domestic dogs and humans of the Ngorongoro district in Tanzania
2. To discern pathogenic and zoonotic viruses present in rodents, domestic dogs, and humans inhabiting the Ngorongoro district of Tanzania
3. To evaluate the community's knowledge, attitudes, and practices (KAP) concerning rodent-borne diseases within the Ngorongoro district of Tanzania
4. To assess the knowledge, attitudes, and practices of the community regarding Rift Valley Fever (RVF) within the Ngorongoro district of Tanzania

1.4 Research Questions

1. What is the composition and diversity of bacteria and viruses in rodents, domestic dogs and humans from the Ngorongoro district?
2. Are there any potential zoonotic pathogens present in the study populations?
3. What is the level of community understanding regarding rodent-borne zoonoses, particularly Rift Valley fever in the Ngorongoro district?

1.5 Methodological and Conceptual Frameworks

The study was cross-sectional involved five villages (Orgosorok, Malambo, Engarasero, Pinyinyi and Sale) of the Ngorongoro district. The study villages were selected intentionally based on the availability of domestic animals including dogs and wild animals. A purposeful sampling method was used in the selection of households based on the willingness of individuals, and the availability of rodents, domestic dogs and other animals. The study used targeted sampling method, where 200 blood samples from humans, 230 blood samples from rodents, and 100 blood samples from domestic dogs (Plate 5) were collected. In each village, a deliberate sampling effort was undertaken, encompassing 40 households from which human blood samples were collected. Additionally, 46 rodents were meticulously trapped and sampled, while 20 blood samples were obtained from domestic dogs. The relatively limited number of dog samples is attributed to the inherent challenges associated with capturing these animals, as many of them roam freely and lack close bonds with their owners. Live trapping of rodents was conducted using Sherman(R) and wire cage traps baited with peanut butter mixed with maize bran (Mgode *et al*., 2014). Rodents were humanely killed using isoflurane in anesthetic chamber. The ventral surface of euthanized rodents was disinfected using 70% alcohol, and the blood was collected via the cardiac puncture method.

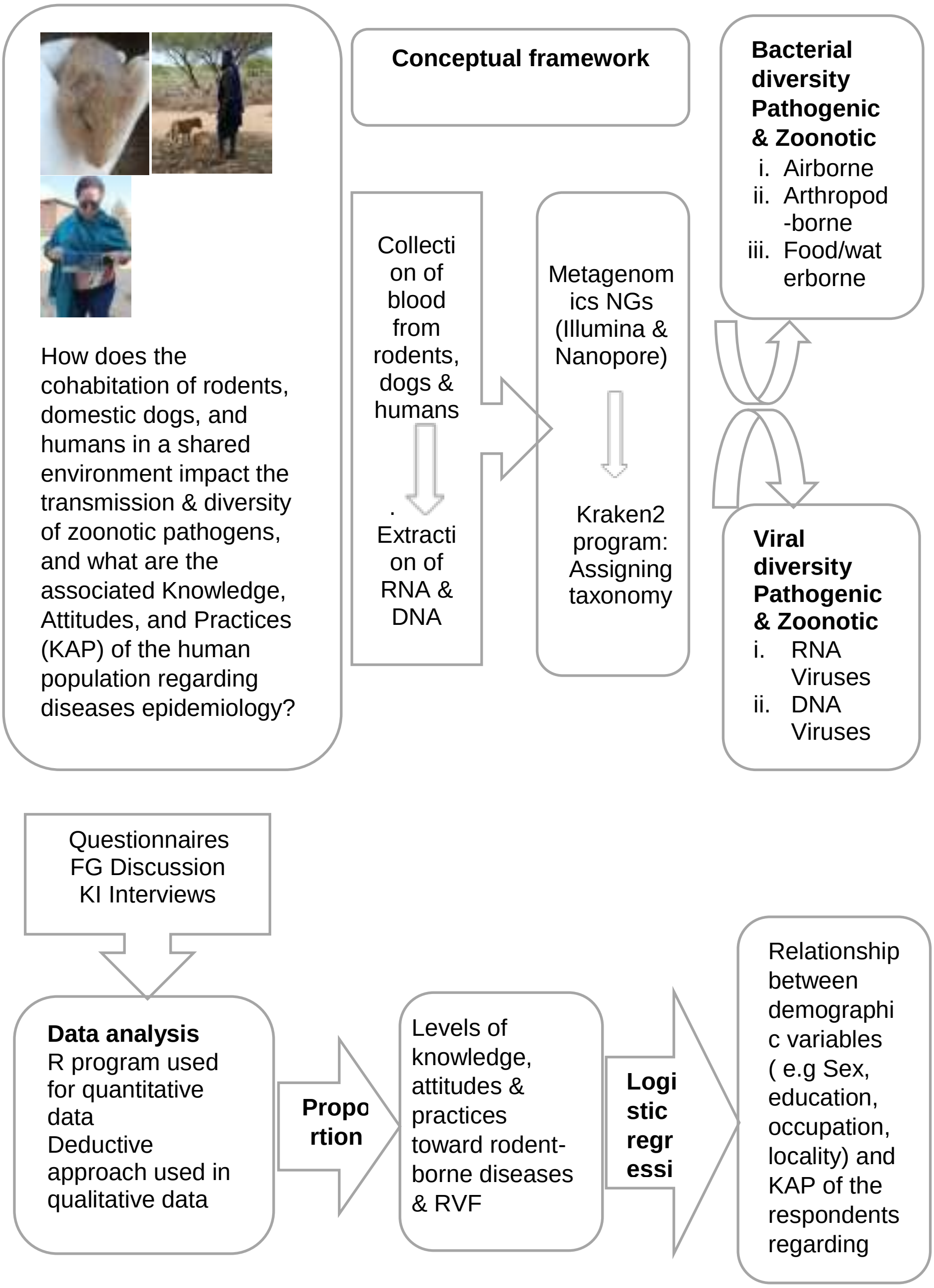
Before collecting blood samples from humans and dogs, the professional personnel ensured hand hygiene by thoroughly washing with soap and water or using an alcohol rub, paying careful attention to wrists and spaces between the fingers for at least 30 seconds (WHO, 2010). Following hand cleaning, they donned protective gloves to shield themselves from infections. The participants' skin was disinfected using 70% alcohol, starting at the needle-insertion site and moving outward in several expanding circles (WHO, 2010). Blood was then drawn from the cephalic veins, which are the most preferred veins for this procedure (Lima-Oliveira *et al*., 2015).

Metagenomics Next-generation sequencing Illumina (used MiSeq) and Nanopore (used MinION sequencer (Plate 6) technologies were applied. Nucleic acids were extracted using the QIAamp® RNA/DNA Blood Mini Kit following Qiagen's instructions. For MinION sequencing library preparation, the PCR-cDNA sequencing-barcoding kit (SQK-PCB109) was utilized. Meanwhile, sequencing libraries for the MiSeq platform were prepared using the Nextera™

XT DNA Library Prep Kit following the supplier's procedures. These methods ensured comprehensive and accurate genomic analysis, laying the foundation for robust molecular insights. To identify and classify bacteria and viruses in metagenomic data, the Kraken2 program version 2.1.2 was used (Wood *et al*., 2019) with database constructed from the National Center for Biotechnology Information (NCBI) reference sequence. This step ensured the precise identification of zoonotic bacteria and viruses present in the blood samples.

To gain a holistic understanding of the community on rodent-borne diseases and Rift Valley fever, a mixed-methods approach was employed. Questionnaires (Appendix 2) were administered to the communities, focus group discussions (Appendix 3) were held with various stakeholders, and key informant interviews (Appendix 4) (Abdi *et al*., 2015). The household sample size was estimated using the formula described by Chulaluk (2009); $n = \frac{Z^2 p(1-P)}{d^2}$ assuming a response distribution of 50% and a precision of 5%. Initially calculated at 384, accounting for a 5% markup for missing samples, the adjusted sample size for an infinite population became 403. Correcting for finite population yielded a final sample size of 392 households. However, only 352 households were reached due to various fieldwork challenges. Despite this, the sample achieved a statistical power of 0.9, indicating a scientifically acceptable level of significance. Additionally, twenty key informant interviews were conducted to collect insights into rodent-borne diseases and Rift Valley fever. Using a targeted sample technique, individuals were identified voluntarily by local collaborators. Participants included various community health workers, nurses, livestock officers, and local leaders. The interviews were also recorded using a phone recorder and the audio records were transcribed. Moreover, three focus group discussions (FGDs) were conducted, each comprising 12 respondents. Purposeful sampling targeted adults aged 18 years and above, who were confident communicators, permanent residents, and animal keepers. Facilitated by trained community health workers or livestock officers alongside the researcher, each FGD included two note takers. Discussions followed a semi-structured guide. In parallel with the above data collection methods, direct observation was conducted to assess rodents' in-habitation indications around human households (Plate 4). The observation provided the critical context to understand the ecological interaction between rodents, domestic animals and humans.

The collected data were inputted into Microsoft Excel 2010 and subsequently refined to eliminate invalid variables. Following this, the data were exported to R software version 4.1.0 (2021) for analysis. The qualitative data collected from Focus Group Discussions (FGD) and Key Informant interviews were manually summarized based on themes of discussions and interviews. The findings were presented in the text along with pertinent quotations.



How does the cohabitation of rodents, domestic dogs, and humans in a shared environment impact the transmission & diversity of zoonotic pathogens, and what are the associated Knowledge, Attitudes, and Practices (KAP) of the human population regarding diseases epidemiology?

R program used for quantitative data
Deductive approach used in qualitative data

Levels of knowledge, attitudes & practices toward rodent-borne diseases & RVF

Figure 1: Diagram showing conceptual framework of the current study done in the Ngorongoro district in Arusha, Tanzania

1.6 Study Limitations

The use of metagenomics next-generation sequencing technologies can have drawbacks, like unintentionally sequencing host genomes, which reduce the sensitivity in detecting low-abundance pathogens (Gu *et al*., 2019). Additionally, there is a lack of universally recognized standard program or software for metagenomic data analysis. Pooling blood samples in metagenomics have advantage and disadvantages in terms of statistical validity and generalization of findings. The advantage of pooling is that it reduces the number of samples to be processed, saving time and resources. Some disadvantages include loss of individual-level data, making it difficult to attribute specific findings to particular samples or individuals. Also pooling dilute the concentration of target sequences, reducing the sensitivity of detection, especially for low-abundance pathogens.

1.7 Brief Description on the Organization of the Thesis

This thesis conforms to the following format: Chapter two which comprised a published paper titled as “Exploring pathogenic and zoonotic bacterial species from wild rodents, domestic dogs, and humans of the Ngorongoro district in Tanzania using Metagenomic Next-generation sequencing”. Chapter three comprise a published paper titled as “Metagenomic screening of viruses in Arusha, north Tanzania, a one health perspective across wild rodents, domestic dogs and humans”. Chapter four contain a published paper titled as “Knowledge, Attitude and Preventive practices towards rodent-borne diseases in the Ngorongoro district”. Chapter five comprised a published paper titled as “Knowledge, Attitudes and Practices on Rift Valley fever among pastoral and agropastoral communities of Ngorongoro in the Rift Valley ecosystem, Tanzania”. Chapter six, consisting a broad discussion rooted in the findings. Chapter seven, which includes the general conclusion, contribution, and recommendations. At the end of this thesis the following appendices are attached i) various images ii) questionnaire tool, ii) guidelines for focus group discussion, iii) guideline for key informants’ interviews and, iv) form for collecting rodents inhabitation data.

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CHAPTER TWO

PAPER ONE

Exploring Pathogenic and Zoonotic Bacteria from Wild Rodents, Dogs, and Humans of the Ngorongoro District in Tanzania Using Metagenomics Next-Generation Sequencing

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zoonotic diseases

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Article

Exploring Pathogenic and Zoonotic Bacteria from Wild Rodents, Dogs, and Humans of the Ngorongoro District in Tanzania Using Metagenomics Next-Generation Sequencing

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Simple Summary: This study explored pathogenic and zoonotic bacteria in blood samples of wild rodents, domestic dogs, and humans in the Ngorongoro District in Tanzania. The district is inhabited by wildlife, domestic animals, and humans. Previous studies carried out on the livestock documented the existence of zoonotic bacterial diseases in the district. The role played by wild rodents and domestic dogs in the transmission of pathogenic and zoonotic bacteria was unknown. Therefore, the objective of this study was the detection and identification of pathogenic and zoonotic bacteria circulating among wild rodents, domestic dogs, and humans. The study concluded that a variety of zoonotic bacteria are present in wild rodents, domestic dogs, and humans sharing the same environment. Wild rodents carried numerous pathogenic and zoonotic bacteria compared to domestic dogs and humans. These results emphasize the importance of sustained investigations and unified health efforts to alleviate zoonotic disease transmission in this ecosystem.

Abstract: Globally, zoonoses have serious consequences due to their socioeconomic impacts. Ngorongoro District is home to a diverse range of wildlife and domestic animals, including rodents and dogs, which often coexist in close proximity with humans. The aim of the study was to identify the zoonotic bacteria present in wild rodents, domestic dogs, and humans using metagenomics next-generation sequencing technology. A cross-sectional study was conducted in 2022. This study used both Illumina and Oxford Nanopore sequencing technologies to identify bacteria in 530 blood samples collected from humans ($n = 200$), wild rodents ($n = 230$), and dogs ($n = 100$). Several zoonotic airborne/contagious bacteria, including *Mycobacterium* spp., *Mycoplasma* spp., *Bordetella* spp., and *Legionella* spp., were detected in wild rodents, domestic dogs, and humans. Arthropod-borne zoonotic bacteria such as *Bartonella* spp., *Borrelia* spp., and *Rickettsia* spp. were detected in all three hosts, while *Orientia* spp. was found in wild rodents and domestic dogs. *Yersinia pestis*, *Streptobacillus* spp. and *Anaplasma* spp. were found only in wild rodents. Other zoonotic bacteria found shared among wild rodents, domestic dogs, and humans are *Leptospira* spp., *Brucella* spp., and *Salmonella* spp. Generally, wild rodents had the highest prevalence of zoonotic bacterial species when compared to domestic dogs and humans. The detection of zoonotic bacteria in rodents, dogs, and humans supports the hypothesis that infections can spread between animals and humans sharing the same environment.

Keywords: integrated disease surveillance; arthropod-borne zoonoses; airborne zoonoses; rodent-borne diseases; domestic dogs; humans; Ngorongoro District; Tanzania

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1. Introduction

Zoonotic diseases have a significant socioeconomic impact globally [1]. Wildlife populations, by forming the reservoirs from which zoonotic agents can arise, have long been considered a link in the chain of pathogen emergence [2]. Approximately 75% of emerging infectious diseases affecting humans are zoonoses of animal origin [3]. Rodents are important reservoirs of numerous pathogenic and zoonotic bacteria including *Leptospira* [4], *Bartonella* [5], *Mycobacteria* [6], and *Campylobacter* [7]. It has been found that activities like crop cultivation and livestock farming bring wild rodents into close contact with humans [8]. Studies have documented that wild rodents are usually attracted to crops and stored grains, which can increase the interaction with humans and domestic dogs [8]. Additionally, improper waste disposal practices attract wild rodents to human settings [9]. These activities can facilitate the cross-species transmission of pathogenic bacteria [9]. In addition, many African countries, including Tanzania, are reporting a growing proportion of cases of fever of unknown origin [10,11]. Probably, some of these cases are associated with rodent-borne infections, which are under-reported in Tanzania. Therefore, surveillance studies are important in the determination and justification of the socioeconomic impact of rodent-borne diseases in Tanzania.

Keeping dogs in underdeveloped countries, such as Tanzania, can present unique challenges due to limited resources and infrastructure [12]. However, with proper planning and care, it is possible to maintain a healthy and safe environment for dogs. This study constitutes a comprehensive overview of the dog-keeping system in underdeveloped countries, focusing on Tanzania. In Tanzania, dog ownership is often influenced by cultural and traditional practices. Dogs are kept for various purposes such as security, herding, hunting, companionship, and even as status symbols [12]. There is limited awareness about responsible dog ownership including proper healthcare, a routine feeding system, and basic housing [12]. Tanzania's dogs cannot access conventional dog houses or dedicated shelters. Instead, they live in makeshift shelters, such as small huts, outdoor enclosures, and open spaces, or live as stray dogs [12]. Stray dogs can play a significant role in the transmission of bacterial diseases [13]. Due to a lack of proper veterinary care, stray dogs are more susceptible to infections, and their scavenging and roaming behavior can contribute to the spread of bacterial pathogens to humans through direct contact or a contaminated environment. Some bacterial diseases commonly associated with stray dogs are Leptospirosis [13], Salmonellosis, Campylobacteriosis, Pasteurellosis, and a strain of *Staphylococcus aureus* bacteria that has developed resistance to several antibiotics [14]. Despite the large population of domesticated dogs in the Ngorongoro District, no study has been conducted to evaluate the health status and pathogenic bacteria of dogs.

Previous studies conducted in the Ngorongoro District documented the occurrences of bacterial diseases in livestock, including Anthrax [15], Bovine Tuberculosis [16], Leptospirosis [17], and Brucellosis [18]. On the human side, the studies documented seroprevalence of *Brucella* infection in pregnant women receiving antenatal care [19] and the genetic diversity of *Mycobacterium tuberculosis* from TB patients attending health facilities in the Serengeti ecosystem [20] bordering the Ngorongoro District. The above-mentioned studies were performed around human–livestock–wildlife interfaces. It has been found that areas of interfaces generate unique hotspots of numerous infectious diseases including bacterial infections [21].

Ngorongoro District in Tanzania is home to a diverse range of wildlife and domestic animals, including rodents and dogs, which often coexist in close proximity with humans [22]. While previous studies have examined the transmission of pathogens in livestock and hospital-based research in humans within the district, the specific role played by rodents and dogs in the transmission of pathogens remains largely unknown. This research gap highlights the need for a comprehensive investigation into the contribution of rodents and dogs in pathogen transmission in the Ngorongoro District, allowing for a more comprehensive understanding of disease dynamics and potential risks to public health.

High throughput methods, such as metagenomics, can analyze multiple genomes of bacterial species [23]. This allows the identification of bacteria genomes directly from samples and can reveal information related to the diversity of microbes that circulate among different hosts in the communities [24,25]. The main objective of the study was to identify pathogenic and zoonotic bacteria present in wild rodents, domesticated dogs, and humans of the Ngorongoro District by using metagenomics next-generation sequencing techniques.

2. Materials and Methods

2.1. Description of the Study Area

This study was conducted in the Ngorongoro District (Figure 1). The district was selected based on areas of the interface of wild animals, domestic animals, and humans, as well as the previous seroprevalence studies of bacterial infection in livestock. Ngorongoro District is located in Arusha Region, northern Tanzania. It is bordered by the Manyara region to the West, the Karatu district to the south, and the Monduli district to the east. It has an area of 14,036 square kilometers, is located between latitudes 30.30′s and longitudes 35.42′, and it is between 1009 and 3645 m above sea level [26]. The district has a population of 174,278 as of the 2012 Tanzania National Census [27]. Ngorongoro, Loliondo, and Sale are the 3 administrative divisions of the district, together with 28 wards and 65 villages. The district experiences tropical weather with moderate temperatures and an average rainfall of 800 to 1000 mm. The predominant vegetation in the study area is grass and bushes of several acacia species, as well as open, dense forests.

2.2. Study Design and Sampling Procedures

A cross-sectional study was conducted in the Ngorongoro District in 2022 to explore bacteria of public health importance found in wild rodents, domestic dogs, and humans. The study population was made up of all the households in the selected villages, and the sample frame was a list of households in each village.

The study villages were selected intentionally based on the availability of domesticated animals, including dogs and wild animals, and accessible areas. Five villages (Orgosorok, Malambo, Sale, Engarasero, and Pinyinyi) were included in this study. A purposeful sampling method was used in the selection of households based on the willingness of individuals and the availability of wild rodents, domestic dogs, and other animals.

The selection of participants was based on voluntary willingness and adult humans of 18 years and older. Adults were selected because they have a longer history of exposure to various environments and animals, which can provide more comprehensive insights into the transmission of zoonotic pathogens. Also, dogs of 6 months and above were selected for the study because, at this age, dogs do not have maternal antibodies which could prevent infections. Before starting the sampling of humans and domestic dogs and the trapping of wild rodents, written consent from the head of the household was sought.

2.3. Trapping of Rodents

Live rodents were captured using Sherman LFA live traps (HB Sherman Traps, Inc., Tallahassee, FL, USA) and wire cage traps baited with peanut butter mixed with maize bran and sardines [4]. Trapping was carried out in specifically defined places such as areas surrounding livestock farms, fallow land around houses, as well as areas with green vegetation and marshes near homes. In each study village, 30 to 50 houses were selected to set traps indoors and in their surroundings. Based on the size of the household, 2 to 4 modified wire cage traps were placed in each house for the purpose of trapping indoor rodents. Depending on rodents' activities, 2 to 6 Sherman traps were set for peri domestic purposes. In each village, a total of 30 modified wire cage traps and 70 Sherman traps were used indoors and in the surroundings, respectively. For the remaining habitats (crop fields and grass-covered vegetation), the maximum number of Sherman traps set was 30–70 depending on the size of the selected habitat; thus, a total of 100 traps were used in each village. All traps were baited and set for 5 to 7 days in each village. Traps were set at 5 pm and checked in the morning at 8 am every day.

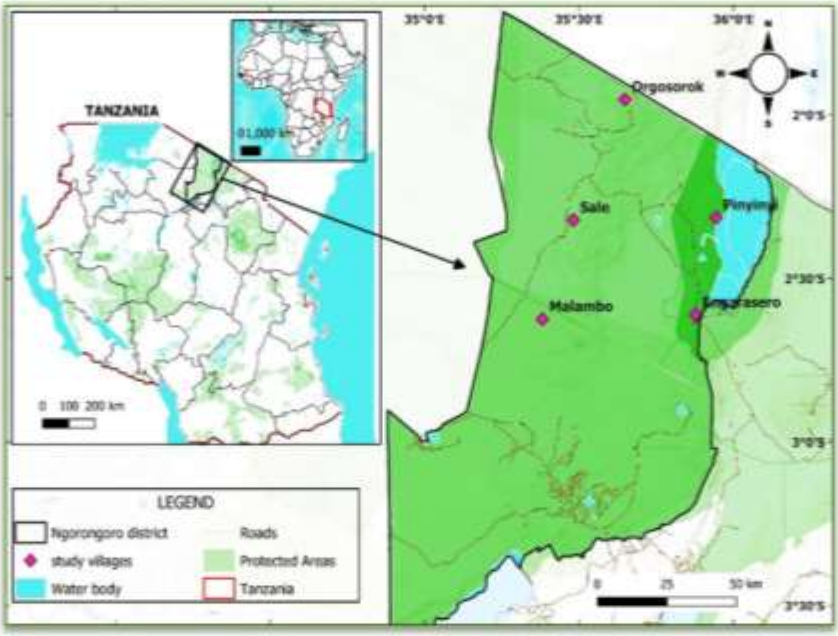

Figure 1. The map of Tanzania and the Ngorongoro District showing the study villages. The map was developed using QGIS software version 3.26.1 and shapefiles from DIVA-GIS and The Humanitarian Data Exchange (HDX), freely accessible at https://www.diva-gis.org/datadown (accessed on 3 July 2023) and https://data.humdata.org/dataset/cod-ab-tza (accessed on 3 July 2023), respectively.

2.4. Collection and Handling of Samples (from Wild Rodents)

Trapped rodents were anaesthetized and humanely killed using isoflurane (volatile inhalation agent). The rodents were placed into an anesthetic chamber with cotton wool soaked in isoflurane, as described in the previous study. The animal was removed from the chamber after cessation of respiration and heartbeats, and morphometric characteristics of rodents were recorded prior to dissection. The ventral surfaces of the rodents were disinfected using 70% methylated spirit to kill external germs. Using proper protecting gears to safeguard the health of the researcher, almost 1 ml of blood samples was collected from the rodent's ventral surface using hypodermic needles and syringes. The cardiac puncture technique was used in blood sample collection [10]. The blood sample was mixed with 2 mL of DNA/RNA shield reagents in cryogenic tubes, labelled, and kept in liquid nitrogen. Samples were transported to the Sokoine University of Agriculture in the Department of Veterinary Microbiology, Parasitology and Biotechnology Laboratory for detailed analysis. DNA/RNA shield reagent was used to maintain the integrity of the nucleic acids due to inhibition of DNase and RNase activities.

2.5. Collection of Blood Samples from Humans and Domestic Dogs

Before taking a blood sample from humans and dogs, the human laboratory scientist and veterinary officer thoroughly washed their hands with soap and water, and alcohol rub, for at least 30 s [28]. To prevent infections, the personnel put on safety gloves after cleansing his/her hands. The skin of the individual and domestic dog was disinfected using 70% alcohol, beginning at the needle-insertion site and making several outwardly expanding circles [28]. The cephalic vein was used for blood collection [29]. A blood sample of 1 mL was taken using a 21-gauge needle. To avoid contaminations that could lead to infections, the needle entry site was wrapped with gauze and sellotape immediately after sample collection. The obtained blood samples were mixed with 2 mL of DNA/RNA shield reagents in cryogenic tubes. All blood tubes were labelled, transported to SUA in liquid nitrogen, and stored at −80 °C until further analysis.

2.6. Preparation of Pools of Blood Samples

A total of 200 blood samples from human subjects were collected and then pooled into 22 pools and grouped by sexes [30]. Likewise, 230 blood samples were collected from rodents and then pooled into 16 pools [6], as shown in Table 1 below. Similarly, a total of 100 blood samples were collected from domestic dogs and then grouped into 10 pools based on their sexes [31] (Table 1). The study involved 5 villages and, in each village, 46 samples of rodents, 40 samples of humans, and 20 samples of domestic dogs were collected.

Table 1. Summary of the sample's description, sample size, and procedures for pooling of blood samples.

Sample Type	Sex	Number of Samples Studied	Number of Pools	Number of Samples (s) per Pool	Pooling Volume (µL) per Sample	Total Volume (mL) per Pool
Human	Female	130	13	10	100	1
	Male	70	9	7–8	100	0.7–0.8
	Total	200	22			
Wild Rodents	Total	230	16	14–15	80	1.12–1.2
Domestic Dogs	Female	57	5	11–12	100	1.1–1.2
	Male	43	5	8–10	100	0.8–1
	Total	100	10			

2.7. Nucleic Acids Extraction, Libraries Preparation, and Sequencing

The QIAamp® RNA blood Mini Kit and QIAamp® DNA blood Mini Kit were used for the purification of RNA and DNA, respectively, as per the manufacturer's instructions (Qiagen, Valencia, CA, USA). The extracted RNA genomes were converted into complementary DNA (cDNA) using Omniscript RT Kit based on the supplier's protocol (Qiagen, Valencia, CA, USA).

Two methods of next-generation sequencing (Illumina and Nanopore) were employed in this work: the MiSeq sequencing platform (Illumina) and MinION sequencing technology (Nanopore). The MinION sequencing libraries were generated by using the PCR-cDNA sequencing-barcoding kit (SQK-PCB109-Oxford Nanopore Technologies) following the manufacturer's protocol.

The Illumina Nextera™ XT DNA Library Prep Kit (Illumina, San Diego, CA, USA) was used to prepare sequencing libraries for the MiSeq platform following the manufacturer's protocol. The quality of the libraries generated was assessed by using a qubit high-sensitivity quantification assay, following the manufacturer's protocol (Thermo Fisher Scientific technology). Subsequently, the nucleic acids were pooled in equimolar amounts, and the resulting libraries were sequenced on a single lane (paired-end, 151 bp read-length) on an Illumina MiSeq$^{\text{TM}}$ machine (Illumina, San Diego, CA, USA).

2.8. Bioinformatics Analysis

The sequencing data obtained from MinION were processed using ONT Guppy version 6.4.2 and the 9.4.1 450 bps SUP model was used for base calling. The reads that were demultiplexed were identified by ONT Guppy barcoder version 6.4.2. Unclassified reads from each run were saved as distinct pseudo-samples. FastQ Screen version 0.14.1 with GRCh38 and UniVec Core was used to screen reads for human and vector contamination [32]. The reads underwent quality filtering and trimming using fastp version 0.20.1 using settings -5 -3 -M 8 -q 6 -e 10 -l 64 [33]. Fundamental quality control measurements (read counts, base counts, and quality scores) were obtained using fastq-stats from fastq-utils 1.3.0 [33].

MiSeq reads were base called and demultiplexed with Illumina BCL Convert 3.9.3. Reads that were not classified were reserved as a separate pseudo-sample. The FastQ Screen version 0.14.1 was used with GRCh38 and UniVecCore to screen the reads for any contamination from human or vector sources [32]. Reads were trimmed with fastp 0.20.1 using default settings plus front and tail trimming (-5 -3). Important quality control measurements were acquired through the utilization of the fastq-stats function from fastq-utils version 1.3.0 [33].

The process of assigning taxonomy was accomplished by employing Kraken2 version 2.1.2 [34] using the Kraken2 standard databases plus fungi, constructed from NCBI Reference Sequence data. The analyses were carried out on 2 separate occasions. The first analysis categorized the quality-filtered and trimmed MiSeq and MinION reads using Kraken2's paired-end mode. The second analysis classified the merged MiSeq and MinION reads for each sample, which was quality-filtered and trimmed, using Kraken2's default mode.

3. Results

3.1. Bacterial Families and Genera Identified

This study detected 24 families of potentially pathogenic and zoonotic bacteria in wild rodents, domestic dogs, and humans (Figure 2).

3.2. Airborne, Contagious, and Arthropod-Borne Zoonotic Bacteria

The study detected five and nine genera of airborne and arthropod-borne zoonotic bacteria species, respectively (Table 2). Among the five genera of airborne bacteria species, *Mycobacterium* species were detected in high proportion in wild rodents (56.25%) (Table 2). Among the arthropod-borne bacteria detected, *Bertonella* species were found in high proportion in wild rodents (68.75%) compared to domestic dogs and humans. Among the nine arthropod-borne bacteria, three genera (*Borrelia, Bartonella*, and *Rickettsia*) were found in humans, wild rodents, and domestic dogs (Table 2). Generally, wild rodents have the highest proportion of zoonotic bacterial species, followed by domestic dogs, and then humans (Table 2). Various airborne and arthropod bacterial species identified in wild rodents, domestic dogs, and humans are presented in Tables 3 and 4, respectively.

3.3. Pathogenic and Zoonotic Bacteria Detected in Humans, Wild Rodents, and Domestic Dogs

The results have shown that ten genera of zoonotic bacteria species were identified in humans and domestic dogs while nine genera were found in wild rodents (Tables 5 and 6). Some of the pathogenic and zoonotic bacteria species are presented in Table 6.

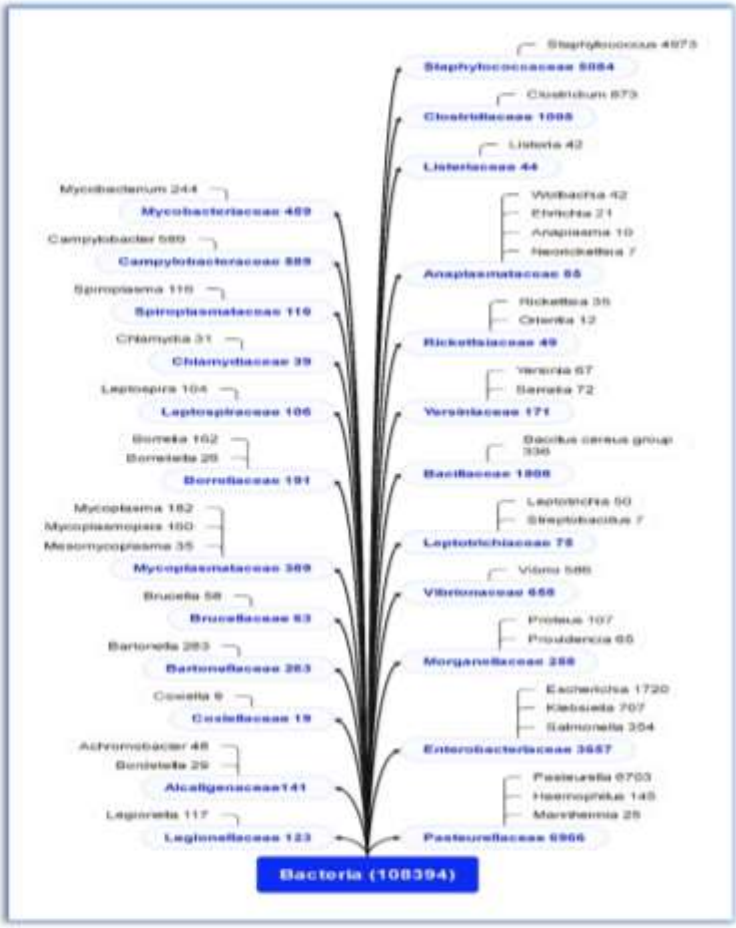

Figure 2. Tree diagram indicating the names of the bacterial families as well as genera and their overall reads (abundances).

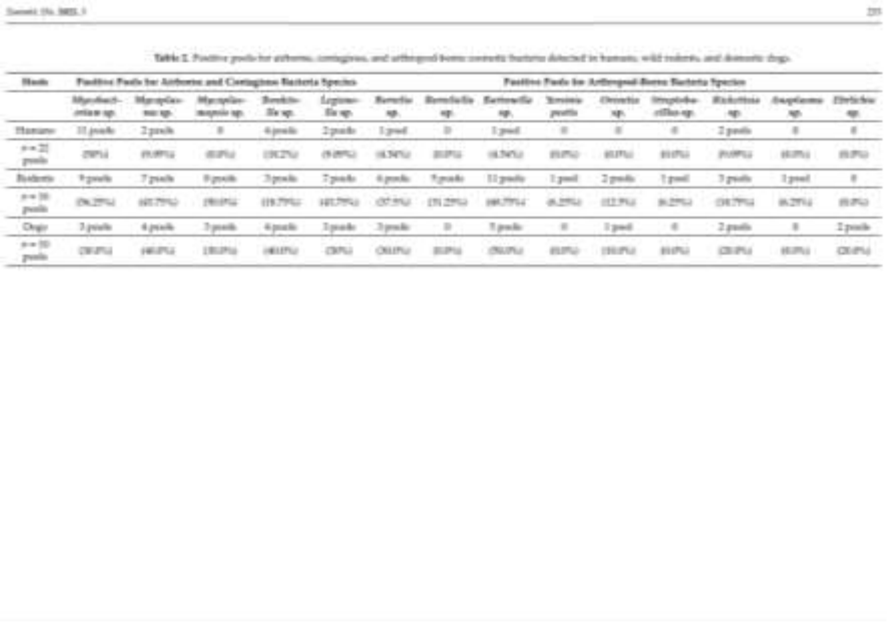

Table 2. Positive pools for airborne, contagious, and arthropod-borne zoonotic bacteria detected in humans, wild rodents, and domestic dogs.

Hosts	Positive Pools for Airborne and Contagious Bacteria Species					Positive Pools for Arthropod-Borne Bacteria Species								
	Mycobacterium sp.	*Mycoplasma* sp.	*Mycoplasmopsis* sp.	*Bordetella* sp.	*Legionella* sp.	*Borrelia* sp.	*Borreliella* sp.	*Bartonella* sp.	*Yersinia pestis*	*Orientia* sp.	*Streptobacillus* sp.	*Rickettsia* sp.	*Anaplasma* sp.	*Ehrlichia* sp.
Humans	11 pools	2 pools	0	4 pools	2 pools	1 pool	0	1 pool	0	0	0	2 pools	0	0
n = 22 pools	(50%)	(9.09%)	(0.0%)	(18.2%)	(9.09%)	(4.54%)	(0.0%)	(4.54%)	(0.0%)	(0.0%)	(0.0%)	(9.09%)	(0.0%)	(0.0%)
Rodents	9 pools	7 pools	8 pools	3 pools	7 pools	6 pools	5 pools	11 pools	1 pool	2 pools	1 pool	3 pools	1 pool	0
n = 16 pools	(56.25%)	(43.75%)	(50.0%)	(18.75%)	(43.75%)	(37.5%)	(31.25%)	(68.75%)	(6.25%)	(12.5%)	(6.25%)	(18.75%)	(6.25%)	(0.0%)
Dogs	3 pools	4 pools	3 pools	4 pools	3 pools	3 pools	0	5 pools	0	1 pool	0	2 pools	0	2 pools
n = 10 pools	(30.0%)	(40.0%)	(30.0%)	(40.0%)	(30%)	(30.0%)	(0.0%)	(50.0%)	(0.0%)	(10.0%)	(0.0%)	(20.0%)	(0.0%)	(20.0%)

Table 3. Airborne and contagious zoonotic bacteria species found in humans, rodents, and domestic dogs.

Na	Genus	Isolated Species	Host
1	*Mycobacterium avium* complex	*M. avium* subsp. *Paratuberculosis*, *M. intracellulare* subsp. *chimaera*	Rodents, humans, and dogs
		M. avium subsp. *Hominissuis*, *M. kansasii*, *M. koreense*, *M. diernhoferi*, *M. paragordonae*, and *M. mantenii*	Rodents
	Mycobacterium tuberculosis complex	*M. canettii* and *M. tuberculosis*	Rodents, dogs, and humans
		M. grossiae, *M. colombiense*, *M. mantenii*, *M. virginiense*, *M. basiliense*, *M. paragordonae*, *M. diernhoferi*, *M. marseillense*, and *M. senriense*	Rodents
	Mycobacterium simiae complex	*M. simiae*, and *M. rufum*	Rodents and humans
		M. kubicae, *M. lentiflavum*, and *M. saskatchewanense*	Rodents
	Mycobacterium ulcerans group	*M. ulcerans* subsp. *Shinshuense*. *M. spongiae* *M. paraseoulense*, *M. dioxanotrophicus*, *M. shinjukuense*, *M. extraniense*, *M. kansasii*, *M. holsaticum*, *M. leprae*, and *M. goodii*	Rodents
		M. seoulense, *M. lacus*, and *M. cookii*	Rodents and humans
		M. xenopi	Rodents, dogs, and humans
		M. virginiense and *M. heidelbergense*	Humans
2	*Mycoplasma*	*M. miroungigenitalium*, *M. fastidiosum*, *M. hyopneumoniae* *M. putrefaciens*, *M. haemofelis*, *M. wenyonii*, *M. parvum*, and *M. iguanae*	Rodents
		M. crocodyli, *M. pneumoniae*, *M. suis*, and *M. tauri*	Rodents and humans
		M. mycoides subsp. *Capri* and *M. haemocanis*	Dogs
3	*Mycoplasmopsis*	*M. arginini*	Rodents and dogs
		M. bovirhinis, *M. gallopavonis*, *M. agalactiae* *M. synoviae*, *M. felis*, *M. equigenitalium*, and *M. meleagridis*	Rodents
		M. glycophila, *M. canis*, *M. bovis*, and *M. gallinacea*	Dogs
4	*Bordetella*	*B. bronchiseptica*	Rodents, dogs, and humans
		B. bronchialis, *B. parapertussis*, *B. avium*, and *B. pseudohinzii*	Dogs
		B. genomosp. 6, *B. flabilis*, and *B. trematum*	Dogs and humans
		B. hinzii	Humans
5	*Legionella*	*L. pneumophila* and *L. sainthelensi*	Rodent and humans
		L. antarctica and *L. lytica*	Dogs

Table 4. Arthropod-borne zoonotic bacteria species found in humans, wild rodents, and domestic dogs.

Na	Genera	Species	Hosts
1	*Bartonella* (21 species)	*B. krasnovii* and *B. tribocorum*	Rodents and dogs
		B. taylorii	Rodents and humans
		B. quintana, *B. ancashensis*, *B. henselae*, *B. machadoae*, *B. clarridgeiae*, *B. vinsonii*, *B. bovis*, *B. birtlesii*, *B. elizabethae*, *B. taylorii*, *B. alsatica*, *B. bacilliformis*, *B. harrusi*, *B. grahamii*, *B. australis*, *B. schoenbuchensis*, *B. kosoyi*, and *B. apihabitans*	Rodents

Table 4. *Cont.*

Na	Genera	Species	Hosts
2	*Borrelia* (6 species)	*Borrelia miyamotoi*,	Rodents, humans, and dogs
		B. turcica, *B. parkeri*, *B. anserina* *B. coriaceae*, and *B. crocidurae*	Rodents
3	*Borreliella* (5 species)	*B. burgdorferi*, *B. afzelii*, *B. bissettiae* *B. valaisiana*, and *B. mayonii*	Rodents
4	*Streptobacillus*	*S. moniliformis*	Rodents and dogs
5	*Rickettsia*	*R. rhipicephali*	Rodents and humans
		R. typhi and *R. prowazekii*	Rodents and dogs
		R. tillamookensis, *R. asiatica*, *R. slovaca* *R. australis*, and *R. bellii*	Rodents
6	*Spiroplasma*	*S. corruscae*	Rodents, dogs, and humans
		S. cantharicola	Humans
7	*Mycoplasma*	*M. suis*	Humans
8	*Anaplasma*	*A. platys*, *A. phagocytophilum*, and *A. marginale*	Rodents
9	*Ehrlichia*	*E. canis* and *E. muris*	Dogs
10	*Yersinia*	*Y. pestis* subsp. *Pestis*	Rodents
11	*Orientia*	*O. tsutsugamushi*	Rodents

Table 5. Positive pools for pathogenic and zoonotic bacteria detected in humans, wild rodents, and domestic dogs.

Hosts	**Bacteria Genera**									
	Leptospira	***Brucella***	***Bacillus***	***Vibrio***	***Listeria***	***Campylobacter***	***Salmonella***	***Clostridium***	***Pasteurella***	***Chlamydia***
Humans	4 pools	2 pools	7 pools	6 pools	1 pool	3 pools	5 pools	6 pools	9 pools	2 pools
n = 22 pools	(18.18%)	(9.09%)	(31.81%)	(27.27%)	(4.54%)	(13.63%)	(22.72%)	(27.27%)	(40.9%)	(9.09%)
Wild rodents	6 pools	7 pools	3 pools	2 pools	8 pools	4 pools	7 pools	9 pools	11 pools	0
n = 16 pools	(37.5%)	(43.75%)	(18.75%)	(12.5%)	(50%)	(25%)	(43.75%)	(56.25%)	(68.75%)	(0.0%)
Domestic dogs	3 pools	2 pools	6 pools	5 pools	4 pools	4 pools	3 pools	4 pools	4 pools	3 pools
n = 10 pools	(30%)	(20%)	(60%)	(50%)	(40%)	(40%)	(30%)	(40%)	(40%)	(30%)

Table 6. Pathogenic and zoonotic bacteria species detected in humans, wild rodents, and domestic dogs that can spread via contaminated fomites, food, and water.

Bacterial Communities			
Na	**Genus**	**Species**	**Host**
1	*Leptospira*	*L. santarosai*, *L. kmetyi*, and *L. weilii*	Rodents and dogs
		L. interrogans	Rodents, dogs, and humans
		L. kobayashii	Rodents and humans
		L. kirschneri, *L. mayottensis*, *L. borgpetersenii*, *L. tipperaryensis*, and *L. noguchii*	Rodents

Table 6. *Cont.*

		Bacterial Communities	
Na	**Genus**	**Species**	**Host**
2	*Brucella*	*B. anthropi*	Rodents and humans
		B. suis	Rodents and dogs
		B. pseudogrignonensis	Dogs
3	*Bacillus*	*B. cereus*	Humans, rodents, and dogs
		B. cytotoxicus and	Rodents and dogs
4	*Vibrio*	*V. anguillarum*	Humans, rodents, and dogs
		V. vulnificus	Rodents
5	*Listeria*	*L. monocytogenes*	Rodents and humans
6	*Campylobacter*	*C. jejuni*	Rodents and dogs
7	*Salmonella*	*S. enterica subsp. Enterica*	Humans, rodents, and dogs
8	*Clostridium*	*C. botulinum*	Rodents, dogs, and humans
9	*Pasteurella*	*P. multocida subsp. multocida*	Humans, rodents, and dogs
10	*Chlamydia*	*C. crocodile* and *C. abortus*	Humans
		C. gallinacean, C. trachomatis, C. pecorum, and *C. felis. C. avium*	Dogs

4. Discussion

This study focused on the identification of various pathogenic and zoonotic bacteria circulating among wild rodents, domestic dogs, and humans in the Ngorongoro District. Numerous zoonotic bacteria that pose a threat to public and animal health were found, whereby some species were detected in either one, two, or three hosts involved in the study. This showed the possibility of cross-species transmission of different bacterial species in the study area.

Several airborne and contagious zoonotic bacteria species were found in wild rodents, domestic dogs, and humans in this study. Most of them are transmitted from one host to another through inhalation of infected aerosol droplets or through direct contact with infected animals or contaminated surfaces [6,35,36]. The current study found several species of *Mycobacteria* and *Bordetella* in domestic dogs, wild rodents, and humans. This indicated that the interaction between humans, wild rodents, and domestic dogs increases the chance of cross-transmission of pathogens among different host species. The importance of the *Mycobacterium* species for public health is based on its capacity to cause tuberculosis, leprosy, and ulcerations in humans [35]. A previous study carried out in Morogoro, Tanzania, also reported findings on the occurrence of nontuberculous *Mycobacteria* species in wild rodents and *Crocidura* species [6]. Most human cases caused by *Bordetella* spp. were documented in immunocompromised patients and presented in a variety of ways, from moderate coughing and tracheobronchitis to sepsis and death [36]. These results necessitate unified health surveillance of pathogens among communities in order to safeguard public health.

Bordetella species found in this study were previously linked with pulmonary infection in humans. *B. bronchiseptica* and *B. pertussis* are the causative agents of pneumonia and whooping cough in humans [37]. This study revealed the occurrence of *B. bronchiseptica* infection in wild rodents, domestic dogs, and humans and *B. genomosp, B. flabilis*, and *B. trematum* infection in both domestic dogs and humans. Contrary to the case in rodents and humans, most *Bordetella* species were detected in domestic dogs. This suggests that domestic dogs can play a role in the transmission of *Bordetella* spp. infection to humans and other domestic and wild mammals. The occurrences of *Bordetella* species in more than one host justify the possibility of cross-species transmission of these pathogens in the study area.

Additionally, some of the airborne and arthropod-borne *Mycoplasma* species were also identified in wild rodents, domestic dogs, and humans. *M. pneumoniae*, *M. crocodyli*, *M. suis*, and *M. tauri* species were all found in both humans and wild rodents in this study. In contrast to humans and dogs, the majority of *Mycoplasma* species were identified in wild rodents. And most of the species discovered in this work have been isolated in other animals, including cattle (*M. tauri*), goats (*M. mycoides*), pigs (*M. suis*, *M. hyopneumoniae*), and crocodiles (*M. crocodyli*) [38–41]. Some of the *Mycoplasma* species identified in this study have been reported to cause numerous animal fatalities and huge economic losses globally. For instance, the swine sector suffers financial losses because of hemolytic anemia and swine enzootic pneumonia caused by *M. suis* and *M. hyopneumoniae*, respectively [39,40]. Moreover, *M. mycoides* subsp. *Capri* caused a severe mortality outbreak of respiratory mycoplasmosis in goats in Mexico [42]. In general, these findings showed the possibility of the occurrence of inter-species cross-transmission of *Mycoplasma* in the study area. Moreover, this study recognized *Legionella* species that cause fatal pneumonia (Legionnaires' disease) in humans after inhalation of airborne droplets containing viable bacteria (Cunha et al., 2016). The aforementioned *Legionella* species were found in wild rodents, domestic dogs, and humans. Perhaps the infections were acquired from contaminated natural water or aquatic environments. It was reported that water is the major natural reservoir for *Legionella* species [43].

This study presents the first report of *Borrelia miyamotoi* infection among wild rodents, domestic dogs, and humans in Tanzania. A high proportion of infection was found in rodents, followed by domestic dogs and, lastly, humans. This indicates that wild rodents are the main reservoirs of *Borrelia* spp. in Tanzania. Most of the identified arthropod-borne bacteria have been widely reported to cause infections in humans and animals [44–48]. For example, *Borrelia* spp. causes Lyme borreliosis in humans [45,46]. These pathogens use both rodents and ticks as reservoirs and vectors, respectively [46]. *Bartonella* species linked to human illnesses were found, including *B. tribocorum*, *B. elizabethae*, *B. grahamii*, and *B. taylorii*. Bilateral retinal branch occlusions or neuro retinitis have been linked to *Bartonella grahamii* [49]. *Bartonella elizabethae* was isolated in individuals with endocarditis illness [49]. *Bartonella tribocorum* was revealed in patients with fever in Thailand [50]. The discovery of these zoonotic *Bartonella* species in wild rodents, domestic dogs, and humans calls for increased awareness of these infections among healthcare professionals, particularly in cases of unexplained febrile illness.

Yersinia pestis, a causative agent of plague (a zoonotic disease which has stable foci throughout Africa, America, and Eurasia) was detected in wild rodents [47]. The main mode of transmission from one host to another is by infected flea bites that result in painful, swollen lymph nodes known as buboes and septicemia [47]. A bacterium *Orientia tsutsugamushi* was found in wild rodents in this study. This causes scrub typhus (tsutsugamushi sickness), an acute infectious disease in humans [51,52]. *Streptobacillus moniliformis* responsible for rat-bite fever was also found in wild rodents and domestic dogs in this study. Based on these findings, education on rodent management is important in order to protect the community from zoonotic bacteria.

Furthermore, the Rickettsia typhus group (TG) and Rickettsia spotted fever group (SFG) were identified in wild rodents, domestic dogs, and humans in this study. Eight species of *Rickettsia* were detected, including *R. australis* (a causative agent of Queensland tick typhus), *R. typhii* (a causative agent of murine typhus), and *R. prowazekii* (a causative agent of epidemic typhus) [53]. The rickettsia species discovered in this work had previously been found in ticks, dogs, and humans in Brazil [54,55], Wisconsin [44], and Australia [53]. The genus *Anaplasma* comprises different zoonotic species which cause diseases in animals and humans [56]. *Anaplasma marginale* cause bovine anaplasmosis in tropical and subtropical regions and other areas globally [56]. *Anaplasma phagocytophilum* cause animal and human granulocytic anaplasmosis [48]. *Anaplasma platys* have been reported to cause febrile illness associated with headache and fever in humans in Venezuela [57]. These pathogens parasitize red blood cells in susceptible hosts and are transmitted by ticks and biting insects [56]. This study documents, for the first time, the occurrence of these three *Anaplasma* species in wild rodents in Tanzania. Additionally, tick-borne bacteria, such as *Ehrlichia canis* and *Ehrlichia muris*, which cause life-threatening diseases, including canine ehrlichiosis in dogs, were observed in this study. These two species of *Ehrlichia* are zoonotic bacteria and were mainly detected in domestic dogs [44,58].

This study detected zoonotic *Leptospira* spp. shared among wild rodents, domestic dogs, and humans. *Leptospira interrogans* was found in all three hosts, while *Leptospira kobayashii* was detected in domestic dogs and humans and *Leptospira santarosai*, *Leptospira kmetyi*, and *Leptospira weilii* were identified in wild rodents and domestic dogs. The majority of *Leptospira* species were identified in domestic dogs and wild rodents compared to humans. This supports the possibility that dogs and rodents in the study area are the sources of human leptospirosis. Humans acquire the infection through direct contact with the urine of infected animals or a contaminated environment [59]. Human infection is associated with various symptoms ranging from asymptomatic fever to complex illnesses with significant morbidity and mortality rate, like Weil's disease [13]. The current study revealed the occurrences of *Brucella* spp. among wild rodents, domestic dogs, and humans. *B. anthropic*, an emerging, opportunistic, nosocomial human pathogen [60,61], was found in humans and wild rodents. Moreover, the detection of *B. suis* in wild rodents and domestic dogs indicates the possibility of occurrences of cross-species transmission in the study area. Dogs that had been pig-hunting and those fed raw, feral pig meat were both confirmed to contract Brucellosis from *B. sui* [62]. *Brucella* is mostly transmitted through contact with contaminated fetal tissues, body fluids, and consumption of raw milk/blood [61].

Additionally, our study identified a number of zoonotic bacteria linked to gastrointestinal diseases that cause diarrhea and human mortality. These included *Campylobacter jejuni* (rodents and dogs), *Salmonella enterica* subsp. *Enterica* (rodents, dogs, and humans), *Listeria monocytogenes* (rodents and humans), and *Clostridium botulinum* (rodents, dogs, and humans). The main route of transmission of these pathogens is through contaminated food and water and via direct contact with animals or contaminated environments [63]. It has been found that *Campylobacter jejuni* and *Salmonella* are among the leading causes of foodborne bacterial illness worldwide [63]. *Listeria monocytogenes* is a causative agent of listeriosis, a foodborne illness with a mortality rate of 20% to 30% in immunocompromised individuals [64]. Worldwide, the incidence of foodborne botulism continues to increase more than the incidence of any other type of botulism [65]. Therefore, the identification of these pathogenic bacterial species in wild rodents supports the probability of rodents being the source of transmitting infections to humans and domestic dogs.

Livestock such as ruminants, pigs, and poultry carry and shed bacteria such as *Salmonella* spp., *Campylobacter* spp., *Vibrio* spp., and *E. coli* which can contaminate the environment, including feed and water sources [66]. Irrigated vegetable gardens provide a suitable environment for microbial growth. In general, bacteria thrive in moist environments, and regular irrigation can provide the necessary moisture for their growth [66]. Rodents that have access to contaminated environments can easily become carriers of bacteria and transmit them to other areas, including human environments.

Authors' Reflection Based on the Finding

The presence of genetic material in a host does not mean that the host will transmit the disease immediately. Some pathogens can be present in a host without causing disease or being transmissible to others. Other factors, such as the host's immune response and the pathogen's ability to replicate and spread, also play a role in determining whether a pathogen can be transmitted to other hosts.

5. Conclusions

The finding of genetic material of several zoonotic bacteria in rodents, dogs, and humans sharing the same environment allows the hypothesis that infections may spread between species. Zoonotic airborne bacteria, including *Mycobacterium* spp., *Mycoplasma* spp., *Bordetella* spp., and *Legionella* spp., were found in rodents, dogs, and humans. Arthropod-borne zoonotic bacteria, such as *Bartonella* spp., *Borrelia* spp., and *Rickettsia* spp., were detected in all three hosts, while *Orientia* spp. was found in rodents and dogs. *Yersinia pestis*, *Streptobacillus* spp., and *Anaplasma* spp. were found in rodents. Other zoonotic bacteria found in both wild rodents, domestic dogs, and humans are *Leptospira* spp., *Brucella* spp., and *Salmonella* spp. Generally, wild rodents harbored more zoonotic bacteria species compared to dogs and humans. Hence, a unified, multidisciplinary health care approach is recommended in order to safeguard public health and animal health from acquiring zoonoses. Additional research should be carried out to investigate the presence of antibacterial resistance and virulence genes and their distribution in all observed pathogens in various animal species and environments. Lastly, studies pertaining to the identification of zoonotic bacteria in livestock (cattle, sheep, goats, cats, and donkeys) should be carried out in order to identify those carrying infectious agents. The government and private sectors are requested to increase the allocation of research funds for sustained surveillance and management of zoonotic diseases for the well-being of humans and animals.

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Institutional Review Board Statement: The proposal of this study was revised by the Ethical Review Committee of the Tanzania Medical for Research Institute (NIMR) (Ref. No. NIMR/HQ/R.8a/Vol. IX/3676; 19 May 2021). The Tanzania Commission of Science and Technology (COSTECH) issued the research permit after Tanzania Wildlife Research Institute (TAWIRI) approved the proposal (Ref. No. 2023-38-NA-2022-480). Also, Sokoine University of Agriculture provided the permission letter for carrying out the study (Ref. No. SUA/ADM/R.1/8A/718; 3 February 2021). Likewise, the local administrative authorities of Arusha region (Ref. No. FA.132/95/01/38; 12 February 2021) and Ngorongoro District (Ref. No. AB.114/354/01/134; 1 April 2021) provided consent. Prior to the start of rodent trapping and blood sample collection from dogs and humans, the household heads provided written informed consent. In case he or she was unable to write and read, verbal assent was obtained. Additionally, participation in the study was voluntary.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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CHAPTER THREE

PAPER TWO

Metagenomic Screening of Viruses in Arusha, North Tanzania: A One Health Perspective Across Wild Rodents, Domestic Dogs and Humans

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Original Article

Metagenomics screening of viruses in Arusha, north Tanzania: a one health perspective across wild rodents, domestic dogs and humans

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Abstract

Background: One of the most significant and perilous challenges faced by African societies, including Tanzania, is the public health threat posed by zoonotic viruses. This study aimed to ascertain the presence of viruses in wild rodents, domestic dogs, and humans who shared the same environment.

Methods: A cross-sectional study was conducted in the Ngorongoro district from January to February 2022. Metagenomics next-generation sequencing technologies, including Illumina (MiSeq) and Oxford Nanopore (MinION), were employed to identify viruses in 530 blood samples collected from 200 humans, 230 wild rodents, and 100 domestic dogs. The captured wild rodents belonged to various species: Mastomys spp (n=87), Ratus spp (n=45), Avicanthis spp (n=40), Arcomys spp (n=28), Mus spp (n=16), and Field mice (n=14). The taxonomic classification of viruses was carried out using the Kraken2 program.

Results: A total of 20 RNA and 20 DNA viral families were detected. Zoonotic RNA families identified in rodents included Peribunyaviridae, Hantaviridae, Flaviviridae, Coronaviridae, Orthomyxoviridae, Paramyxoviridae, Retroviridae, Picornaviridae, Arenaviridae, Togaviridae, and Tobaniviridae. The zoonotic DNA families detected in rodents were Adenoviridae, Poxviridae, Herpesviridae, Anelloviridae, and Circoviridae. Peribunyaviridae and Hataviridae were identified in both humans and rodents. Unclassified +ssRNA viruses were found in dogs and humans. Herpesviridae was present in all three hosts. When compared to dogs and humans, rodents harbored zoonotic viruses that pose a significant public health safety concern.

Conclusion: The study unveiled a noteworthy pattern where the majority of RNA and DNA viruses with zoonotic potential were detected in wild rodents, as opposed to dogs and humans. These findings underscore the pivotal role played by wild rodent populations in the transmission and maintenance of such viruses.

Keywords: Metagenomics Next-Generation Sequencing, Zoonotic Viruses, Wild Rodents, Domestic Dogs, Humans, Ngorongoro, Tanzania

Background

Zoonotic infections exert a considerable strain on healthcare systems globally, particularly in less developed countries [1]. Diseases stemming from rodents have always been a global public health issue [2]. Many rodent species, such as rats and mice, are renowned carriers of multiple viruses [2]. Research in sub-Saharan Africa has highlighted the presence of zoonotic viruses like Hantaviruses and Lassa fever viruses in wild rodents [3-4]. Furthermore, domestic dogs have been implicated in transmitting zoonotic viruses to humans and other animals [4], with instances of rabies in domestic dogs being reported throughout Tanzania [4]. Both rodents and dogs can transfer pathogenic and zoonotic viruses to humans and other animals via bites, or by contact with infected surfaces, food, or water [3-5]. Research from around the world indicates that over half of human pathogens are zoonotic, and a significant portion of emerging infectious diseases arise from zoonotic viruses [6-7]. Historically, global pandemics have often been traced back to pathogens from wildlife [6-10]. Including rodents, dogs, and humans in virus surveillance adheres to the One Health model, which emphasizes the interconnectedness of human, animal, and environmental health [10]. Many infectious diseases involve intricate ecological interactions spanning multiple species, making it essential to comprehend these relationships for effective disease prevention [10]. Concurrent monitoring of infections in animals and humans offers a holistic view of disease patterns, transmission dynamics, and associated risk factors, ultimately promoting improved public health outcomes.

Tanzania boasts a diverse range of small mammal species scattered across various regions [11]. With its plethora of Wildlife National Parks and conservation areas, Tanzania has seen increased human intrusion into these zones due to expanding agricultural practices, hunting, tourism, and the quest for herbal remedies [11]. Such activities heighten the risk of infectious pathogen transmission from wildlife to humans and animals. Although Tanzania is abundant in areas where wildlife, livestock, and humans coexist, there is a dearth of data on rodent-related zoonotic viruses. The Ngorongoro district, with its rich wildlife, domestic animals, and migratory species, is a potential hotspot for the spread of rodent-borne viruses to animals and humans [11-12]. Past studies on livestock and human patients in the district have revealed evidence of infections like the Rift Valley fever virus [13]. A study in Arusha even found the presence of Hantaviruses in asymptomatic adults visiting hospitals [3]. However, the role of wild rodents in viral transmission within the district remains unexplored. Hence, this study aimed to screen and identify viruses in wild rodents, domestic dogs, and humans, with a focus on zoonotic variants. This research serves as foundational data, shedding light on the RNA and DNA viruses in rodents, dogs, and humans.

Methods

Study design and setting

This research was conducted in five villages within the Ngorongoro district (Fig.1). Situated in the northern part of Tanzania within the Arusha region, the district spans an area of 14,036 km2 and is geographically positioned between longitude 35°30"E and 36°23"E, and latitude 02°45"S and 4°0"S [13]. It's bordered by Kenya to the north, Serengeti National Park to the west, and the Longido and Moduli Districts to the east, while Karatu District lies to the south. The district is divided into three divisions, comprising 28 wards and 65 villages, with an estimated human population of 174,278 [14].

Rodent trapping protocol

Wild rodents were trapped using Sherman LFA live traps (HB Sherman Traps, Inc., Tallahassee, FL) and wire cage traps, baited with a mix of peanut butter, maize bran, and sardines [15]. Habitats like houses, human settlements' peripheries, livestock pens, and nearby agricultural plots served as trapping grounds. After capture, rodents were identified morphologically to genus level following the Happold manual [16]. Depending on a home's size, 2 to 4 wire cage traps were placed indoors, while Sherman traps were strategically placed around pathways and burrows for peri-domestic rodents. Each village had 40 modified wire cage traps and 80 Sherman traps designated for indoor and surrounding trapping, respectively. Each village's trapping lasted seven days, setting traps between 4-5 p.m. and collecting them the next morning, around 8-10 a.m.

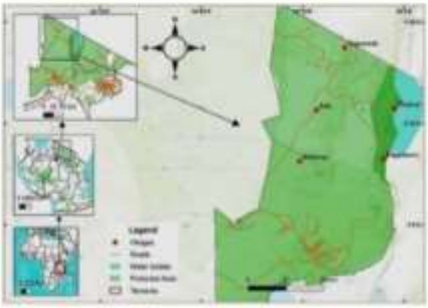

Figure 1: A map that displays Tanzania and studied villages within the Ngorongoro district created by Issae et al. [15]

Sample size and pooling procedures

Human blood samples (n=200) were pooled into 22 pools based on their sex [15]. Rodent samples (n=230) were pooled into 16 groups according to genera, while dog samples (n=100) were divided into 10 pools based on sex.

Sample collection and handling procedures

From Rodents: After being humanely anesthetized and euthanized using Isoflurane [15], rodents were prepared for sampling. Their ventral surface was disinfected with 70% alcohol, and researchers collected approximately 0.5 ml of blood using hypodermic needles and syringes [17]. This blood, obtained using the cardiac puncture method, was mixed with 1 ml of DNA/RNA shield reagents in cryovials to preserve nucleic acids [15]. The samples were kept in liquid nitrogen and transported to the Sokoine University of Agriculture where they were stored at -80°C until molecular analysis.

From Humans and Dogs: Expert personnel from the Ngorongoro district collected blood samples, ensuring sanitary precautions. After disinfecting the skin with 70% alcohol, 1 ml of blood was drawn from the cephalic veins using a 21-gauge needle [17]. The blood was mixed with DNA/RNA shield reagents in cryovials to prevent and stored at -80°C until analysis [15]. Molecular studies were conducted at the Kilimanjaro Clinical Research Institute (KCRI) biotechnology laboratory at the Kilimanjaro Christian Medical Centre (KCMC) in Moshi.

Extraction of RNA and DNA, cDNA synthesis, and sequencing library preparation

The QIAamp RNA Blood Mini Kit was used for RNA extraction and the QIAamp DNA Blood Kit for DNA extraction, both by the manufacturer's instructions (Qiagen, Valencia, CA, USA). The extracted RNA was then reverse transcribed into complementary DNA (cDNA) using the Omniscript Reverse Transcription Kit (Qiagen, Valencia, CA, USA), following the manufacturers' guidelines. Two Next-Generation Sequencing techniques were applied: Oxford Nanopore with the MinION sequencer and Illumina with the MiSeq sequencer. For the MiSeq platform, libraries were prepared using the Illumina Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA), based on the supplier's recommended procedures. For the MinION platform, the PCR-cDNA sequencing-barcoding kit (SQK-PCB109) from Oxford Nanopore Technologies was followed as per the manufacturer's instructions. The concentration of nucleic acids at various stages of library preparation was determined using Thermo Fisher Scientific's Qubit high-sensitivity RNA and DNA assays. Subsequently, equimolar quantities of nucleic acids were combined, and the libraries were sequenced in a single lane on an Illumina MiSeq sequencer (Illumina, San Diego, CA).

Statistical analysis

The MiSeq reads were processed for base calling and demultiplexing using Illumina BCL Convert software, version 3.9.3. For the MinION reads, base calling was performed using the Guppy Software version 6.4.2 from Oxford Nanopore Technologies (ONT), with the 9.4.1_450bps_SUP model. Segregation of reads based on their specific barcodes was achieved using ONT Guppy barcoder, version 6.4.2. Reads that were not classified were maintained as separate pseudo-samples. Following this, the reads were scanned for contamination from humans and vectors using FastQ Screen version 0.14.1, with GRCh38 and UniVec Core as the reference sources [18]. The reads were then trimmed with fastp, using default parameters as well as trimming the front (-5) and tail (-3) ends. Basic quality metrics were generated using fast-stats from the fastq-util tool, version 1.3.0 [19]. For taxonomic classification, Kraken2 version 2.1.2 was used [20], leveraging standard Kraken2 databases augmented with fungi. The databases were formulated using reference sequences from the National Center for Biotechnology Information (NCBI), gathered between November 15th and 18th, 2022. The quality-filtered and trimmed MiSeq and MinION reads were then analyzed in Kraken2's paired-end mode. Notably, Kraken2 is a bioinformatics package renowned for its high accuracy and sensitivity in microbial classification, even detecting those in minimal abundance [20].

Results

This study identified 34 viral families in rodents, 5 in dogs, and 7 in humans. Notably, the study pinpointed 20 RNA and 20 DNA viral families, with rodents demonstrating the highest viral prevalence (Figure 2).

Table 1: Inclusive explanation of rodent genera, size of the sample, and formation of blood pools

Category of samples	Species	Sex	Samples counts	Pools counts	Samples count per pool	Volume (µl) pooled per sample	Final volume (ml) per pool
Human	NA	F	130	13	10	100	1
	NA	M	70	9	7-8	100	0.7-0.8
	Total samples		**200**	**22**			
Rodents	*Mastomys spp*	F	67	6	11-12	100	1.1-1.2
		M	20	1	20	80	1.6
	Rattus spp	F	27	2	13 and14	100	1.3-1.4
		M	18	1	18	80	1.44
	Arvicanthis spp	F	20	1	20	80	1.6
		M	20	1	20	80	1.6
	Arcomys spp	F	15	1	15	100	1.5
		M	13	1	13	100	1.3
	Mus spp	M & F	16	1	16	80	1.3
	Field mice spp	M & F	14	1	14	100	1.4
	Total samples		**230**	**16**			
Dogs	Mongrels	F	57	5	11-12	100	1.1-1.2
	Mongrels	M	43	5	8-10	100	0.8-1
	Total samples		**100**	**10**			

RNA Viral families in rodents

From the wild rodents sampled, 14 RNA families were identified. Notably, the Retroviridae family was prevalent in 9 out of 16 pools, accounting for 56.25% of the pools. The remaining viral families each appeared in a single pool, constituting a 6.35% representation. Of all rodent species, Mastomys spp showed the most RNA viral presence (Table 2).

DNA viral families in rodents

This study identified five DNA viral families within the rodent samples (Table 3). It's noteworthy that some viruses outlined in Table 3 have zoonotic implications.

DNA and RNA viruses in domestic dogs

The findings in domestic dogs included three distinct viral families and an array of unclassified +ssRNA viruses (Table 4). Within these, the Retroviridae family was present in 3 out of 10 pools, translating to a 30% occurrence. Each of the other viral families was detected in a single pool, each with a 6.25% representation.

DNA and RNA viruses in humans

In the human samples, three viral families and unclassified +ssRNA viruses were detected in 5 out of the 22 pools (Table 5). Specifically, Peribunyaviridae was observed in two out of the 22 pools, equating to a 9.09% presence. All other viral families appeared once, each having a 4.54% proportion.

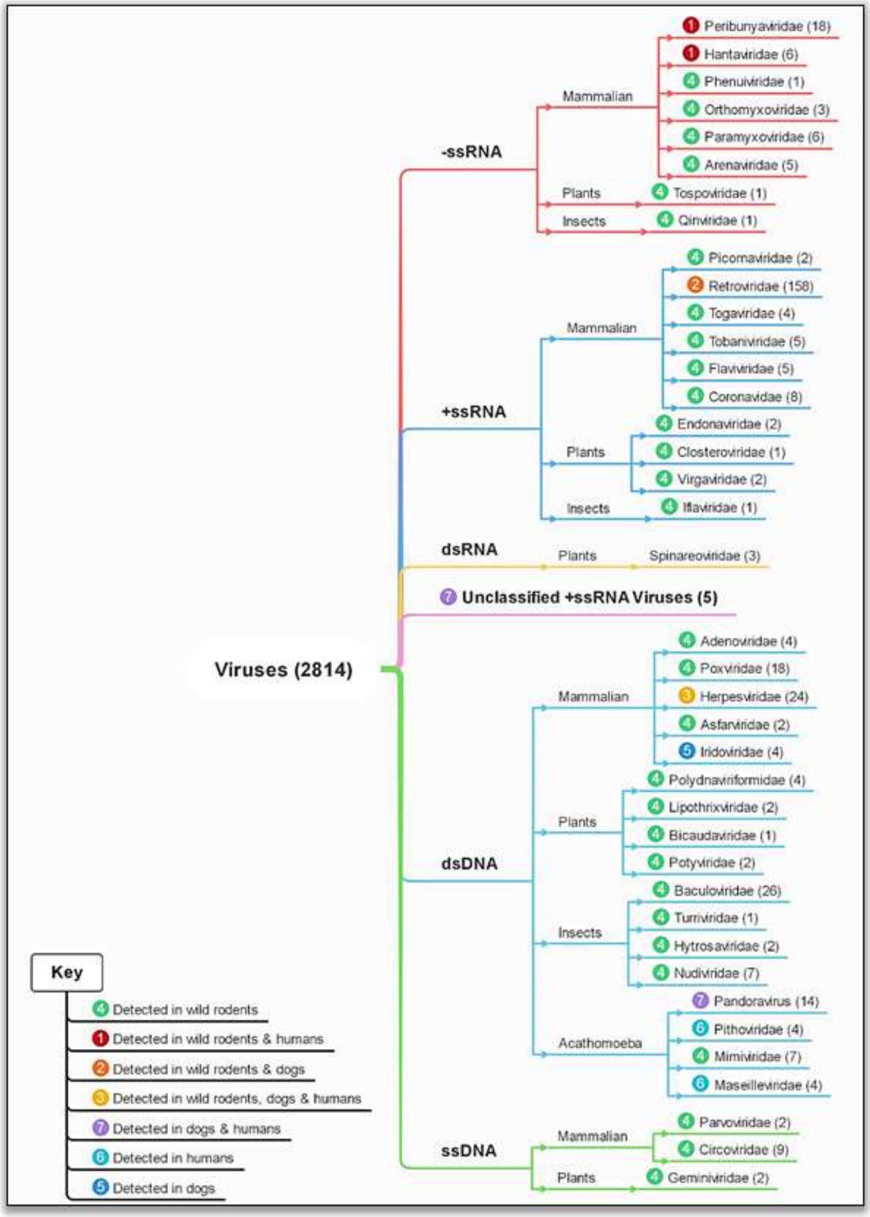

Figure 2: Distribution of viral families in wild rodents, domestic dogs, and humans, with their respective read counts indicated in brackets

Table 2: RNA viruses present in rodent blood samples from Ngorongoro District

Host	Family	Genus	Species and strains	Reads	Genome
Mastomys spp	*Peribunyaviridae*	*Orthobunyavirus*	*California encephalitis orthobunyavirus*	7	-ssRNA
			M'Poko orthobunyavirus	5	-ssRNA
		Herbevirus	*Tai herbevirus*	2	-ssRNA
	Hantaviridae	*Orthohantavirus*	*Hantavirus Z10*	4	-ssRNA
			Choclo orthohantavirus	4	-ssRNA
			Seoul orthohantavirus	4	-ssRNA
Mastomys spp	*Picornaviridae*	*Sicinivirus*	*Sicinivirus A*	2	+ssRNA
		Kunsagivirus	*Kunsagivirus A*	2	+ssRNA
Mastomys spp	*Satellites*	*RNA satellites*	*Olive viral satellite RNA*	3	
Rattus spp	*Orthomyxoviridae*	*Alphainfluenzavirus*	*Influenza A virus*	2	-ssRNA
	Paramyxoviridae	*Orthorubulavirus*	*Human orthorubulavirus 4*	3	-ssRNA
		Jeilongvirus	*Tailam jeilongvirus (Tailam virus)*	2	-ssRNA
Mastomys spp	*Retroviridae*	*Betaretrovirus*	*Mason-Pfizer monkey virus*	23	-ssRNA
			Ovine enzootic nasal tumor virus	15	-ssRNA
			Simian retrovirus 8	6	-ssRNA
			Simian retrovirus 4	4	+ssRNA
Arvicanthis spp			*Enzootic nasal tumor virus of goats*	1	+ssRNA
			Desmodus rotundus endogenous retrovirus	10	+ssRNA
			Mouse mammary tumor virus	6	+ssRNA
Rattus spp		*Alpharetrovirus*	*Avian myelocytomatosis virus*	3	+ssRNA
		Gamma retrovirus	*Murine leukemia virus*	2	+ssRNA
			Abelson murine leukemia virus	5	-ssRNA
			XMRV-related retroviruses	5	+ssRNA
Mus spp	*Arenaviridae*	*Reptarenavirus*	*Giessen reptarenavirus (University of Giessen virus)*	3	+ssRNA
	Togaviridae	*Alphavirus*	*Middelburg virus*	4	+ssRNA
Arvicanthis spp	*Tobaniviridae*	*Torovirus*	*Hainan hebius popei torovirus*	3	+ssRNA
Field mice	*Flaviviridae*	*Flavivirus*	*T'Ho virus*	5	+ssRNA
	Coronaviridae	*Alphacoronavirus*	*Porcine epidemic diarrhea virus*	3	+ssRNA
			Rat coronavirus	2	+ssRNA
			Feline coronavirus	2	+ssRNA

Table 3: DNA viruses found in rodent blood samples from Ngorongoro District

Host	**Family**	**Genus**	**Species and strains**	**Reads**	**Genomes**
Rattus spp	*Adenoviridae*	*Mastadenovirus*	*Human mastadenovirus* C *Bat mastadenovirus* F *Bat mastadenovirus* WIV17	4 2 2	dsDNA
Acomys spp	*Poxviridae*	*Centapoxvirus*	*Yokapox virus*	1	dsDNA
			Murmansk microtuspox virus	1	dsDNA
			Murmansk poxvirus	1	dsDNA
		Avipoxvirus	*Pigeonpox virus*	1	dsDNA
			Canarypox virus	1	dsDNA
		Oryzopoxvirus	*Cotia virus SPAn232*	1	dsDNA
		Cervidpoxvirus	*Mule deerpox virus*	1	dsDNA
			Deerpox virus W-848-83	1	dsDNA
		Yatapoxvirus	*Yaba monkey tumor virus*	1	dsDNA
		Capripoxvirus	*Sheeppox virus*	1	dsDNA
		Orthopoxvirus	*Raccoonpox virus*	1	dsDNA
Mastomys spp	*Herpesviridae*	*Percavirus*	*Equid gammaherpesvirus 5*	2	dsDNA
		Muromegalovirus	*Murid betaherpesvirus 8*	1	dsDNA
		Proboscivirus	*Elephant endotheliotropic herpesvirus*	1	dsDNA
		Quwivirus	*Tupaiid betaherpesvirus 1*	1	dsDNA
		Macavirus	*Bovine gammaherpesvirus 6*	1	dsDNA
		Rhadinovirus	*Saimiriine gammaherpesvirus 2*	1	dsDNA
			Rhinolophus gammaherpesvirus 1	1	dsDNA
		Varicellovirus	*Felid alphaherpesvirus 1*	1	dsDNA
			Cercopithecine alphaherpesvirus 9	1	dsDNA
Field mice spp	*Anelloviridae*	*Alphatorquevirus*	*Torque teno virus 22*	2	ssDNA
	Circoviridae	*Circovirus*	*Rodent-associated circovirus 7*	4	ssDNA
			Rodent-associated circovirus 3	2	ssDNA

Table 4: List of RNA and DNA viruses in domestic dogs from the Ngorongoro district

Viral family	**Genus**	**Species**	**Reads**	**Genome**
Retroviridae	*Gammaretrovirus*	*Baboon endogenous virus* *(Baboon endogenous virus strain M7)*	2 2	+ssRNA
Unclassified Riboviria	unclassified RNA viruses ShiM-2016	*Sanxia water strider virus 19*	1	+ssRNA
		Wenzhou tombus-like virus 13	1	+ssRNA
		Wenzhou picorna-like virus 3	1	+ssRNA
		Wuhan house centipede virus 2	1	+ssRNA
		Hubei picorna-like virus 52	1	+ssRNA
		Hubei picorna-like virus 34	1	+ssRNA
		Wenzhou picorna-like virus 39	1	+ssRNA
		Beihai Charybdis crab virus 1	1	+ssRNA
Herpesviridae	*Simplexvirus*	*Aveline alphaherpesvirus 1*	4	dsDNA
Iridoviridae	*Lymphocystivirus*	*Lymphocystis disease virus Sa*	2	dsDNA
		Lymphocystis disease virus 4	2	dsDNA

Table 5: Catalog of RNA and DNA viruses identified in human samples from the Ngorongoro district

Viral family	**Genus**	**Species**	**Reads**	**Genome**
Peribunyaviridae	*Orthobunyavirus*	*Simbu orthobunyavirus*	5	-ssRNA
		Shamonda orthobunyavirus	3	-ssRNA
Hantaviridae	*Orthohantavirus*	*Oxbow orthohantavirus*	4	-ssRNA
		Oxbow virus	4	-ssRNA
Unclassified RNA viruses	Unclassified +ssRNA viruses	*Wuhan centipede virus*	3	+ssRNA
Herpesviridae	*Roseolovirus*	*Human betaherpesvirus 6B*	4	dsDNA
		Human betaherpesvirus 5	5	dsDNA
	Cytomegalovirus	*Panine betaherpesvirus 2*	3	dsDNA
		Cercopithecine betaherpesvirus 5	2	dsDNA

Discussion

In our research, we conducted an in-depth examination of the study revealed the presence of 19 RNA viral families in rodents, 2 in dogs, and 3 in humans, marking their first identification in the study area. Moreover, the majority of DNA viruses were found in rodents. This indicates that rodents host a wider variety of viruses compared to dogs and humans. One contributing factor is that most rodents, even when infected, don't necessarily die from these infections. Instead, they often become virus carriers due to non-obvious illnesses and their established role as reservoirs for various pathogens [21]. Furthermore, rodents are hosts to ectoparasites, such as ticks, mites, and fleas, which serve as vectors for disease transmission [22]. This research also offers the inaugural evidence of Orthobunyavirus in both rodents and humans in Tanzania. Specifically, the California encephalitis orthobunyavirus and MPoko orthobunyavirus were identified in Mastomys spp, while the Simbu and Shamonda orthobunyaviruses were discovered in humans. Notably, the two Orthobunyaviruses detected in rodents had previously been recognized as human pathogens [23]. The California encephalitis virus is linked to encephalitis in humans [23]. However, the natural hosts and transmission dynamics of the MPoko orthobunyavirus remain unclear, necessitating more research to decipher its potential ramifications on both human and animal health. Meanwhile, Simbu Orthobunyavirus infections are known to cause congenital malformations and abortions in ruminants [24-25]. These discoveries carry significant implications in the context of holistic health due to the potential for these viruses to spread among various mammalian hosts, their widespread arthropod vectors, and the grave nature of the diseases they cause. The study also uncovered the presence of Tai herpesvirus in Mastomys spp. Previously, this virus was isolated from Culex sp. mosquitoes in Côte d'Ivoire and was later detected in the same mosquito species in Ghana [25]. It was then categorized as an insect-specific virus [25]. However, its detection solely in rodents raises questions. Does Tai herpesvirus naturally infect rodents, or did the rodents ingest insects carrying the virus? A deeper understanding of its epidemiology and potential implications for public health necessitates further research and surveillance. This research marked the inaugural identification of Orthohantavirus in both rodents and humans in Tanzania. Specifically, the Choclo orthohantavirus and Seoul orthohantavirus species were identified in wild rodents (Mastomys spp), while the Oxbow orthohantavirus (or Oxbow virus) was found in humans. Seoul orthohantavirus is linked with hemorrhagic fever with renal syndrome (HFRS) in Eurasia and hantavirus pulmonary syndrome (HPS) in America [26-27]. The Oxbow virus was first detected in the American shrew mole in 2003, with its genome analysis revealing a low sequence similarity to rodent-borne hantaviruses [28]. These discoveries represent the first records of Choclo and Seoul orthohantaviruses in wild rodents, and the Oxbow orthohantavirus in Tanzanian humans. Thus, the detection of Orthohantaviruses in both wild rodents and humans highlights the potential risk of cross-species virus transmission between wildlife and humans. Furthermore, the Picornaviridae viral family was detected in rodents. Viruses within this family are recognized as zoonotic [29]. The study identified both Sicinivirus A and Kunsagivirus A in rodents. Remarkably, this is the inaugural report of Sicinivirus A's presence in wild rodents. Nonetheless, Sicinivirus A had previously been isolated from commercially raised chickens exhibiting severe infections in China [29]. On the other hand, Kunsagivirus A was first found in migratory bird feces and subsequently in wild yellow baboon blood [30]. This study now documents its presence in Mastomys spp. Given these discoveries, it's plausible that Sicinivirus A and Kunsagivirus A could undergo cross-species transmission between birds and mammals. This underscores the importance of further research to determine the prevalence and zoonotic potential of these viruses in mammals.

In the current study, four species of unclassified +ssRNA viruses were detected in dogs and one in humans. These viruses have been characterized previously as the unclassified RNA viruses ShiM-2016, novel Picorna-like viruses, found in Culex spp. mosquitoes from the Zambezi Valley in Mozambique [31]. These viruses were likely transmitted to dogs and humans via mosquito bites. Further investigations are essential to elucidate their potential to cause illnesses in animals and humans. The study pinpointed the presence of the Flavivirus, specifically the T'Ho virus. Members of the Flavivirus genus are known to cause severe endemic infections in humans, such as Yellow Fever, Dengue Fever, Encephalitis, and various hemorrhagic illnesses [32]. The T'Ho virus was initially isolated from Culex quinquefasciatus mosquitoes in the Yucatan Peninsula of Mexico [33]. As of now, there are no documented diseases in vertebrates attributed to this virus worldwide. This research marks the first instance of the T'Ho virus being identified in wild rodents, specifically field mice, indicating they could serve as a potential reservoir for this flavivirus. The study also recognized the presence of the Influenza A virus, part of the Orthomyxoviridae family, known to cause respiratory infections in both mammals and birds [33]. Further, the Human Parainfluenza virus 4a and Tailam jeilongvirus, members of the Paramyxoviridae family, were detected in Ratus spp. It's noteworthy that the Human parainfluenza virus 4 had earlier been identified in African bats [34]. Such findings accentuate that rodents can act as reservoirs for a plethora of viruses, posing significant threats to public health. The Retroviridae family was identified in both rodents and dogs. Retroviruses can cause an array of diseases in animals, including lymphomas, leukemias, sarcomas, immunodeficiencies, neurological ailments, and more [35]. The detection of retroviruses with origins traceable to Ovine, Caprine, Avian, and Baboons in rodents and dogs hints at possible cross-species transmission among animals sharing an environment. The presence of livestock, poultry, and baboons in the study area might contribute to the introduction of these viruses in dogs and rodents. It has been documented that certain viruses can be transmitted between animal hosts through aerosolized infectious particles [35]. Moreover, dogs could contract these viruses by consuming baboons and smaller mammals. This study also revealed several families of zoonotic viruses with public health significance in rodents, including Arenaviridae. Arenaviruses are associated with hemorrhagic fever in humans and are endemic to West Africa [36]. Notably, the Giessen reptarenavirus was identified in Mus spp. within this study. Previously, this virus was linked to the Boid inclusion body disease in captive snakes in Brazil, with Boid snakes identified as the primary reservoirs [36]. These findings suggest that rodents might also serve as reservoirs for this virus.

The Middelburg virus, belonging to the Alphavirus genus, was also detected in rodents. Alphaviruses, primarily transmitted by mosquitoes, are of significant public health concern and are prevalent across Africa [37]. The Middelburg virus was first isolated from Aedes mosquitoes in 1957 in South Africa and has been attributed to neurological diseases in various animals, including cattle, sheep, horses, wildlife, and humans [37]. This research marks the inaugural evidence of Middelburg virus infections in Mus spp. in Tanzania, underscoring the need for further research into its role as a source of infections in both animals and humans. Additionally, the study reported the discovery of the Hainan hebius popei torovirus, a member of the Tobaniviridae family, in wild rodents (Arvicanthis spp.). Toroviruses are known to cause gastroenteritis in both animals and humans [38]. Notably, the Hainan torovirus was initially isolated from freshwater snapping turtles in Australia in 2015 and was linked to significant mortality rates among these turtles in a New South Wales coastal river [39]. The presence of this virus in Arvicanthis spp. indicates potential cross-species transmission. Interestingly, the study also detected Coronaviruses in Field mice, specifically identifying the Porcine epidemic diarrhea virus and the Feline infectious peritonitis virus. These viruses traditionally cause diseases in pigs and cats, respectively [40-41]. While previous studies did not label them as zoonotic, an experimental study involving mice suggested the potential for the Porcine epidemic diarrhea virus to cause neuropathogenic effects [42]. This study thus introduces the notion that the Porcine epidemic diarrhea virus, along with the Feline infectious peritonitis virus, might possess the capability to infect a broader range of animals. Also, the research found the presence of Human mastadenovirus C and Bat mastadenovirus F in rodents (Ratus spp). Specifically, Human mastadenovirus C (HAdV-C) is known to cause respiratory infections in children [43]. Mastadenovirus is known to induce diseases across a range of animals [43]. It's plausible that rodents could act as a source, transmitting these viruses to humans and domestic animals. In a surprising turn, the Lymphocystis disease virus was found in dogs. This virus, traditionally associated with lymphocyte disease in fish, has significant implications for the global fish industry [44-45]. This research marks the first documentation of the Lymphocystis disease virus in domestic dogs within Tanzania. Further investigations are essential to understand its transmission dynamics and its broader health implications. The study also revealed the presence of various herpesviruses in rodents, dogs, and humans. The identified herpesviruses had origins traceable to both humans and animals [46]. Such findings reinforce the potential for cross-species transmission of herpesviruses. Given a recent incident in China where a death was attributed to the herpes B virus (Cercopithecine e Herpesvirus 1) [46], it's a pressing reminder of the risks associated with this virus. Further studies are warranted to discern the transmission routes of these viruses within the study area. Notably, apart from viruses affecting animals, the study also identified plant and insect viruses. A majority of these viral families were found in wild rodents. It's well-established that rodents consume both insects and plants [47]. Additionally, viruses that infect free-living amoebae are prevalent in soil and water sources [48]. A recent Australian study indicated that these amoebae might be conduits, transferring pathogenic viruses like Adenoviruses to humans [48]. This revelation demands more in-depth research to understand the public health ramifications of viruses associated with amoebae. While metagenomics next-generation sequencing was utilized, it had inherent drawbacks. These include sequencing host genomes, which potentially reduces the sensitivity in detecting viruses, and the absence of a standardized program for sequence analysis [49].

Conclusion

This research offers a comprehensive view of the viral families present in various animal species, with a particular focus on rodents, dogs, and humans. The data underscores rodents' pivotal role as virus carriers in contrast to dogs and humans. Notably, zoonotic RNA viral families like Peribunyaviridae and Hantaviridae were identified in both rodents and humans, hinting at cross-species transmission potential. This necessitates vigilance in monitoring these viral families across species to curb the spread of zoonotic diseases. The concurrent presence of Herpesviridae in rodents, dogs, and humans suggests mutual vulnerability to this DNA viral family, reinforcing the urgency for robust surveillance and prevention strategies. Intriguingly, Retroviridae was detected in both rodents and dogs, indicating potential viral exchanges within the study area's fauna. Moreover, the discovery of Unclassified +ssRNA viruses in dogs and humans highlights the need for continued monitoring and investigation to grasp their transmission dynamics and implications for public health. In essence, these insights enrich our understanding of viral diversity, and potential zoonotic transmission pathways, and highlight the critical role of a collaborative approach encompassing both veterinary and human health sectors to address virus-associated risks. Future endeavors should intensify efforts in examining prevalence, transmission routes, and the broader impact of viruses on livestock and human populations. The ultimate goal is to architect efficient strategies to preclude the spread of zoonotic diseases.

Abbreviation

RNA: Ribonucleic acid; DNA: Deoxyribonucleic acid; cDNA: Complementary Deoxyribonucleic acid; BCL: Binary Base call; QC: Quality control; NCBI: National Center for Biotechnology Information

Declaration

I, Amina Ramadhani Issae, declare that the manuscript titled "Metagenomics screening of viruses in Arusha, north Tanzania: A One Health Perspective across wild Rodents, Domestic Dogs and Humans" contains materials related to a previously published article. I hereby acknowledge and reference the following published article titled "Exploring Pathogenic and Zoonotic Bacteria from Wild Rodents, Dogs and Humans of the Ngorongoro District in Tanzania Using Metagenomics Next-Generation Sequencing" authored by Issae et al., 2023, published by MDPI Zoonotic Diseases Journal. I confirm that the current manuscript builds upon and provides additional insights into the context of the above-published paper.

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Availability of data and materials

Data will be available by emailing amina.issae@sua.ac.tz

Authors' contributions

Amina Ramadhani Issae (ARI) is the principal researcher who designed the study methodology, performed field work, performed data analysis, and wrote the first draft of the manuscript. Abdul Selemani Katakweba (ASK) is a co-supervisor who assisted in improving the study methodology, fund acquisition, reviewing and editing the manuscript. Rose Peter Kicheleri (RPK) is a co-supervisor, reviewing and editing the manuscript. Augustino Alfred Chengula (AAC) is a co-supervisor, reviewing and editing the manuscript. Christopher Jacob Kasanga (CJK) is the main supervisor who assisted in designing the study methodology and reviewing and editing the manuscript. All authors have read and agreed to publish the final version of the manuscript.

Ethics approval and consent to participate

The ethical clearance to perform this study was granted by the Ethical Review Committee of the Tanzania Medical Research Institute (NIMR) (Ref. No. NIMR/HQ/R.8a/Vol. IX/3676; 19th May 2021). Also, the Tanzania Commission of Science and Technology (COSTECH) granted the research authorization following approval of the proposal by the Tanzania Wildlife Research Institute (TAWIRI) (Ref. No. 2023-38-NA-2022-400). Likewise, the Sokoine University of Agriculture granted permission to conduct this study (Ref. No. SUA/ADM/R.1/8A/718; 3rd February 2021). Furthermore, the permission was issued by the Arusha Regional Authority (Ref. No. FA.132/95/01/38; 12th February 2021) and the Ngorongoro district administration (Ref. No. AB.114/354/01/134; 1st April 2021). Before rodent capturing and collection of blood samples from humans and dogs, the heads of the households provided their written agreement. All individuals participated voluntarily.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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CHAPTER FOUR

PAPER THREE

Knowledge, Attitude, and Preventive Practices Toward Rodent-Borne Diseases in Ngorongoro District, Tanzania

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Knowledge, attitude, and preventive practices toward rodent-borne diseases in Ngorongoro district, Tanzania

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Key words: knowledge, attitude, practice, rodents, diseases, human-wildlife, Ngorongoro.

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Ethical approval and consent to participate: the procedure to conduct this study was revised by the Ethical Review Committee of the Tanzania Medical Research Institute (NIMR) (Ref. No. NIMR/HQ/R.8a/Vol. IX/3676; 19th May 2021). Similarly, Sokoine University of Agriculture gave an approval letter for leading this study (Ref. No. SUA/ADM/R.1/8A/718; 3rd February 2021). Additionally, the local administrative authorities of Arusha region (Ref. No. FA.132/95/01/38; 12th February 2021) and Ngorongoro district (Ref. No. AB.114/354/01/134; 1st April 2021) provided permission too. Before the commencement of the face-to-face interview, the respondent gave written informed assent. In case the respondent can't write and read, verbal consent was obtained.

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Abstract

In addition to their economic significance, rodents are hosts and transmit diseases. Most of rodent-borne diseases are endemic in rural Africa and sporadically lead to epidemics. Ngorongoro district is inhabited by humans, livestock, and wild animals. Therefore, a cross-sectional study was conducted to assess the level of knowledge, attitudes, and practices toward rodent-borne diseases among communities. The study used 3 focus groups, 20 key informant interviews, and the questionnaire (N=352) to collect data. The study found that 8.52% of respondents had good knowledge, 35.5% had a positive attitude and 94.3% had good practices toward rodent-borne diseases. The study revealed that only 28.13% of participants were aware of rodent-borne zoonoses. The majority of them (77.27%) believe that rodents are pests that destroy crops and do not transmit pathogens. Moreover, the results showed that the majority of them (82.9%) live in dilapidated huts that serve as rodent breeding places. Additionally, except for education and religion, the level of knowledge had no significant relationship with most of the participants' demographic variables. When compared to individuals who didn't attend school, those with secondary education (OR=7.96, CI=1.4-45.31, P=0.017) had greater knowledge of rodent-borne diseases and management. Similarly, to how attitude and practice were found to be considerably (r=0.3216, P=0.000) positively correlated, general knowledge and general practice scores were found to be significantly (r=0.1608, P=0.002) positively correlated. Despite showing good practices, the communities still lack knowledge of rodent-borne zoonosis. Rodent-borne disease education should be considered in Ngorongoro and other places.

Introduction

Rodents are mammals that are found worldwide except in Antarctica and are approximately divided into 2277 species globally.[1] These are the most abundant mammals that make up 42% of all mammals around the world. Rodents have the ability to survive in various habitats such as semi-arid, semi-aquatic, and aquatic and produce large litters in a short period of time thus making them abundant in the ecosystem.[1] Rodents are divided into three families including *Muridae*, *Microtidae*, and *Sigmodontidae*. Species of rodents under the *Muridae* family are omnivorous and most abundant in Africa and Australia, while in *Microtidae* family are mostly found in Eurasia and members of *Sigmodontidae* are found in America.[1,2]

Rodents are potential hosts and reservoirs of several zoonotic disease agents that are transmissible to humans.[3,4] About 143 different infectious agent genera were discovered, including 14 viral genera, 31 bacterial genera, 83 parasitic species, and 15 fungal genera.[5,6] Over 75% of these infectious agents were zoonotic.[4] Commonly reported rodent-borne zoonoses include: bacteria (Leptospirosis, Plaque, Lyme disease, relapsing fever), viruses (*e.g.*, Hantavirus diseases, Lassa fever, Rift Valley fever), protozoan (*e.g.*, Toxoplasmosis, Leishmaniasis) and Helminths (*e.g.*, Echinococcosis, Trichinosis).[2,3] Transmission of rodent-borne diseases occurs through direct and indirect ways.[7] Direct transmission can be through arthropod bites or inhalation of germs in rodent feces.[7] Indirect transmission occurs through the consumption of food and/or water contaminated by feces or urine from rodents.[7] Additionally, rodents act as amplifier hosts for the pathogens that spread to humans via arthropod vectors.[8]

Most rodent-borne zoonotic diseases are endemic in rural Africa and sporadically lead to serious epidemics.[9] But more often than not, rodent-borne diseases are not identified, and as such, are generally poorly diagnosed and managed.[7] Consequently, the majority of poor rural individuals die from rodent-borne diseases across Africa each year.[3] Based on the fact that rodents spread more than 50% of diseases to humans and animals,[7] besides crops and home items destructions, rodents are a vital concern to the community's health. Despite this, studies on knowledge about rodent-borne zoonoses and management are limited in Tanzania. Various studies carried out worldwide have shown that knowledge about the impact of rodent-related diseases on public health continues to be extensively under-reported.[3,4,10]

Over time, population growth and demographic shifts have increased contact with wild rodents and enhanced disease transmission possibilities.[7,8,10] Numerous studies carried out around the world have shown that community awareness of rodents is essential for the government to formulate measures for the control of rodent-borne diseases.[11,12] So, the objective of this study was to assess the knowledge, attitude, and practice (KAP) levels of the communities toward rodent-borne infections and control in the Ngorongoro district

Materials and Methods

Description of the study area

The proposed study was conducted between July 2021 and January 2022 in the Ngorongoro District (Figure 1) where wild animals interact with humans in the grazing and residential areas. For example, humans, livestock, and wildlife live together in the Ngorongoro crater, making this district to be unique in the world. Ngorongoro District is one of the seven districts of the Arusha Region of Tanzania.[13] It is bordered to the east by Monduli District, to the south by the Karatu District, and to the west by the Mara Region. The district has an area of about 14,036 square kilometers located between latitudes 30.30'south of the equator and longitudes 35.42 'east of Greenwich and it is between 1,009 and 3,645 meters above sea level.[13] According to the 2012 Tanzania National Census, the population of the district was 174,278. Administratively the district is divided into three divisions (Ngorongoro, Loliondo, and Sale) and 20 Wards (villages). The district has a moderate temperature and tropical climate with an average rainfall of 800 mm to 1,000 mm.[13] The majority of residents are Maasai and Sonjo who are pastoralists and agropastoralists.[14] The district is characterized by low undulating plains with low-lying altitudes. The main vegetation is shrubs of acacia species and grass and open and thick forests.[14]

Study design and sampling procedures

A cross-sectional study was conducted to assess knowledge, attitudes, and practices about rodent management and rodent-borne diseases among communities of the Ngorongoro district. The study population was all households in each study village. The villages were selected based on the factors like population density, ease of access, human-wildlife interfaces, and rodent availability. Additionally, the selection of the households in the villages was done purposively based on indications of rodents' inhabitation. Prior to conducting the questionnaire survey at the household level, the household head was requested to sign an agreement. Based on the following presumptions: i) knowledge of diseases; ii) ability to decide whether or not to participate in the study, the household head or any resident person (18 years or older) was interviewed.

Sample size determination

The sample size of the interviewed households was calculated by this formular,[15] where n= sample size approximation, Z= 1.96; standard normal variety at 5% error ($P<0.05$), and assuming a response distribution of 50%, (1-p) = the probability of knowledge and d= absolute error or precision (5%). The calculated household sample size (n) was 384, however; due to reluctance of participants and difficulties in reaching various parts of the villages, only 352 households were reached. A proportional formula (N=HV1+HV2+HV3) was used to calculate the number of surveyed households in each village; where N = total number of interviewed households in the district, and HV= number of households in the selected village.

Data collection

Before doing the fieldwork, researchers secured the necessary approval to conduct the research from the Ethical Review Committee of the Tanzania Medical Research Institute (NIMR) (Ref. No. NIMR/HQ/R.8a/Vol. IX/3676 and date 19th May 2021), to guarantee adherence to Tanzanian and international research guidelines and regulations. Researchers explained the purpose of the study to participants, including community leaders. Participation in the study was voluntary and all participants gave written consent prior to interviews. All data and information collected from respondents were confidentially stored.

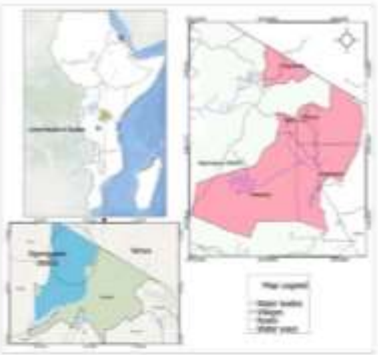

Figure 1. Map of Arusha region and Ngorongoro district showing the villages where the study was done (by the researcher using the QGIS).

Questionnaire survey

A semi-structured questionnaire tool was created based on a literature review of rodents and rodent-borne diseases. The questionnaire tool embraced five groups: i) respondent demography; ii) household characteristics; iii) knowledge of rodent control and rodent-borne diseases; iv) attitude toward rodents; v) practices against prevention of rodent-borne diseases. The questionnaire was pretested in 15 families and changed in accordance with the issues identified to ensure that questions were appropriate. Enumerators fluent in English and Swahili languages were deployed in the administration of the questionnaire. To buy time for retranslation, questions were posed in Swahili, and answers were written down in English. The researchers took great effort to observe the administration of surveys and to check the completed forms in order to ensure the integrity of the data acquired. Also, local translators were employed to interpret the Masai language into Swahili for the respondents who did not speak the Swahili language.

Focus group discussions

Three focus group discussions were conducted with a total of 36 participants. A purposive sampling technique was used to select members for the focus group discussion (FGD). Criteria for selection included adult individuals of 18 years and above and permanent inhabitants of the Ngorongoro district. The focus group discussion was facilitated by trained community health workers of the Ngorongoro district together with the researcher and there were two note-takers. A semi-structured FGD guide was used during the discussions.

Key informants' interview

A total of 20 key informants (KI) interview was conducted to collect data about rodent-borne diseases in the Ngorongoro district. A purposive sampling technique was used and participation was voluntary. All participants were identified by local leaders in each selected ward: two to three personnel per ward. The KI members were health workers and selected local authority leaders. Participants were asked about awareness of rodent-borne zoonoses and control of the rodent population. Trained researchers carried out the interviews and keynotes were taken by a notetaker. Additionally, the interviews were recorded using a phone recorder and the audio records were transcribed. The KI interview information was summarized and analyzed manually based on themes. Themes were explained in the text and speech marks were used where necessary.

Personal observation

The researcher collected information about rodent inhabitation in households and surrounding using the observation form. The observation form included indications like the presence of runways, burrows, droppings, and live captured rodents.

Data analysis

Quantitative data analysis

For each question in the knowledge part, scores ranging from 1 to 4 were given to correct responses based on the kind of question. Moreover, wrong and don't know responses were assigned zero scores. A knowledge score for each respondent was calculated by summing the number of correct answers out of the total scores. Attitudes regarding rodent management and diseases transmitted by rodents were evaluated by using a Likert scale approach.[16] Responses ranged from 1 (completely disagree) to 5 (completely agree). Responses were divided into two categories for cross-tabulation analysis: i) completely disagree/disagree/neutral; ii) completely agree/agree. Respondents who responded, "completely disagree/ disagree/neutral" were thought to have negative attitudes toward rodents, whereas those who "completely agreed or agreed" were believed to have positive attitudes. In the practices section, the score of each correspondent was computed by summing the number of correct answers out of the four questions posed. The respondent was regarded to have good practice if the score was 50% and above and bad practice when the score was below 50% of the total score points.

The variables in the data were coded for easy entry and analysis. Data were entered into Microsoft Excel 2010 and edited to remove the invalid variable and thereafter, exported to R software version 4.1.0 (2021) for analysis. Findings were presented in descriptive statistics like means, proportions, and frequencies. The relationship between demographic characteristics and knowledge was done by using a logistic regression model. The outcome variables were knowledge and attitude toward rodent management and rodent-borne diseases. Odds ratios and their corresponding 95% confidence interval were calculated and were considered statistically significant at $P<0.05$.

Qualitative data analysis

A deductive analytical method was applied for qualitative information collected from FGD and KIs interviews.[17] The topics for discussions and interviews were developed from the literature review on rodent-borne diseases and management. The FGD and KIs data were manually analyzed based on topics presented during the discussions and interviews. In the text, the results are described together with any pertinent speech marks.

Ethical consideration

The procedure to conduct this study was revised by the Ethical Review Committee of the Tanzania Medical Research Institute (NIMR) (Ref. No. NIMR/HQ/R.8a/Vol. IX/3676; 19th May 2021). Similarly, Sokoine University of Agriculture gave an approval letter for leading this study (Ref. No. SUA/ADM/R.1/8A/718; 3rd February 2021). Additionally, the local administrative authorities of Arusha region (Ref. No. FA.132/95/01/38; 12th February 2021) and Ngorongoro district (Ref. No. AB.114/354/01/134; 1st April 2021) provided permission too. Before the commencement of the face-to-face interview, the respondent gave written informed assent. In case the respondent can't write and read, verbal consent was obtained.

Results

Questionnaire

This study involved 352 people in all, most of them were men (67.61%). The respondents were aged 18 to 65, with 54.5% being adults. The majority of the people (58.9%) were pastoralists and attended primary school in high numbers (39.5%) (Table 1).

Household characteristics

In the Ngorongoro district, a high percentage (48%) of households had one to five individuals. The majority of respondents possessed homes that were built using animal feces or mud (Table 2). The grass was found to be the main thatching material for roofs, and 41.2% of homes had open windows. Finally, the findings revealed that 53.4% utilize pit latrines, as shown in Table 2. More information about households' characteristics is well described in Table 2.

The source of information among the study communities

The findings showed that the communities in the study area prefer the use of phones (42.8%) and radio (29%) as the major source of communication (Figure 2). Only 5% and 6% of respondents receive information regarding diseases from health and veterinary servants respectively (Figure 2).

Knowledge about rodent control and rodent-borne zoonoses

The analysis of the knowledge score showed that out of the maximum of 19 points, the respondents' scores ranged from 3 to 12. Only 30 respondents (8.81%) scored 50% and above 50% of the total score, indicating a low level of knowledge of the communities on rodent diseases and management. Among 352 respondents, only 99 (28.13%) were aware of rodent-borne zoonoses as indicated in Table 3.

Relationship between demographic factors and knowledge

The findings showed that most of the participants' demographic factors do not have a significant (P≥0.05) influence on knowledge about rodent-borne diseases and management (Table 4). However, level of education and religion had a significant (P≤0.05) positive influence on knowledge about rodent management and diseases. The detailed description is well narrated in Table 4.

The attitude of the communities toward contracting rodent-borne diseases

In the present study, 35.5% (n=125) and 65.5% (n=227) of respondents had positive and negative attitudes toward rodent-borne diseases, respectively. The proportion of responses on each statement is well illustrated in Figure 3.

Table 1. Respondents' demographic variables.

Variable	Female n (%)	Male n (%)	Total N (%)
Age categories			
Youth (18-34 years)	57(16.2)	89(25.3)	146 (41.5)
Adult (35-64 years)	54(15.3)	138(39.2)	192(54.5)
Elderly (≥65 years)	3(0.9)	11(3.1)	14(4.0)
Total number of participants	114(32.4)	238(67.6)	352(100)
Education levels			
Not attended school	58(16.0)	71(20.2)	129(37.2)
Did not complete school	7(2.0)	23(7.0)	30(9.0)
Primary	40(11.4)	99(28.0)	139(39.4)
Secondary	8(2.3)	35(10.0)	43(12.3)
College	3(0.8)	10(2.8)	11(3.1)
Total number of participants	114 (32.8)	238(68.0)	352(100)
Marital status			
Single	8(2.3)	15(4.2)	23(6.5)
Married	106(30.1)	223(63.4)	329(93.5)
Total number of participants	114 (32.4)	238(67.6)	352(100)
Occupation			
Agropastoral	53(15.1)	94(26.7)	147(41.8)
Pastoralists	61(17.3)	144(40.9)	205(58.2)
Total number of participants	114 (32.4)	238(67.6)	352(100)
Locality (villages)			
Orgosorok	37(10.5)	74(21.0)	111(31.5)
Malambo	16(4.5)	38(10.8)	54(15.3)
Sale	27(7.7)	64(18.2)	91(25.9)
Engaresero	15(4.3)	30(8.5)	45(12.8)
Piyaya	19(5.4)	32(9.1)	51(14.5)
Total number of participants	114 (32.4)	238(67.6)	352(100)

Relationship between attitude and respondents' demographic factors

The results indicate that among the demographic characteristics of respondents; gender, level of education, and occupation have a significant (P<0.05) positive influence on attitude toward rodent-borne diseases and rodent management (Table 5).

Practices of the communities toward rodent-borne diseases

The overall results indicated that about 94.3% (n=332) of respondents showed good practices in rodent-borne disease prevention. All participants do not consume rodents and shrews as shown in Figure 4. Though, the general results showed good practices, only 0.57% of respondents reported wearing protective gear during environmental cleanliness (Figure 4).

General proportions of knowledge, attitude, and practices score among the study communities in Ngorongoro

Although the majority of participants described good practices still, they demonstrated low knowledge and poor attitude toward rodent-borne diseases as shown in Figure 5.

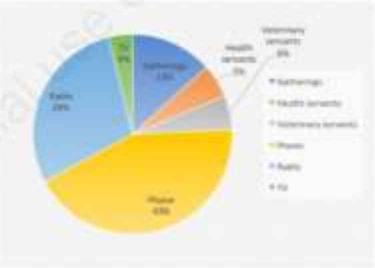

Figure 2. The source of information dissemination among communities in the Ngorongoro district (N=352).

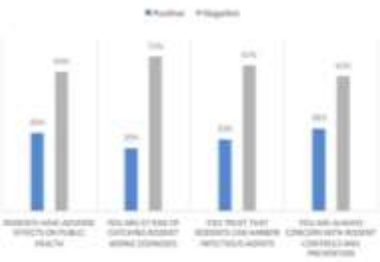

Figure 3. Proportion of communities' attitude toward rodent-borne diseases transmission and prevention (N=352).

Focus group discussions

Communities' knowledge about rodent-borne diseases and transmission

In the current study, three FGD were conducted with a total of 36 participants (Table 6). All FGD participants explained the presence of rodents in their living environment. They see rodents at night and during daytime inside the house, around home compounds, and in the farms or bushes. Most of them were able to describe rodents by their body size and color, whereby they mentioned black, brown, and straw colors. Most individuals complained that rodents are destructing animals because they destroy crops, eat clothes and other household stuff, and store food or cooked food. Most FGD participants were not aware of rodent-borne diseases, so their level of knowledge was regarded as low. The disease transmission through rodents was observed to be of minimal concern in the study communities. Only, three men and four women out of 36 respondents believed that rodents could harbor and transmit pathogens to humans through contamination of food or water, nevertheless, among them, none was able to describe any disease. One female participant described that rodent cause fever and diarrhea. Two male participants said rodents cause wounds because of bites during the night. The majority of FGD participants did not associate rodents with any zoonotic diseases. The majority of the participants insisted that they have been intermingling with wild animals including rodents for years and no person has contracted a disease from them.

Key informants' interviews

Information collected from KIs about rodents' control and rodent-borne diseases differs from the FGDs' discussions. Most of the KIs participants were community health workers and health professionals so they had knowledge about diseases. All KIs participants described that rodent may harbor and spread infectious agents to their communities. Most participants reported that they regularly see rodents around home compounds and farms during the daytime and evening. The majority mentioned that rodents may spread infectious agents to humans through food or water contaminated with feces or urine because of unhygienic activities. One community health worker mentioned that some of the community members do not cover food and water properly. The majority of participants mentioned Plaque as the key disease associated with rodents. Additionally, two clinical officers mentioned Leptospirosis, Salmonellosis, rat bite fever, and toxoplasmosis.

When probed about the possibilities of their communities acquiring rodent-borne diseases, two nurses and one medical officer reported that the possibility is high because humans and rodents live in the same compound. This was also observed by the researchers during the survey because rodents were trapped inside the houses and around home surroundings. The most common

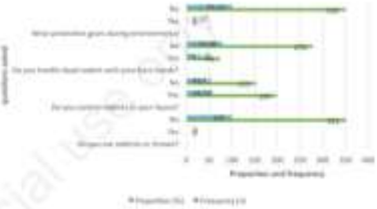

Figure 4. Proportions of communities' preventive practices toward rodent-borne diseases (N=352).

Table 2. Proportions of participants' household characteristics based on the study villages.

Variable	Engaresero n (%)	Orgosorok n (%)	Malambo n (%)	Pinyinyi n (%)	Sale n (%)	Total N (%)
Household size						
1-5	20(11.8)	71(42.0)	30(17.8)	19(11.2)	29(17.2)	169(48.0)
6-10	17(12.7)	33(24.6)	13(9.7)	21(15.7)	50(37.3)	134(38.1)
Above 10	8 (16.3)	7 (14.3)	11 (22.4)	11(22.4)	12(24.5)	49(13.9)
House walls						
Block	1 (2.1)	23(47.9)	5(10.4)	2(4.2)	17(35.4)	48 (13.6)
Concrete block	0(0.0)	9(75.0)	0(0.0)	3(25.0)	0(0.0)	12(3.4)
Mud	0(0.0)	48(28.6)	1(0.6)	46(287.4)	73(43.5)	168(47.7)
Livestock feces	44(35.5)	31(25.0)	48(38.7)	0(0.0)	1(0.8)	124 (35.2)
Window types						
No window	24(15.7)	43(28.1)	15(9.8)	9(5.9)	62(40.5)	153(43.5)
Open window	14(9.7)	44(30.3)	35(24.1)	32(22.1)	20(13.8)	145(41.2)
Net window	5(21.7)	9(39.1)	1(4.3)	3(13.0)	5(21.7)	23(6.5)
Shuttered windows	2(6.5)	15(48.4)	3(9.7)	7(22.6)	4(12.9)	31(8.8)
House roof						
Grass	33(16.8)	42(21.4)	38(19.4)	28(14.3)	55(28.1)	196(55.7)
Mud and grass	0(0.0)	27(90.0)	3(10.0)	0(0.0)	0(0.0)	30(8.5)
Iron sheet	12(9.5)	42(33.3)	13(10.3)	23(18.3)	36(28.6)	126(35.8)
Electrical power						
None	38(16.0)	67(28.3)	38(16.0)	38(16.0)	56(23.6)	237(67.3)
Solar panels	7(6.2)	42(37.2)	16(14.2)	13(11.5)	35(31.0)	113(32.1)
TANESCO	0(0.0)	2(100.0)	0(0.0)	0(0.0)	0(0.0)	2(0.6)
Toilet types						
No toilet	24(14.6)	27(16.5)	31(18.9)	6(3.7)	76(46.3)	164(46.6)
Pit toilet	21(11.2)	84(44.7)	23(12.2)	45(23.9)	15(8.0)	188(53.4)

Table 3. Knowledge of the communities about rodent-borne diseases and control measures.

Variable	Frequency (n)	Proportion (%)
Have you seen rodents?		
Yes	352	100.00
Why do rodents get inside the house?		
Hide from predators	5	1.42
Looking for food	342	97.16
Do you know that rodents harbor zoonotic diseases?		
Yes	99	28.13
Mentioned zoonotic diseases transmissible from rodents to humans		
Bite fever	28	7.95
Plague	49	13.92
Applied control measures of rodents		
Rodenticides	122	34.66
Set traps	9	2.56
Use of cats	134	38.07
Form of rodenticides used		
Rat poison pellet	1	0.28
Rat poison powder	157	44.60
Described the health risks of using rodenticides in your house		
Can kill animal	1	0.28
Kill chicken	43	12.22
Risk to kids	79	22.44
Mentioned the adverse effects of rodents in the communities		
Bites on humans	10	2.84
Destroying crops	272	77.27
Eat clothes	100	28.41
Eat stored or cooked food	53	15.06
Transmission of diseases	7	1.99

Table 4. Logistic regression analysis of the relationship between demographic factors and knowledge about rodent-borne diseases and control measures.

Variable	OR	Confidence interval (95%)	P-value
Gender			
Female	Reference		
Male	1.27	0.41-4.22	0.334
Age			
Elderly	Reference		
Adult	0.23	0.04-1.23	0.082
Youth	0.28	0.05-1.64	0.129
Level of education			
Not attended school	Reference		
Did not complete school	1.07	0.23-4.46	0.931
Primary	3.11	0.98-10.67	0.060
Secondary	7.96	1.48-45.31	0.017**
College	7.56	0.33-73.79	0.111
Occupation			
Agropastoral	Reference		
Pastoralist	1.79	0.52-6.79	0.367
Marital status			
Single	Reference		
Married	2.36	0.43-21.73	0.286
Ward (locality)			
Orgosorok	Reference		
Engaresero	0.23	0.05-1.28	0.109
Malambo	0.44	0.05-2.32	0.376
Pinyinyi	0.41	0.02-2.88	0.443
Sale	1.88	0.46-8.07	0.378

**= Moderately significant at P<0.05.

route of disease transmission was reported to be the contamination of food with feces or urine of rodents. Lastly, they mentioned the signs of rodent invasions at home like the presence of droppings, burrows, and runways in surroundings, and the unpleasant odor of urine from rodents.

Personal observation

The researchers conducted rodent assessments through direct observation of runways, droppings, burrows, and live trapping inside the houses, stores, and around home compounds. Observational results are presented in Figure 6.

Correlation between knowledge, attitude, and practices

In general, the results showed a significant positive correlation among the scores. Knowledge was significantly positively correlated to practice likewise to attitude and practice (Table 7).

Discussion

Based on literature reviews this is the first KAP study to be conducted in Tanzania on rodent-borne diseases. Rodents have a considerable effect on crops and public health in several locations

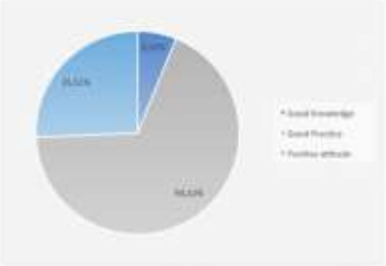

Figure 5. Percentage of respondents' scores on knowledge, attitude, and practices regarding rodent-borne diseases and prevention (N=352).

Figure 6. The various indications used in the assessment of rodents' inhabitations in the households visited (N=352) in the Ngorongoro district.

Table 5. Logistic regression analysis of the relationship between various factors and attitudes toward rodent-borne diseases and control measures.

Variable	OR	Confidence interval (95%)	P-value
Gender			
Female	Reference		
Male	1.89	1.07-3.34	0.003**
Age			
Elderly	Reference		
Adult	1.34	0.36-4.98	0.245
Youth	0.92	0.24-3.55	0.125
Level of education			
Not attended school	Reference		
Did not complete school	2.29	0.85-6.19	0.027*
Primary	1.63	0.87-3.056	0.005**
Secondary	7.77	3.85-19.78	0.001**
College	34.13	3.71-314.0	0.000***
Occupation			
Agropastoral	Reference		
Pastoralist	1.44	0.76-2.75	0.047*
Marital status			
Single	Reference		
Married	2.81	0.65-4.21	0.094
Ward (locality)			
Orgosorok	Reference		
Enguserosambu	0.62	0.27-1.43	0.661
Malambo	0.21	0.09-0.51	0.196
Piyaya	0.48	0.22-1.04	0.957
Sale	0.67	0.29-1.54	0.668

*= Significant at P≤0.05; **= Moderately significant at P≤0.005; ***= Highly significant at P≤0.001.

in Tanzania.[1,18] The recent study was aimed at assessing the level of knowledge, attitudes, and practices toward rodent management and zoonoses among communities in the Ngorongoro district. The majority of participants were adults between the ages range of 35-64, and the majority had only primary education. Men appeared to be more than women because the questionnaire targeted the heads of households. Also, due to the traditional customs of the Maasai tribe, men are the heads of the household and are the ones who give information to the respective household.[19] Women may give information but they often have to ask permission from household heads.[19]

Dissemination of health information among communities is a fundamental factor in disease prevention. Our study found that most of the respondents use phones and radios as preferred methods of information dissemination at the proportion of 42.8% and 29% respectively. It has been reported that mobile phones contributed to the improvement of rural livelihood by providing the fastest and easy means of communication among households.[20] Therefore, information dissemination among communities through phones and radios should be well organized in order to increase health education deliverance. Additionally, planned dissemination of information about rodent-borne diseases will probably help in reducing the population of rodents through the application of proper rodent control methods in the communities.

Rodent-borne diseases are a major threat to residents of poor housing areas which can lead to severe public health issues. The results of this study have revealed that the majority (82.9%) of the respondents live in poorly constructed houses or huts which can facilitate the entry of rodents. Most houses were constructed by using muds and thatched with grasses and had open windows. Furthermore, the study revealed that 67.3% of households had no access to power, implying that the majority of respondents live in dark environments. These findings could be attributed to poverty and lack of awareness of health safety measures, as the majority of people do not communicate with health care providers. Begon's (2003) findings are in agreement with the results of this study. Begon's (2003) study reported that most rural communities live in poor environments with a high risk of contracting rodent-borne diseases. Furthermore, rodent interaction activities were reported among villagers, such as hunting, transportation, preparation for food, and selling processes in countries like Thailand, Zambia and Southern Tanzania.[21-23]

According to this study, only 8.52% of respondents were found to have good knowledge about rodent-borne diseases and management. Participants with a secondary education who had a substantial (P=0.017) high level of knowledge about rodent-borne infections and management were likely responsible for this proportion. This level of knowledge indicates that the communities of Ngorongoro district lack good knowledge about rodent-borne diseases and control measures. Similar findings were observed in Trinidad, where less than half of the population was knowledgeable about rodent-borne diseases and their transmission routes.[4] Also, Begon (2003) and Suwannarong *et al.*, (2022), reported that a high population of individuals in rural settings lack knowledge of rodent-borne illnesses as well as proper control measures. Moreover, Banda *et al.* (2022), documented that some community members may have awareness of zoonotic diseases, although they lack knowledge of specific rodent-borne diseases.

The living condition of rural communities was found to be harsh which may increase the risk of acquiring infections from rodents.[23] For instance, this study revealed that 67.3% of respondents live in dark unhygienic houses which are good for hiding rodents. In direct eye observations, rodent paths were identified in 78% of the households. Additionally, rodent tunnels, droppings, and live captured rodents were discovered inside the houses. These results demonstrate the populations' vulnerability to diseases transmitted by rodents. The improper storage of agricultural products like maize and a lack of hygiene may be the cause of rodent activity in residential areas. Whereby, a high percentage of rodents were trapped in the maize stores and sometimes in the kitchens. This was due to the fact that food was more readily available in these areas than in other parts of the house but was not protected. Last but not least, 97.16% of respondents reported seeing rodents in their houses hunting for food. Our findings somehow reflect the previous study by Mulungu *et al.* (2015) in Morogoro-Tanzania, where poor hygiene and improper storage of cereals were found to enhance rodent invasion in homes.

The majority of participants were not aware that rodents may harbor and transmit infectious agents to humans and livestock. The majority of them (77.27%) think that rodents are pests that solely damage homes' properties, stored cereals, and crops only. These results are very similar to those recorded in other studies carried

Table 6. Composition of focus group discussions in Ngorongoro district.

FGD	Number of participants		Total number of participants
	Male	Female	
FGD 1	6	6	12
FGD 2	6	6	12
FGD 3	6	6	12
Total participants	18	12	36

Table 7. Correlation between knowledge, attitude, and practice scores of respondents.

Scores	Mean	SD	n	Correlation coefficient (r)	P
Total knowledge score	7.14	1.65	352	0.1600	0.002*
Total practice score	1.33	1.64	352	0.1600	0.002*
Total knowledge score	7.14	1.65	352	0.0609	0.255
Total attitude score	2.36	0.59	352	0.0609	0.255
Total attitude score	2.36	0.59	352	0.3216	0.000**
Total practice score	1.33	1.64	352	0.3216	0.000**

*= Moderately significant at P<0.005; **= Highly significant at P<0.0001.

out in Tanzania and elsewhere in the world, where it was known that communities lack adequate knowledge of rodent management.[18,24,25] The results of this study can be used as an effective tool for encouraging the people of the Ngorongoro district to implement proper rodent control measures.

Knowledge about rodent control is crucial for preventing the spread of diseases because rodents carry pathogens of public health significance. This study revealed that the majority (38.08%) and (34.6%) of participants use cats and rodenticides as the preferred rodents control measures respectively. The use of rodenticides was also reported by Mulungu *et al.* (2015) whereby 53% of farmers used this technique. In addition, based on the researcher's direct observation many of these cats were found free roaming around the home compounds, which could also increase the risk of transmitting zoonoses like Toxoplasmosis, Leptospirosis, and Plague. This observation may suggest the need for training the communities on the proper and continuous use of rodenticides instead of using cats to control rodents. This result is somehow similar to the reports documented in other studies in Trinidad and Zimbabwe countries where individuals use cats as the main rodent control strategy and most cats were found to be Feral or semi-feral cats.[5,24]

The findings showed that most of the demographic factors didn't have a significant ($P>0.05$) influence on knowledge about rodent-borne diseases and prevention except education and religion ($P<0.05$). When compared to individuals who did not attend school, those with secondary education ($P=0.017$) had greater knowledge of rodent-borne diseases and control measures. Individuals who have completed their secondary education and/or college may be able to obtain information via books, magazines, radio, and/or television. It has been reported that education provides the opportunity for acquiring knowledge and skills that enable individuals to be potential members of the communities.[26] General knowledge and general practice scores were found to be significantly ($P=0.002$) connected, similar to how attitude and practice were found to be significantly ($P=0.000$) correlated. Therefore, based on these findings, it is reasonable to draw a conclusion that an increased level of knowledge will also influence how the communities perceive the prevention of rodent-borne diseases. Moreover, the study found that 94.3% of respondents have good practices in rodent-borne zoonosis prevention. Good rodent management practices noted include not eating rodents, not touching dead rodents with bare hands, and using cats and rodenticides. Despite receiving a good score in general practice, 99.43% of respondents admitted not using safety gear during cleaning the environment. This habit predisposes communities to rodent-borne diseases through direct contact with droppings or urine. The study conducted in America and elsewhere in the world reported that rodents transmit pathogens to humans through excreta and consumption.[4,6,23] In contrast to this study, previous studies conducted in Thailand,[23] Zambia,[21] Ghana,[26] and Tanzania,[22] reported that the majority of the villagers had direct contact with rodents and their ectoparasites through hunting, killing, and eating. Therefore, education about rodent-borne diseases should be delivered in the Ngorongoro district and in other places across the world.

In the present study, 35.5% of respondents showed a positive attitude toward rodent-borne diseases and 65% of respondents did not believe that they were at risk of acquiring rodent-borne zoonosis. These recent observations are probably attributed to poor knowledge of the communities on rodent-borne diseases (Table 4). The observations showed that there was a positive correlation ($r=0.0609$) between knowledge and attitude scores toward rodent-borne diseases and rodent control measures. Furthermore, among the demography of respondents, gender, level of education, and occupation all showed a significant ($P<0.05$) favorable impact on attitudes about rodent-related diseases and management (Table 5). Respondents who went to school had a much higher ($P<0.05$) positive attitude about rodent-borne diseases and prevention than those who didn't. It has been documented that education often adopts positive views of actions.[26]

Lastly, this study discovered that individuals with health education were more knowledgeable about rodent-borne infections compared to those without health education. This can be proved by the KIs participants as were able to mention rodent-borne diseases like plaque, leptospirosis, salmonellosis, rat bite fever, and toxoplasmosis. Whereas the FGD members were unable to describe any disease. Similarly, to our finding, the study by Salmón-Mulanovich *et al.* (2016) of Peru, documented that most of the participants could not mention any rodent-borne infection except for healthcare personnel. Additionally, KIs participants managed to describe the common routes of pathogens transmission from rodents to humans such as contaminated food and water by rodents' feces or urine. Moreover, they mentioned indications of rodent invasions at home including rodents' droppings, burrows, and runways in surroundings and unpleasant odor of urine from rodents. These findings suggest that improving public education will probably increase knowledge and practices towards rodent-borne diseases in the Ngorongoro district.

Conclusions and recommendations

According to this study, it was found that few participants had good knowledge and positive attitude toward rodent borne-diseases due to a lack of education. However, the communities showed good practices especially in avoiding consuming rodents, avoiding touching dead rodents with bare hands, and use of some rodent control methods particularly the use of cats and rodenticides. The majority didn't know that rodents may harbor and transmit infectious agents to humans and livestock. Therefore, it is justifiable to say that limiting human interaction with all wild and peri-domestic rodents, wearing protective gear during cleanliness, good hygiene, proper storage of food and cereals, and improving building designs to prevent rodents' inhabitation should all be part of the public health education initiatives in the Ngorongoro district. Although this study was done in a single district, it has shown that further needs to be done to educate communities in the Ngorongoro district about rodent controls and the associated risks of zoonoses. Further studies are required to understand the common rodent-borne diseases present in the population and their transmission dynamic among communities in places with a human-animal interface. The recent study has given a piece of useful baseline information that will help in planning the surveillance of rodent-borne zoonoses in the Ngorongoro district and other places worldwide.

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CHAPTER FIVE

PAPER FOUR

Knowledge, Attitudes and Practices on Rift Valley Fever Among Pastoral and Agropastoral Communities of Ngorongoro in the Rift Valley Ecosystem, Tanzania, Conducted In 2021/2022

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PLOS NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Knowledge, attitudes and practices on rift valley fever among pastoral and agropastoral communities of Ngorongoro in the rift valley ecosystem, Tanzania, conducted in 2021/2022

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Abstract

Epidemics of Rift Valley fever (RVF), a mosquito-borne zoonotic disease caused by RVF virus, have been linked to exceptionally heavy rainfall and widespread flooding. The disease is endemic in most African countries and pose a major global health risk. Given that the disease was reported in various districts of Tanzania, we hypothesized a lack of knowledge about RVF epidemiology among agropastoral and pastoral communities. The research took place in a period of 7 months, from July, 2021 to January, 2022. The aim of this study was to assess the knowledge, attitudes, and practices (KAP) among the agropastoral and pastoral communities of Ngorongoro district towards RVF. The survey employed a mixed method system, which included 3 focus groups (each comprised 12 individuals), 20 key informant interviews and administration of questionnaire (N = 352) in agropastoral and pastoral community members of Ngorongoro district. The relationship between demographic characteristics and communities' knowledge, attitudes, and practices regarding RVF was observed using a multiple logistic regression model. A total of 352 participants were interviewed, with the majority (67.61%) being male and 32.39% being female, majority (39.5%) attending primary school, and majority (58.2%) being pastoralists. The findings showed that only 36.1%, 38.64% and 16.19% of participants had good knowledge, positive attitude and good practices regarding RVF respectively. Significant demographic factors related with knowledge included: gender (OR = 1.9, CI = 1.03–3.56, P = 0.041), education levels (primary: OR = 3.97, CI = 2–8.16, P = 0.000; secondary: OR = 15.27, CI = 5.5–46.23, P = 0.000 and college: OR = 34. 23, CI = 5.4–67.22, P = 0.000), and locality (Pinyinyi: OR = 0.14, CI = 0.05–0.38, P = 0.000 and Sale: OR = 0.14, CI = 0.04–0.44, P = 0.001). Male participants showed significant positive attitude towards RVF compared to female (OR = 2.37, CI = 1.35–4.17, P = 0.003). Individuals with formal education showed a significant positive attitude toward RVF compared to informal (OR>1, P<0.05). Agropastoral members showed a significant negative attitude toward RVF compared to pastoralists (OR = 0.51, CI = 0.26–0.99, P = 0.048).

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The calculated RVF prevention practices values were insignificantly (P = 0.853) correlated with knowledge values. The significant correlation between knowledge and attitude, as well as attitude and practice were found (P<0.05). In general, the study revealed poor knowledge, negative attitude and poor practices of communities towards RVF. The lack of regular education programs to make the communities aware of the disease was implicated for these findings. This recommends that provision of health education should be a long-term practice among agropastoral and pastoral communities in order to prevent further RVF outbreaks in Tanzania.

Author summary

In the current study, we demonstrated that the KAP for RVF transmission and prevention is low among the pastoral and agropastoral community members in Ngorongoro district, Tanzania. The causes, route of transmission and signs of RVF in both humans and animals were mostly unknown to the respondents. The population was unaware that the high mortality rate of young animals and the miscarriage of pregnant animals were severe signs of RVF. The crucial preventive measures against RVF were not being used, which created a gap between recommended and real practices. Low level of knowledge about the RVF was linked to poor preventive efforts, so raising awareness could launch public awareness in a positive way. We suggest that important authorities should launch public awareness campaigns to help bridge these knowledge gaps and strengthen people's understanding about RVF. It might be necessary to use radio and mobile applications to provide the public with access to such health information. In order to alter behavioral patterns in the study communities to avert RVF occurrences, barriers to change must be identified and eliminated. The livelihoods of agropastoral and pastoralist communities will benefit if these procedures are put into action since they may prevent regular disruptions of livestock market chains that occur whenever RVF outbreaks happen.

Introduction

Rift Valley fever (RVF) is an ecologically complex arthropod-borne viral zoonotic disease caused by RVF virus, which belongs to order *Bunyavirales*, family *Phenuiviridae*, genus *Phlebovirus* [1]. The disease was originally documented in 1930 following a storm of abortions and sudden deaths in sheep along the coasts of Lake Naivasha in Kenya's greater Rift Valley [2]. Following infected livestock trade from the horn of Africa, RVF spread in September 2000 to Saudi Arabia and Yemen [3,4]. The largest outbreak in East Africa occurred in 1997–1998 and 2006–2007, which encompassed Kenya, Somalia, and Tanzania, with over 1000 confirmed positive cases and 300 deaths [5,6]. Between 1947 and 1978, four Tanzanian districts (Ngorongoro, Simanjiro, Monduli and Hai) experienced epidemics, as well as further outbreaks from1997 to 2007.

The RVF virus is maintained in infected mosquitoes and transmitted to animals via mosquito bites. Mosquito species in the genera *Aedes*, *Anopheles*, *Culex*, *Eretmapodites*, *Coquillettidia* and *Mansonia* have been associated with RVF virus transmission [7]. These vectors have been found in various regions throughout Africa [8]. Transmission of RVF virus by hematophagous (blood-feeding) flies is also possible [9]. In 1997–98, a major outbreak occurred in Kenya, Somalia, and Tanzania following an El Niño event causing extensive flooding [10]. Flooding triggers the hatching of eggs of *Aedes* mosquito species, which are the RVF virus's main reservoirs. According to studies conducted in various locations, the RVF virus is transmitted to domestic animals either through mosquito bites or direct contact with infected tissues or body fluids, particularly when abortions are involved [10].

The majority of human infections result from direct or indirect contact with the blood or organs of infected animals [10,11]. Human infections have also resulted from the bites of infected mosquitoes, most commonly the *Aedes* and *Culex* mosquitoes. The female mosquito is also capable of transmitting the virus directly to her offspring via eggs (transovarial) leading to new generations of infected mosquitoes hatching from eggs [10,11]. Consuming raw meat and milk, caring of sick animals, assisting parturition, and sheltering livestock inside residential houses are all reported risk factors for RVF virus infection in humans [11]. Affected animals, both clinically and sub clinically, are an important source of infection to humans; to date, no human-to-human transmission has been documented [12].

The RVF virus can cause mild to fatal illness in wildlife, domestic animals, and humans [12]. Studies reported high morbidity and mortality approaching 100% in lambs and kids of less than 12 weeks of age [5,6,13]. Hemorrhagic fever, mucopurulent nasal discharge, abortions, salivation, anorexia, fetid diarrhea, and uncoordinated gait are some of the symptoms in infected animals [5,6,13]. In humans, RVF virus can induce flu-like symptoms that can progress to a severe disease characterized by hemorrhagic fever and encephalitis, with a 1–5 percent fatality rate [14]. For a successful early warning monitoring system to respond to outbreaks in a timely manner, the communities must have appropriate knowledge of how to identify RVF indications in advance [14].

The socio-economic consequences of the RVF include animal and human fatalities, disruption of livestock market chains, human hunger, malnutrition, and monetary losses at the individual and national levels [13]. As a result of its socioeconomic implications, both veterinary and public health officials in Tanzania are concerned about the disease [15].

Apart from the disease's socioeconomic impact in Tanzania, just one study was undertaken to assess communities' knowledge about the RVF infections [16]. Because communities' knowledge and attitudes are associated with diseases management, Knowledge, Attitude and Practices (KAP) research is becoming increasingly important in infection prevention and control efforts [16,17]. Also, provided that RVF had occurred in several districts in Tanzania, we hypothesized a lack of sufficient knowledge about RVF epidemiology among agropastoral and pastoral communities. Therefore, the goal of a recent study was to identify and contribute to bridging information gaps by examining the level of knowledge, attitudes, and practices of agropastoral and pastoral communities in Ngorongoro district about RVF. The findings will contribute in building effective and long-term effective RVF prevention efforts. This would benefit pastoral and agropastoral societies whose livelihoods have been threatened by RVF outbreaks since they rely on the sale of livestock and livestock products.

Materials and methods

Ethics statement

The protocol to do this study was reviewed by Ethical Review Committee of the National Institute for Medical Research (NIMR) (Ref. No. NIMR/HQ/R.8a/Vol. IX/3676; 19th May, 2021). Also, Sokoine University of Agriculture provided the permission letter for conducting this study (Ref. No. SUA/ADM/R.1/8A/718; 3rd February, 2021). Moreover, the local administrative authorities of Arusha region (Ref. No. FA.132/95/01/38; 12th February, 2021) and Ngorongoro district (Ref. No. AB.114/354/01/134; 1st April, 2021) also gave permission. Prior to the start of face-to-face interviews, participant provided written informed consent. In cases where the participant was unable to write and read, verbal assent was obtained.

Description of the study area

The proposed study was conducted in five villages (Orgosorok, Malambo, Sale, Pinyinyi and Engarasero) of Ngorongoro district where animal and human cases of RVF were reported in the previous outbreaks [18]. The district is in the eastern Rift Valley ecosystem that suffered the outbreaks from 1947 to 1978 and 2006/2007 [18]. Ngorongoro District is one of the seven districts of the Arusha Region of Tanzania. It is bordered to the east by Monduli District, to the south by the Karatu District and to the west by the Mara Region. The district has an area of about 14,036 square kilometers located between latitudes 30.30'south of the equator and longitudes of 35.42 'east of Greenwich and is between 1,009 and 3,645 meters above sea level [18]. According to the 2012 Tanzania National Census, the population of the Ngorongoro district was 174,278 [19]. Administratively, the district is divided into three divisions (Ngorongoro, Loliondo and Sale) and 20 Wards. The district has a moderate temperature and tropical climate with average rainfall of 800 mm to 1,000 mm [19]. The major ethnic tribes are Masai and Sonjo, who depends on livestock keeping and sometimes on crop farming for their livelihoods [20]. The district is characterized by low undulating plains with low-lying altitude [20]. The main vegetation in the study area is shrubs of acacia species and grass; open and thick forests [20].

Study design and sampling procedures

A cross-sectional study was done in Ngorongoro district to investigate RVF knowledge, attitudes and practices among agropastoral and pastoral residents. The study population comprised of all households in selected villages and the sampling frame was the list of the households in each village. Villages were purposefully selected based on the following criteria: population density, accessibility of the area, located at Great Rift Valley, livestock and wild animal availability. The study was conducted in five villages namely Orgosorok, Malambo, Sale, Engarasero and Pinyinyi. Purposeful sampling was also used in the selection of households based on availability of animals like ruminants, dogs and rodents. At household level, the written consent of the household head was sought before the interview. The household head or any other resident person (18 years and above) was interviewed based on the assumptions like knowledge on diseases and ability of deciding on their participation in the study.

Sample size determination

This formula $n = \frac{Z^2 p(1-p)}{d^2}$ was used for estimation of the households' sample size [21], where: n = sample size estimate, Z = 1.96; standard normal variety at 5% error ($P<0.05$), and assuming a response distribution of 50%. (1-p) = the probability of knowledge and d = absolute error or precision (5%). The calculated household sample size (n) is 384. By considering the 5% mark up for missing samples; the infinite population size becomes 403. Then, the sample size adjusted to finite population (n_f) was computed using the equation; $n_f = n/(1+f)$ where; n = estimated sample size (403) and f = sampling fraction (403/14,195), this gave 392 number of households however only 352 households were interviewed in Ngorongoro district due to various challenges encountered during field work (unwillingness of individuals, difficult accessibilities of areas and reallocation of pastoralists). However, the 352 households interviewed had a statistical power of 0.9 value. This indicated that the results are statistically significant by 90% which is scientifically acceptable. The proportionate formula was applied (N = HW1 + HW2+ HW3) to determine the number of households to be interviewed in each village, where: N = total number of households interviewed in the district and HW1 + HW2 + HW3 = number of households in the selected village. Selection of hamlets in each village was done purposefully based on history of RVF cases and availability of animals as well as areas of humans-wildlife interface (word of mouth from the villages leaders).

Data collection

The researchers explained the study's purpose to all respondents, who included the local communities' leaders and government officials. Before being interviewed, participants gave written or oral consent to participate in the study. All collected data and information were confidentially handled and stored.

Questionnaire survey. Based on literature review of Rift Valley fever, a semi-structured questionnaire tool was established [22]. The survey was divided into five categories: (i) respondent demographics; (ii) household characteristics (iii) knowledge on RVF virus vectors, transmission modes in humans and animals, and clinical signs; (iii) attitude toward RVF management and (v) practices against prevention of RVF. To ensure that questions were appropriate, the questionnaire was pretested in 20 households and amended according to the problems recognized. The questionnaire was filled by visiting one house after another in each study village. Enumerators who were fluent in English and Swahili languages were deployed in administration of the questionnaire. Questions were asked in Swahili and responses recorded in English to serve time for retranslation. Investigators kept a close eye on the questionnaire administration process and double-checked the completed form to ensure that the acquired data was of high quality.

Interviews with the key informants (KIs). In the Ngorongoro district, a total of twenty KI interviews were done to collect more information about Rift Valley fever. The study employed a targeted sample technique, and participation was entirely voluntary. Local collaborators at each study ward/village identified participants; four individuals per village. The community health workers, nurses, clinical officers, assistant field livestock/ livestock officers, veterinary/medical officers and elected local authority leaders were among those who participated in the interview. Participants were asked on the RVF etiology, transmission, symptoms, prevention, socioeconomic implications, risk factors and dissemination of education among communities. The interviews were conducted by a trained researcher, and notes were collected by the note takers. The interviews were also recorded using a phone recorder and the audio records were transcribed.

Focus group discussions (FGD). We conducted three FGD and each group comprised 12 respondents [22]. Purposeful sampling was used in selecting participants for the FGD, targeting on adults (aged 18years and above) with confidence in talking, permanent residents, animals' keeper and person who didn't participate in questionnaire. The FGD was facilitated by a trained community health worker or livestock officer together with the researcher and there were two note takers. A semi-structured FGD guide was created and used during discussions. FGD participants were asked on transmission of RVF virus, signs of RVF in both humans and animals, control and prevention, risk factors of RVF and socioeconomic impact of RVF in the communities.

Data analysis

Quantitative data analysis. For each question in the knowledge part, scores ranging from 1 to 5 were given to correct responses based on the question type. Furthermore, incorrect and don't know responses were assigned zero scores. A knowledge score for each respondent was calculated by summing the number of correct answers out of the total scores [23]. Correct answers were assigned a score of 1, and answers that were incorrect, or selected as "I don't know", were assigned a score of 0 [23].

Attitudes regarding RVF prevention and control measures were assessed using the Likert scale system, with responses ranging from 1 to 5, with 1 = completely disagree; 2 = disagree; 3 = neutral; 4 = agree and 5 = completely agree [22,24]. During cross tabulations analysis, responses were dichotomized into (1) completely disagree/disagree/neutral and (2) completely agree/agree. Correspondents who completely agreed or agreed were reported to have positive attitudes toward RVF occurrence, while the rest were considered to have negative sentiments [22]. In the Practices section, each correspondent's score was calculated by adding the number of correct responses to the nine questions posed.

The variables in the data were coded for easy entry and analysis. Data were entered into Microsoft Excel 2010 and edited to remove invalid variables and thereafter, exported to R software version 4.1.0 (2021) for analysis. Findings were presented in descriptive statistics like means, proportions and frequencies. Comparison of proportions for categorical variables such as socio-demographics, knowledge and practices were conducted by deploying the Chi-square test. The outcome variables were knowledge about RVF and preventive practices of RVF. Relationship between various factors and knowledge on RVF, attitude or practices on taking into account about the influence was assessed using multiple logistic regression. Odd ratios (ORs) and their corresponding's 95% confidence interval (CIs) were calculated and $P<0.05$ was considered significant for all statistical analyses.

Qualitative data analysis. A deductive analysis approach was used for qualitative data collected from FDG and Key Informants interviews [25], whereas the researcher developed themes from the RVF literature review. FGD and Key Informants information was summarized manually according to themes presented in the discussions and interviews. Results are described in the text together with relevant speech marks.

Results

Quantitative data from questionnaire

Demographic characteristics of the studied communities. In the Ngorongoro district, a total of 352 personnel took part in the survey, with the majority of them being men (n = 238, 67.61%). Their median age group was 25–34 years old and the age ranged from 18–65 years. The majority of the population went to primary school (n = 139, 39.5%). Also, the majority (n = 205, 58.2%) were pastoralists (Table 1).

Household characteristics. Among 352 households visited during this study, 349 (99.15%) were headed by men and 3 were (0.85) headed by woman. A high proportion (48%) of the household size was found to be 1–5 members in each household in Ngorongoro district. About 35.2% of respondents own houses constructed by using animal feces (Table 2). Furthermore, 41.2% of houses have open windows and grasses were found to be the preferred thatching materials of roofs. Lastly, the results show that about 53.4% of households use a pit latrine. With the exception of toilets type, other variables were highly significant different (Table 2).

Sources of various Information among agropastoral and pastoral communities. The study found that communities in the study area prefer use of phone (42.8%) and radio (29%) as the major source of communication (Table 3).

Knowledge about mosquitoes management and their zoonoses. The analysis of the knowledge score indicated that scores of respondents ranged from 0 to 13 out of a total of 17 total points. Among the participants, only 36.1% (n = 127) managed to get above 50% of the total scores, showing poor knowledge of the community. The most known mosquito borne disease is Malaria as stated by 89.22% of respondents. 88.35% of participants reported the use of bed nets as the main preventive measure of mosquito bites (S1 Table).

Table 1. Respondents demographic characteristics in Ngorongoro district.

Variable	Female n (%)	Male n (%)	Total N (%)
Age group			
Youth (18–34 years)	57(16.2)	89(25.3)	146 (41.5)
Adult (35–64 years)	54(15.3)	138(39.2)	192(54.5)
Elderly (≥65 years and above)	3(0.9)	11(3.1)	14(4.0)
Level of education			
Not attended school	58(16.5)	71(20.1)	129(36.6)
Did not completed school	7(2.0)	23(6.5)	30(8.5)
Primary	40(11.4)	99(21.1)	139(39.5)
Secondary	8(2.3)	35(10.0)	43(12.3)
College	1(0.3)	10(2.8)	11(3.1)
Religion			
Christian	84 (24.0)	165 (47.0)	249 (71.0)
Muslim	2 (1.0)	15 (4.0)	17 (5.0)
Traditional	28 (8.0)	58 (16)	86 (24.0)
Marital status			
Single	8(2.3)	15(4.2)	23(6.5)
Married	106(30.1)	223(63.4)	329(93.5)
Occupation			
Agropastoral	53(15.1)	94(26.7)	147(41.8)
Pastoralist	61(17.3)	144(40.9)	205(58.2)
Locality (Villages)			
Orgosorok	37(10.5)	74(21.0)	111(31.5)
Malambo	16(4.5)	38(10.8)	54(15.3)
Sale	27(7.7)	64(18.2)	91(25.9)
Engaresero	15(4.3)	30(8.5)	45(12.8)
Piyaya	19(5.5)	32(9.0)	51(14.5)
Total number of respondents	**114 (32.39)**	**238(67.61)**	**352(100)**

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Relationship between respondent factors and good knowledge about mosquitoes. Most of the respondents' demographic factors had insignificant positive influence on knowledge about mosquito-borne zoonosis and control measures ($P>0.05$). Only education level was found to have significant positive influence on knowledge about mosquito borne diseases as well as on control measures ($P<0.05$), as indicated in Table 4.

Knowledge of the communities about rift valley fever symptoms and transmission. The analysis of knowledge score demonstrated that out of total points (17), respondents score ranged from 0 to 14 points. Whereby, only 37 respondents equivalent to 10.51% scored above the average of total score, implying low level of knowledge of the communities. Among 352 total participants, 52% respondents had heard about RVF and 25% of them knew that the disease is zoonotic (S2 Table). 45.74% of respondents (n = 161) mentioned ruminants as the group of animals that can succumb from RVF infection. Considering signs of RVF infection in humans, febrile fever 13.35% followed by headache 10.23% symptoms were frequently mentioned. In regard to signs of RVF in animals, 30.4% and 17.61% participants mentioned fever followed by abortion as major signs of RVF, respectively. Mosquito bites as the significant known route of RVFV transmission in animals was mentioned by 13.64% of respondents (S2 Table).

Relationship between demographic factors and good knowledge about RVF. The results of this study show that the communities' knowledge on RVF is significantly related to

Table 2. Respondents household characteristics based on the study villages.

Variable	Engarasero n (%)	Orgosorok, n (%)	Malambo n (%)	Pinyinyi n (%)	Sale n (%)	Total N (%)	χ^2 tests	P-value
Household size							64.915	<0.0001
1–5	20(11.8)	71(42.0)	30(17.8)	19(11.2)	29(17.2)	169(48.0)		
6–10	17(12.7)	33(24.6)	13(9.7)	21(15.7)	50(37.3)	134(38.1)		
Above 10	8 (16.3)	7 (14.3)	11 (22.4)	11(22.4)	12(24.5)	49(13.9)		
House walls							171.27	<0.0001
Block	1 (2.1)	23(47.9)	5(10.4)	2(4.2)	17(35.4)	48 (13.6)		
Block with concrete	0(0.0)	9(75.0)	0(0.0)	3(25.0)	0(0.0)	12(3.4)		
Mud	0(0.0)	48(28.6)	1(0.6)	46(287.4)	73(43.5)	168(47.7)		
Animal feces	44(35.5)	31(25.0)	48(38.7)	0(0.0)	1(0.8)	124 (35.3)		
Type of window							169.86	<0.0001
No window	24(15.7)	43(28.1)	15(9.8)	9(5.9)	62(40.5)	153(43.5)		
Open window	14(9.7)	44(30.3)	35(24.1)	32(22.1)	20(13.8)	145(41.2)		
Net window	5(21.7)	9(39.1)	1(4.3)	3(13.0)	5(21.7)	23(6.5)		
Window with shutters	2(6.5)	15(48.4)	3(9.7)	7(22.6)	4(12.9)	31(8.8)		
Roof of the house							118.38	<0.0001
Grass	33(16.8)	42(21.4)	38(19.4)	28(14.3)	55(28.1)	196(55.7)		
Mud and grass	0(0.0)	27(90.0)	3(10.0)	0(0.0)	0(0.0)	30(8.5)		
Iron sheet	12(9.5)	42(33.3)	13(10.3)	23(18.3)	36(28.6)	126(35.8)		
Source of electricity							255.57	<0.0001
Neither	38(16.0)	67(28.3)	38(16.0)	38(16.0)	56(23.6)	237(67.3)		
Solar	7(6.2)	42(37.2)	16(14.2)	13(11.5)	35(31.0)	113(32.1)		
TANESCO*	0(0.0)	2(100.0)	0(0.0)	0(0.0)	0(0.0)	2(0.6)		
Type of toilets							1.636	0.2008
No toilet	24(14.6)	27(16.5)	31(18.9)	6(3.7)	76(46.3)	164(46.6)		
Pit Latrine	21(11.2)	84(44.7)	23(12.2)	45(23.9)	15(8.0)	188(53.4)		

TANESCO = Tanzania Electric Supply Company Limited

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demographic characteristics like sex, level of education and location (P<0.05) as presented in Table 5 below. Males, respondents with primary, secondary and college education demonstrated significant (P<0.05) high knowledge, while inhabitants of Pinyinyi and Sale were found to have significant (P<0.05) less knowledge about RVF compared to those of Engarasero (Table 5).

Communities' attitude toward RVF transmission and mosquitoes' management. The results indicate that among 352 respondents, 269 (76.42%) and 83 (23.35%) showed positive

Table 3. Source of information dissemination among study villages in Ngorongoro district.

Variable	Engarasero n (%)	Orgosorok n (%)	Malambo n (%)	Pinyinyi n (%)	Sale n (%)	Total N (%)	χ^2 tests	P-value
Source of information							289.43	P<0.0001
Gatherings	12(23.5)	15(29.4)	8(15.7)	3(3.9)	13(25.5)	51(13.3)		
Health servants	3(14.3)	8(38.1)	7(33.3)	0(0.0)	3(14.3)	21(5.5)		
Veterinary servants	3(14.3)	8(38.1)	7(33.3)	0(0.0)	3(14.3)	21(5.5)		
Phone	22(13.4)	46(28.0)	25(15.2)	26(15.9)	45(27.4)	164(42.8)		
Radio	6(5.4)	44(39.6)	17(15.3)	18(16.2)	26(23.4)	111(29.0)		
TV	3(20.0)	2(13.3)	0(0.0)	4(26.7)	6(40.0)	15(3.9)		

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Table 4. Multiple logistic regression analysis of the relationship between demographic factors and good knowledge about Mosquitoes borne diseases.

Variable	OR	Confidence interval (CI, 95%)	P-value
Sex			
Female	Reference		
Male	1.13	0.58–2.28	0.693
Age			
Elderly	Reference		
Adult	1.17	0.22–6.32	0.839
Youth	1.08	0.19–6.11	0.092
Level of education			
Not attended school	Reference		
Did not completed school	1.62	0.39–6.86	0.509
Primary	3.55	1.51–8.38	0.004**
Secondary	9.02	3.15–25.83	0.000***
College	17.32	3.82–78.51	0.000***
Occupation			
Pastoralist	Reference		
Agropastoral	0.61	0.29–1.28	0.191
Religion			
Muslim	Reference		
Christian	1.96	0.22–16.76	0.550
Traditional	3.48	0.39–31.13	0.265
Marital status			
Single	Reference		
Married	0.91	0.28–2.91	0.872
Ward (locality)			
Engaresero	Reference		
Malambo	0.60	0.19–1.83	0.368
Pinyinyi	1.43	0.51–4.06	0.497
Sale	0.86	0.27–2.73	0.801
Orgosorok	1.03	0.41–2.55	0.951

* = Significant at P< 0.05.

** = Moderately significant at P <0.005.

*** = Highly significant at P <0.0001

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and negative attitude toward mosquito-borne diseases, respectively. The majority of respondents agreed that mosquitoes have adverse effects on their quality of life and they are at risk of getting diseases through mosquito bites (S3 Table).

Relationship between factors and attitude toward mosquito borne diseases. The results of the logistic regression model indicated that sex, level of education, marital status and locality have significant ($P<0.05$) influence on attitude toward mosquitoes borne diseases and management (Table 6). Male respondents showed more positive attitude compared to female one and the difference was significant ($P = 0.006$). Individuals with primary and secondary level of education were found to have a more positive attitude compared to those didn't attend school, and this difference was statistically significant ($P<0.05$).

Communities' attitudes toward Rift Valley Fever transmission and prevention. Among 352 participants, 136 (38.64%) and 216 (61.36%) respondents showed positive and negative attitudes, respectively. The findings showed that most of participants neither "agrees"

Table 5. Multiple logistic regression analysis of the relationship among demographic factors and good knowledge on RVF signs and transmission.

Variable	OR	Confidence interval (95%)	P-value
Sex			
Female	Reference		
Male	1.90	1.05–3.56	0.041*
Age			
Elderly	Reference		
Adult	0.77	0.22–2.93	0.686
Youth	0.45	0.12–1.80	0.239
Level of education			
Not attended school	Reference		
Did not completed school	1.03	0.21–4.01	0.944
Primary	3.97	2.00–8.16	0.000***
Secondary	15.27	5.50–46.23	0.000***
College	34.23	5.40–67.22	0.001***
Occupation			
Pastoralist	Reference		
Agropastoral	1.72	0.85–3.52	0.135
Religion			
Muslim	Reference		
Christian	1.02	0.20–7.73	0.987
Traditional	0.72	0.14–5.63	0.724
Marital status			
Single	Reference		
Married	2.23	0.67–8.23	0.205
Villages (locality)			
Engaresero	Reference		
Malambo	0.47	0.18–1.20	0.118
Pinyinyi	0.14	0.05–0.38	0.000***
Sale	0.14	0.04–0.44	0.001***
Orgosorok	0.95	0.39–2.27	0.914

* = Significant at P< 0.05.

** and *** = Highly significant at P <0.0001

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nor "disagrees" about the statements posed to them about RVF. The majority of respondents didn't decide on RVF being a hazardous disease of public health importance. The majority of respondents didn't decide on being at risk of getting RVF virus infection. The majority of them didn.t decide on wearing protective gears can prevent transmission of RVF virus, and that the interaction between humans and animals can facilitate RVF transmission (S4 Table)

Relationship between demographic factors and attitudes toward RVF transmission. The results indicated that sex, education and occupation have a significant ($P<0.05$) influence on communities' attitudes toward RVF transmission and prevention. Males have a significant ($P = 0.003$) positive influence on attitude towards RVF. Also, individuals who attended school found to have a significant (P,0.05) positive influence on attitude toward RVF transmission and prevention (Table 7). Moreover, agropastoral were found to have a significant ($P = 0.048$) negative influence on attitude toward RVF.

Communities' practices on RVF transmission and prevention. The analysis of participants practices was characterized as effective infection prevention practices or non-effective

Table 6. Multiple logistic regression analysis of the relationship between demographic factors and positive attitude toward Mosquito borne diseases.

Variable	OR	Confidence interval (CI, 95%)	P-value
Sex			
Female	Reference		
Male	2.29	1.26–4.14	0.006**
Age			
Elderly	Reference		
Adult	1.02	0.23–4.11	0.981
Youth	0.93	0.22–3.95	0.917
Level of education			
Not attended school	Reference		
Did not completed school	1.51	0.53–4.34	0.444
Primary	2.44	1.21–4.91	0.013*
Secondary	5.57	1.61–19.26	0.007**
College	1.36e+07	0.00-Inf	0.981
Occupation			
Pastoralist	Reference		
Agropastoral	0.66	0.31–1.41	0.289
Religion			
Muslim	Reference		
Christian	0.34	0.06–1.81	0.206
Traditional	0.31	0.06–1.39	0.139
Marital status			
Single	Reference		
Married	3.41	1.14–10.17	0.028*
Villages (locality)			
Engaresero	Reference		
Malambo	0.53	0.15–1.70	0.270
Pinyinyi	8.65	0.89–83.39	0.062
Sale	0.44	0.12–1.58	0.207
Orgosorok	0.22	0.07–0.65	0.006**

* = Significant at P< 0.05, and

** = Moderately significant at P <0.005.

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infection prevention practices (effective when the score was above 50% and non-effective when the score was below the 50% of total score). The maximum score was 11, and 57 (16.19%) of the 352 respondents showed effective infection prevention practices. The majority of participants (n = 336 or 95.5%) do not avoid contacting sick or dead animals' fluids or handling aborted fetuses with their bare hands (S5 Table). 60.23% drink fresh raw animal blood and 77.27% keep animals in their sleeping area (S5 Table). Bed netting, insecticide sprays, and repellent ointments, as well as the treatment of stagnant water, were reported as being used in the communities to manage mosquito populations and prevent mosquito bites. However, the majority (35.51%) of respondents reported the use of bed nets as the preferred control measure against mosquito bites (S5 Table).

Multiple logistic regression analysis of factors and preventive practices of RVF. Concerning relationships among demographic characteristics and effective preventive practice of RVF, the performed logistic regression model indicated that respondents with primary and secondary education levels had significantly (P = 0.018) positive influence on preventive

Table 7. Multiple logistic regression analysis of the relationship between demographic factors and positive attitude toward RVF transmission and prevention.

Variable	OR	Confidence interval (CI, 95%)	P-value
Sex			
Female	Reference		
Male	2.37	1.35–4.17	0.003**
Age			
Elderly	Reference		
Adult	0.49	0.15–1.63	0.245
Youth	0.37	0.11–1.31	0.125
Level of education			
Not attended school	Reference		
Did not completed school	2.89	1.12–7.43	0.027*
Primary	2.42	1.31–4.47	0.005**
Secondary	4.15	1.74–9.88	0.001**
College	46.25	5.19–411.7	0.001**
Occupation			
Pastoralist	Reference		
Agropastoral	0.51	0.26–0.99	0.048*
Religion			
Muslim	Reference		
Christian	0.43	0.13–1.45	0.174
Traditional	0.35	0.11–1.16	0.087
Marital status			
Single	Reference		
Married	2.70	0.85–8.62	0.094
Villages (locality)			
Engaresero	Reference		
Malambo	0.53	0.22–1.37	0.196
Piyaya	1.03	0.45–2.40	0.957
Sale	1.25	0.46–3.41	0.668
Orgosorok	0.83	0.37–1.88	0.661

* = Significant at P< 0.05, and ** = Moderately significant at P <0.005

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practices (Table 8). On other the other hand, individuals of Malambo and Engaraseru had significant (P<0.05) negative influence on effective preventive measures of RVF transmission compared to those of Orgosorok (Table 8). While those of Sale village had significant (P = 0.000) positive influence on preventive practices of RVF compared to Orgosorok's respondents. Moreover, pastoralists had significant (P = 0.005) positive influence on effective preventive practices of RVF than agropastoral community (Table 8).

Correlation between knowledge, attitude and practice. Results of this study indicated that knowledge about RVF was insignificantly (P = 0.853) negatively correlated with RVF effective preventive practices. Also, results indicates that there is a significant relationship between knowledge and attitude, as well as attitude and practice (P<0.05) as shown in Table 9.

Qualitative information from KI Interviews and FGD

Health education delivery system to the communities in Ngorongoro district. During the Key Informant (KI) Interviews, the participants were probed on how the communities get

Table 8. Multiple logistic regression analysis of the relationship between demographic factors and effective preventive practices of RVF.

Variable	OR	Confidence interval (CI, 95%)	P-value
Sex			
Female	Reference		
Male	0.79	0.46–1.37	0.408
Age			
Elderly	Reference		
Adult	1.41	0.41–4.81	0.586
Youth	1.48	0.41–5.30	0.549
Level of education			
Not attended school	Reference		
Did not completed school	2.74	0.92–8.11	0.069
Primary	2.43	1.33–4.44	0.004**
Secondary	4.12	1.60–10.39	0.003**
College	2.99	0.72–12.43	0.131
Occupation			
Agropastoral	Reference		
Pastoralist	2.53	1.31–4.87	0.005**
Religion			
Muslim	Reference		
Christian	1.86	0.46–7.51	0.383
Traditional	1.62	0.42–6.29	0.483
Marital status			
Single	Reference		
Married	0.46	0.15–1.44	0.188
Villages (locality)			
Orgosorok	Reference		
Malambo	0.47	0.22–0.99	0.043*
Piyaya	1.89	0.85–4.16	0.116
Sale	6.02	2.40–15.07	0.000***
Engaresero	0.28	0.12–0.62	0.002**

* = Significant at P< 0.05.

** = Moderately significant at P <0.005.

*** = Highly significant at P <0.0001

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Table 9. Correlation of total knowledge score, total attitude score and total practice responses score of respondents about RVF disease.

Scores	Mean	SD	N	Correlation coefficient	P- value
Total knowledge score	2.912	2.656	352	-0.089	0.053
Total practice score	4.821	1.099			
Total knowledge score	2.912	2.656	352	0.543	0.000***
Total attitude score	3.716	3.531			
Total attitude score	3.716	3.531	352	0.162	0.002**
Total practice score	4.821	1.099			

** = Moderately significant at P <0.005.

*** = Highly significant at P <0.0001

https://doi.org/10.1371/journal.pntd.0011560.t009 information about diseases and preventive measures to adopt. Health workers often conduct health education campaigns at the community level depending on availability of funds (Key Informants). Health workers deliver health education to the community level but with difficulties due to inadequate facilitation from the government (Key Informants). Health workers in Pinyinyi and Sale villages usually have a regular weekly and monthly outreach training at the community level (Key Informants). They provide health education to individuals or patients who attend clinics in working facilities like dispensaries and health centers or hospitals. They give education to targeted groups of people like pregnant women. Therefore, the provision of health education depends on government's priorities and the availabilities of funds (Key Informants).

Livestock vaccination program in Ngorongoro district. In the last RVF episode in 2007, the government conducted vaccination of small ruminants in the district. The local authority and ministry of livestock together organized the vaccination program in various places of RVF outbreaks (Key informants). The interviews with participants indicated that the intervention was carried out late after livestock acquired the infection (FGD members). Vaccination process to small ruminants was also performed in 2011 and 2012 in RVF outbreak prone villages (Key informants).

Socio-economic impact of RVF in the communities of Ngorongoro district. During the FGD and key informants' interviews, participants described several socio-economic impacts as demonstrated in the following quotes: "During the outbreak, there were high mortality of sheep and goats along the Rift Valley ecosystem in villages like Malambo, Engarasero and Pinyinyi (Key informants). Also, there was a deficiency of food particularly to the pastoralists community that mostly depends on meat and milk diets (FGD respondent). Furthermore, there was lack of income as the government closed the livestock markets and restricted animals' movement (KIs and FGD). During outbreaks, approximately 35 individuals were suspected of dying from RVF infection. These cases involved the livestock keepers who consumed sick or dead animals in their households (Key informants).

Socio-cultural behaviors of the agropastoral and pastoralists of Ngorongoro. Several cultural behaviors were perceived in this study as described by FGD and KIs respondents during the discussions and interviews. The risk behaviors included drinking raw milk and blood, consumption of raw meat (kidneys and livers), sheltering of young animals in human houses, handling of aborted fetuses with bare hands and the use of animal feces for construction of human houses. However, some of the respondents stated that some of the livestock keepers have reformed their habits of consuming dead animals and raw livestock products such as milk, blood, and meat. Changes were due to the government education campaign in the last RVF outbreak intervention where the communities were educated about health risks of using raw livestock products ((Key informants). The government authorities-imposed restriction on eating of dead, sick animals and raw livestock products so as to reduce the risks of contracting RVF (Key informants). Despite education, some of the pastoralists continue with consumption of raw meat, milk and fresh blood on believing that they will be healthy and strong (Key informants). Some of the respondents believe that raw products contain many nutrients compared to cooked ones, thus why they prefer them (FGD members).

Climatic factors associated with RVF. Most Key Informants mentioned high temperature and heavy rainfall as the major climatic factors associated with occurrence of Rift valley fever. In comparison to other villages that are not located along the rift valley, the villages along the rift valley experience extremely hot environments. For instance, it's extremely hot in Engarasero and Pinyinyi villages (Key informants). According to participants of the Engarasero, mosquitoes remain an issue throughout the year in some villages in the rift valley ecosystem (FGD members). Additionally, villages in the Rift Valley region become flooded whenever the year's heavy rains have fallen (Key informants).

Discussion

The aim of our study was to assess the knowledge, attitude and practices toward RVF disease. In this present study, a total of 352 people aged 18–65 years were interviewed, with the majority being men (67.61%). This result was probably due to Masai customs where women are not allowed to give information without permission of the heads of household [26]. Consequently, women hesitated at taking part in this study. As a result, our study has a higher proportion of male respondents than a comparable study conducted in Kenya by Abdi et al. [22], who reported that only 53.06% of respondents were men. Moreover, the majority of participants were found to be at an age group of 25–34 years old. This result was attributed by voluntary participation of this age group in the study compared to other age groups (Researcher's observation).

Similarly, the study found that majority (39.5%) of respondents had primary education and 58.2% were pastoralists. It is known that pastoralist tribes send children to primary school or not at all in order to take care of livestock or to protect their cultures [26]. Only few individuals allowed to join secondary school and higher studies. These results are based on the history of Masai and Sonjo tribes whose origin is livestock keeping. These are ethnic tribes inhabited the Ngorongoro district in high percentages [26]. Our study populations had lower educational levels than a similar study done in Uganda; approximately 12% of our participants had secondary educations, compared to 55% of participants in Uganda [27]. In comparison to a study by Abdi et al. [22], in Kenya, our study identified a lower proportion (36.6%) of respondents with informal education compared to a higher proportion (88%) of respondents who received informal education in Kenya.

Dissemination of information about diseases among the communities is crucial for safeguarding the public's health. According to our findings, the majority of the participants in Ngorongoro had heard of RVF. This was not unexpected as the Ngorongoro district had experienced several RVF epidemics between 1947 to 1978, and from 1997 to 2007 [2,18]. The majority of the participants reported to get information about diseases through radio and calls from friends or family members and other livestock keepers from Kenya. Sometimes they get information from the community meetings and government officials. These results are probably due to the fact that many agropastoral and pastoralists in the study area live in environments that lack a reliable source of electricity. Lack of expertise in livestock and agriculture sectors in some villages of Ngorongoro district can contribute to poor dissemination of information to the communities (Researcher's observations). Additionally, inadequate facilitation from the government may lead to insufficient dissemination of health education among the communities.

The assessment of knowledge and attitude among communities about mosquito-borne infections is important in planning management strategies. This study revealed that 36.1% and 76.42% of participants had good knowledge and positive attitudes on mosquito-borne diseases, respectively. Whereas the majority (89.22%) of them identified malaria as the only disease spread by mosquito bites. These results were probably due to various initiatives campaigns to eradicate malaria carried out here in Tanzania [28]. For instance, the Minister of Health announced the following: "our responsibility is to ensure that the prevalence of malaria decreases up to zero percent. For this reason, we must have a continuous exercise of distributing long-lasting insecticides treated bed nets to all households in Tanzania. Similarly, we must use health centers to reach the primary beneficiaries: mothers who attends clinics and children under one year old" [28]. Our study revealed the benefits of government's initiatives because majority (88.35%) of participants reported the use of mosquito nets. This finding was probably due to health education on prevention of malaria provided to the communities by the health workers.

Knowledge about the modes of transmission of RVF infection is critical for executing proper deterrent measures and thereby lowering the likelihood and magnitude of RVF epidemics. The majority of respondents surveyed in this study were unaware of mosquitoes' role as the principal vectors for transmission of RVF virus to animals. Only 13.64% of respondents knew that RVF virus is transmitted through mosquito bites, in spite of believing that mosquitoes have adverse effects to livelihood. The majority of them reported malaria as the major mosquito-borne disease. These findings are almost parallel to those reported in studies conducted in Tanzania [16], Kenya [22] and Democratic Republic of Congo [29].

Correspondingly, this study evaluated the general knowledge, attitude and practices toward RVF among agropastoral and pastoral communities. The study found that 36.1%, 38.64% and 16.19% of participants had good knowledge, positive attitude and effective infections preventive practices toward RVF, respectively. Generally, these results indicated that the studied communities had poor knowledge, negative attitude and non-effective infection preventive practices toward RVF disease. Similar results were found in various studies done in Africa where agropastoral and pastoral societies found to have low KAP regarding RVF occurrences [16,22,30,31,32,33] The lack of education may have influence on KAP of the communities toward interventions that could reduce risks of RVF virus transmission and RVF epidemics.

Despite the fact that 52% of participants had heard of RVF, the majority were unable to identify the most significant signs of the disease in both humans and animals. Fever (increased body temperature) was the most prevalent symptom reported in both humans and animals. Regarding the signs of RVF in humans, unlike fever and headache, other symptoms (muscle pain, joint pain, backache, blurred vision and lethargy) were quantified by less than 11% of the participants. The outcomes of our study differ from the study by Abdi et al. [22] in Kenya, who reported that the majority (92%) of respondents identified hemorrhage as the key sign of RVF in humans and other signs by less than 40%. These findings suggest that most of participants are unaware of how RVF manifests clinically in humans. This was probably due to lack of regular educational campaigns about RVF in some villages in Ngorongoro district.

Despite the fact that Ngorongoro district had experienced numerous outbreaks of RVF, this study showed that the communities had poor knowledge regarding RVF clinical signs in animals. Apart from hemorrhagic fever, abortion and fetid diarrhea other signs were pronounced by less than 8% of participants. This was in contrast to research conducted among livestock keepers in Tanzania (poor awareness of RVF clinical indications was reported with a score of less than 50%) and in Kenya (knowledge score of less than 25%) [22,32]. This indicates that people forget what they learn about RVF from earlier epidemics as time passes and this reinforces the need for consistent and continued education and sensitization of communities outside of active outbreaks.

Furthermore, the findings revealed that only around 8% of the communities' members were aware that consumption of raw meat and milk, direct contact with contaminated surfaces or handling of aborted fetuses on bare hands, and sheltering animals in their houses are all risk factors for RVF transmission. The results of this study, which showed that raw livestock products are consumed by the highest proportion, reflect findings from previous research conducted in Kenya [22, 32, 33, 34], Uganda [27] and Tanzania [16]. The study conducted in Kenya documented that pastoralist communities shelter livestock in human houses and drink raw milk, regardless of health education provided during RVF epidemics [22,32,34]. These risk factors increase the likelihood of the communities in Ngorongoro district and their livestock to get RVF infections and other zoonoses. Therefore, the government should keep on educating the communities on risks of consuming raw livestock products and use of protective gears during handling of sick or dead animals.

According to this survey, the majority of respondents do not consider Rift Valley Fever to be a dangerous disease of public health concern, despite the fact that RVF outbreaks are common in Ngorongoro district. Also, they don't believe that they are at a high risk of contracting RVF. Moreover, the majority of them do not feel that wearing protective gears during the environmental cleanliness will protect them from catching RVF. Lastly, despite the existence of wild animals in Ngorongoro district, the majority of respondents do not believe that interactions among humans, domestic, and wild animals can facilitate the spread of the RVF virus. The lack of communities' awareness about the disease was linked to the participants' negative attitudes in this study (P = 0.000). This suggests that health education should be a continuous practice in agropastoral and pastoral communities in order to prevent the occurrence of RVF infection. These outcomes correspond well with findings reported in various surveys conducted worldwide, in which communities expressed a negative attitude towards RVF transmission [22,31].

The recent study indicated that general knowledge about RVF is not related with age, marital status and religion but significantly related to sex, education and locality. Males were more likely aware of RVF compared to the females (P = 0.04). This is probably connected with socio-cultural practices of Maasai people, where males are mostly involved in animals' diseases management compared to the females. The finding of this study differs from those reported by Abdi et al. [22], in Kenya, whereby knowledge was not linked to sex. Also, individuals with formal education (primary, secondary and college) were found to be more knowledgeable on RVF than informal ones ($P<0.05$). This result implies that level of knowledge can in fact be influenced by level of education. Similar results were documented by Owange et al. [10] in Kenya where, respondents with tertiary education were more knowledgeable on RVF. Lastly, the residents of Pinynyi and Sale villages demonstrated good practices of RVF prevention compared to those of Orgosorok ($P<0.05$). This finding is attributed by a well-organized health delivery system in these villages (Key Informants in Pinyinyi and Sale). The community health workers usually conduct a monthly field visit for the purpose of health education deliverance in their communities (Key Informants in Pinyinyi). Therefore, improving publics' education will lead into satisfactory knowledge which in turn will enhance good practices.

Moreover, our study revealed that during the last RVF outbreak in 2007, the government conducted vaccination campaign to the small ruminants. Unfortunately, the intervention came after livestock had already developed RVF infection. Since vaccination was done late, the livestock keepers did not see good recovery impact as their livestock continued to die. Therefore, the livestock keepers advised that the vaccine should be delivered before the RVF manifests (between December and June). Additionally, the government continued to vaccinate sheep and goats in 2011 and 2012 even though there were no RVF cases. After that, no further vaccination took place in Ngorongoro, so the livestock keepers stopped vaccinating their livestock. The government was graciously requested by the communities to enhance vaccination initiatives through the private sector so as to prevent RVF epidemics from recurrences in Ngorongoro and the country as a whole.

Lastly, this study found that most of the Key Informants were aware of climatic factors associated with RVF occurrences. Most of the them were managed to associate the last epidemic in 2007 with high temperature and heavy rainfall. They defined that the villages along the Rift valley ecosystem were heavily flooded and there was massive death of livestock during the outbreak. The previous studies conducted in Kenya [22,32,33] and Tanzania [2,16,18] had linked these factors with the RVF epidemics in the eastern ecosystem of Rift Valley. The eastern Rift Valley ecosystem is characterized by bimodal rainfall patterns which give suitable environment for growth and survival of vectors [2,18].

Limitations of the study

Because this study was conducted 14 years since the last RVF epidemic in 2007, the results may have been influenced by failure to recall phenomena. The KAP survey was established to collect baseline information in order to inform the government of the current situation in RVF-prone areas. Finally, because the participants were questioned about their knowledge, attitudes, and practices regarding RVF, there may be bias in responses based on how participants wish to fit in the KAP.

Conclusions and recommendations

According to a recent survey conducted in Ngorongoro district, agropastoral and pastoral residents have inadequate knowledge, unfavorable attitudes, and unsatisfactory practices when it comes to RVF. The recommended preventive measures toward RVF are not followed by these communities. Therefore, the awareness and practices regarding RVF epidemiology needs to be improved so that these communities can protect themselves and their livestock from recurrences of epidemics. Well-planned education campaigns on livestock vaccination and mosquito control would be effective methods to help prevent the spread of RVF. Phone and radio-based information dissemination must be well thought out, as phone and radio have been found to be the most widely used modes for communication. For instance, developing a phone application with the aim of educating the communities on RVF transmission, symptoms and management, could be an easily accessible way to continue to deliver consistent education and sensitization to communities. Health education should be continued and strengthened when there is a high chance of RVF outbreak. Finally, the government should ensure that personal protective gears are available to the agropastoral and pastoral communities, and strongly promote their usage.

Supporting information

S1 Table. Knowledge about Mosquito-borne zoonoses and control measures in Ngorongoro district.
(DOCX)

S2 Table. The knowledge of agropastoral and pastoral communities about RVF signs and transmission in Ngorongoro district, Tanzania.
(DOCX)

S3 Table. Proportion of communities' attitude toward Mosquitoes borne diseases.
(DOCX)

S4 Table. Proportion of communities' attitude toward RVF transmission and prevention.
(DOCX)

S5 Table. Proportions of communities' responses on practices about RVF prevention in Ngorongoro district.
(DOCX)

S1 Data. Excel files of general RVF KAP data collected from agropastoral and pastoral communities of Ngorongoro district.
(XLSX)

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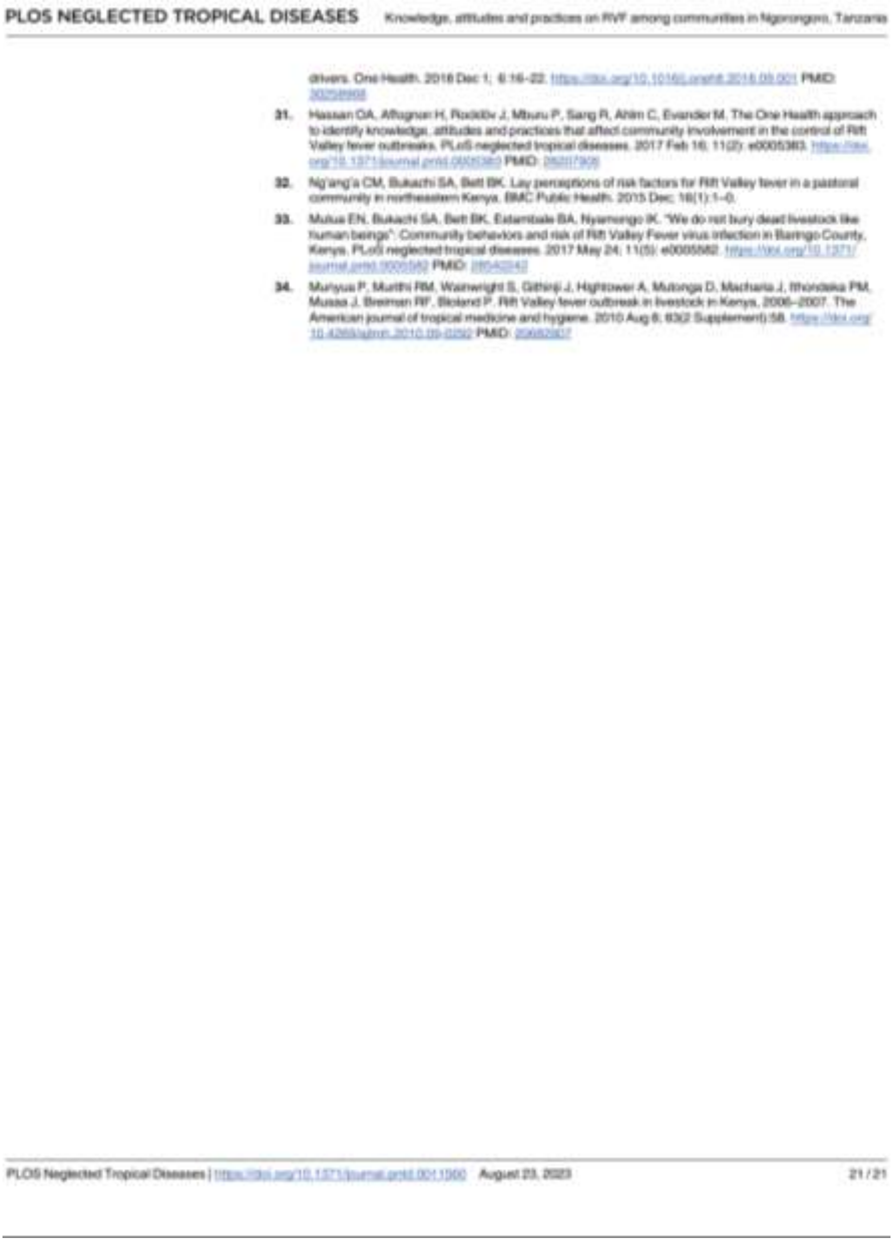

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List of tables provided to PLOS Neglected Tropical Diseases journal as supporting information

S1 Table: Knowledge about Mosquito-borne zoonoses and control measures in Ngorongoro district

Variable	Frequency (n)	Proportion (%)
Know mosquitoes	n=352	
Yes	349	99.15
No	3	0.85
How often have you bitten by mosquitoes	n=352	
Everyday	263	74.72
Rarely	54	15.34
I don't know	35	9.94
What effects do Mosquitoes have to the quality of your life?	n=352	
Transmit pathogen	307	87.23
Cause nuisance	24	6.82
I don't know	21	5.97
Mosquito-borne diseases known/mentioned	n=353*	
Malaria	314	89.22
Dengue	1	0.03
I don't know	38	10.79
Species of mosquitoes known/mentioned	n=364*	
Aedes	2	0.57
Anopheles	77	21.88
Culex	12	3.41
I don't know	273	77.56
Which measures do you use to reduce mosquito population?	n=361*	
Use of insecticides sprays	22	6.25
Eliminate stagnant water	5	1.42
Bush clearance	71	20.17
I do nothing	256	72.72
Kill as noticed	7	1.99
Which control measure do you take to protect yourself and your family from being bitten by mosquitoes?	n=355*	
Mosquito netting	311	88.35
Repellents	3	0.85
Stay indoor	11	3.13
I don't know	30	8.52
Which season of the year mosquito population is high?	n=352	
Rain season	335	95.17
I don't know	17	4.83

***Respondents gave multiple response**

S2 Table: The knowledge of agropastoral and pastoral communities about RVF signs and transmission in Ngorongoro district, Tanzania

Variable	Frequency (n)	Proportion (%)
Have you heard about RVF disease?	n=352	
Yes	184	52.00
No	168	48.00
Is the disease zoonotic?	n=352	
Yes	88	25.00
No	264	75.00
Types of animals can be infected	n=428*	
Humans	71	20.17
Ruminants	161	45.74
Wild animals	9	2.25
I don't know	187	53.13
Common clinical signs of RVF in humans	n=401*	
Hemorrhagic fever	5	1.42
Febrile fever	47	13.35
Muscle pain	18	5.11
Backache	9	2.56
Convulsion	3	0.85
Lethargy	11	3.13
Headache	36	10.23
Blurred vision	5	1.42
Joint pain	15	4.26
I don't know	282	80.11
Common clinical signs of RVF in animals	n=488*	
Hemorrhagic fever/fever	107	30.40
Fetid diarrhea	45	12.78
Lameness	9	2.56
Lacrimation	15	4.26
Abortion	62	17.61
Incoordination	22	6.25
Mucopurulent discharge	22	6.25
Sudden death of animal	27	7.67
I don't know	201	57.10
Routes of transmission of RVFV to animals	n=353*	
Blood feeding flies	28	7.95
Mosquitoes' bites	48	13.64
Ticks' bites	32	9.09
Direct contact with fluid or contaminated fomites	16	4.55
I don't know	229	65.06
Routes of transmission of RVFV to humans	n=373*	
Consumption of raw meat	29	8.24
Direct contact with fluid	28	7.95
Drinking raw milk	20	5.68
Mosquitoes' bite	4	1.14
Sheltering animals in house	10	2.84
I don't know	282	80.11

Respondents gave multiple response

S3 Table: Proportion of communities' attitude toward Mosquitoes borne diseases

Variable	Number (%) of respondents' responses				
	Completely agree	Agree	Neutral	Disagree	Completely disagree
Believing that mosquitoes have impact on their quality of life	57(16)	210(60)	56(16)	25(7)	4(1)
Believing that they at risk of acquiring mosquito borne diseases	28(8)	202(57)	64(18)	34(10)	24(7)
Believing that mosquitoes' bites can transmit diseases	40(11)	221(63)	67(19)	19(6)	5(1)
Always concerned with mosquitoes' management	19(5)	218(62)	59(17)	48(14)	8(2)

S4 Table: Proportion of communities' attitude toward RVF transmission and prevention

Variable	Number (%) of respondents' responses				
	Completely agree	Agree	Neutral	Disagree	Completely disagree
Rift Valley Fever is a hazardous disease of public health importance	22(6)	83(24)	172(49)	71(20)	4(1)
Humans are at high risk of being infected with RVF virus in this district	27(8)	73(21)	132(37)	99(28)	21(6)
Spread of RVF virus infection to both humans and animals can be prevented	41(12)	124(35)	143(41)	37(10)	7(2)
Vaccination of livestock against RFV is crucial.	58(16)	127(37)	132(37)	31(9)	4(1)
Wearing protective gears while cleaning the environment prevents RVF transmission.	25(7)	86(24)	147(42)	64(18)	30(9)
Proper vectors management can prevent RVF outbreak	28(8)	95(26)	150(43)	55(16)	24(7)
Interaction between human, domestic, companion and wild animals can facilitate spreads of RVF virus.	29(8)	92(26)	154(44)	55(16)	22(6)
Health workers can solve the problem of RVF outbreaks in this district.	39(11)	88(25)	174(50)	45(13)	6(2)
You always provide information regarding sick or deceased animals	40(12)	114(32)	103(29)	89(25)	6(2)

S5 Table: Proportions of communities' responses on practices about RVF prevention in Ngorongoro district

Variable	Frequency (n)	Proportion (%)
Avoid contact with blood/fluids from sick or dead animals		
Yes	16	4.55
No	336	95.45
Avoid handling of aborted fetuses on bare hands		
Yes	16	4.55
No	336	95.45
Eat well cooked meat from domestic and wildlife animals		
Yes	279	79.26
No	73	20.74
Drink properly boiled milk		
Yes	269	76.42
No	83	23.58
Eat wild animals		
Yes	113	32.29
No	237	67.71
Drink fresh blood collected from animals		
Yes	212	60.23
No	140	39.77
Wear protective gears during handling sick or dead animals		
Yes	6	1.71
No	346	98.29
Avoid keeping animals inside their houses		
Yes	80	22.73
No	272	77.27
Use of insecticides treated nets		
Yes	125	35.51
No	227	64.49
Use of mosquito repellents		
Yes	21	5.97
No	331	94.03
Treat ponds/stagnant water with insecticides		
Yes	6	1.71
No	346	98.29

CHAPTER SIX

6.0 GENERAL DISCUSSION

The research was performed in the Ngorongoro district, where there is harmonious coexistence of wildlife, domestic animals and humans (Mweya *et al*., 2015). Zoonotic diseases were previously documented in both livestock and human patients within the district (Mellau *et al*., 2009; Mweya *et al*., 2015; Mwakapeje *et al*., 2018; Motto *et al*., 2021). Although prior research had focused on livestock and human cases, the district remained unaware of the contribution of rodents and dogs in transmitting pathogens. Rodents are recognized as natural hosts for a wide range of infectious agents responsible for emerging diseases and global outbreaks (Theonest *et al*., 2019; Dahmana *et al*., 2020). Likewise, domesticated dogs have the potential to transfer pathogens to humans and other animals (Ghasemzadeh and Namazi, 2015). This study concentrated on investigating zoonotic bacteria and viruses present in rodents, dogs, and humans interacting in the same environment. Also, this study established levels of knowledge, attitudes, and practices of communities regarding rodent-borne diseases with a focus on Rift Valley fever.

The study successfully identified 24 bacterial families comprising numerous pathogenic and zoonotic bacteria species in rodents, domestic dogs and humans. The study revealed the occurrence of airborne, arthropod-borne, water or foodborne zoonotic bacteria in all study populations. The detected airborne zoonotic bacteria were *Mycobacterium* spp, *Bordetella* spp, *Mycoplasma* spp, and *Legionella* spp. *Mycobacterium* species are well known to cause several diseases in humans, and the most notable include tuberculosis, leprosy, Buruli ulcer, and non-tuberculous pulmonary infections (Jha *et al*., 2007). *Bordetella* commonly causes respiratory infections in humans and animals, while *Legionella* species can lead to Legionnaires' disease in humans, and *Mycoplasma* infections affect a wide range of tissues in both species

(Wagner *et al*., 2018). These results highlight the need for exhaustive investigation and execution of suitable deterrence and control measures to reduce transmission of airborne bacteria among populations in the district and other places in the country.

The arthropod-borne bacteria found in rodents, dogs and humans were *Borrelia miyamotoi*, *Rickettsia* spp, and *Bartonella* spp. This indicates the likelihood of zoonotic transmission through vectors such as ticks and fleas. Moreover, *Streptobacillus moniliformis* was found in both rodents and dogs, while *Yersinia pestis* and *Anaplasma* spp were detected exclusively in rodents, and *Ehrlichia* spp were solely identified in dogs. Generally, these zoonotic bacteria species are well-known as causative agents of mild to severe illnesses in humans and animals globally (Michelet *et al*., 2016; Kernif *et al*., 2017; Richardson-Boedler, 2020). These results highlight the need for vector control and awareness campaigns to prevent the risk of arthropod-borne infections among hosts in the district.

Furthermore, the study found waterborne, foodborne and contagious bacteria in rodents, dogs and humans, including *Leptospira* spp, *Brucella* spp, and *Salmonella* spp. It has been documented that these bacteria species spread through direct contact with infected animals, contaminated water and food which pose a significant risk to public and animal health (Nthiwa *et al*., 2019). These species of bacteria are of significant public health importance due to their potential to cause zoonotic infections such as leptospirosis, brucellosis and salmonellosis (Tabibi *et al*., 2013; Nthiwa *et al*., 2019). These findings necessitate robust surveillance and control measures to prevent outbreaks and safeguard public health.

The study also detected 20 RNA and 20 DNA viral families, along with multiple species, providing insights into the diversity of viral infections among the three hosts. The study findings showed that rodents had a greater rate of viruses’ detection in comparison to dogs and humans. The results imply that rodents play a crucial role as significant reservoirs for various pathogenic viruses. This research revealed the occurrence of *Orthobunyaviruses* and *Orthohantaviruses* in both rodents and humans, representing the first confirmation of their existence in the study area. Rodents harboured *California encephalitis orthobunyavirus* and *MPoko orthobunyavirus*, while humans were found to be infected with *Simbu* and *Shamonda orthobunyavirus*. It has been found that the *California encephalitis virus* is associated with encephalitis in humans (Putkuri *et al*., 2014). The public health importance of *MPoko orthobunyavirus* is still unknown. The *Simbu orthobunyavirus* is reported to cause infection in ruminants leading to various clinical manifestations including hemorrhagic fever, and reproductive and neurological disorders (Dutuze *et al*., 2018). The *Shamonda orthobunyavirus* is known to cause mild to severe infection associated with hemorrhagic fever, and neurological abnormalities in cattle (Yanase *et al*., 2005). Correspondingly, the *Seoul orthohantavirus* and *Choclo orthohantavirus* were recognized from rodents, while the *Oxbow orthohantavirus* was spotted in humans in this study. Studies have documented that *Seoul orthohantavirus* is mostly spread by rodents and it causes hemorrhagic fever with renal syndrome (HFRS) in humans (Clement *et al*., 2019; Kabwe *et al*., 2020). The *Choclo orthohantavirus* is also transmitted by rodents, and its association with human illness is not well-established. The *Oxbow orthohantavirus* was reported for the first time in American shrew mole and the associated disease is unknown (Kang *et al*., 2009). These results have provided the baseline information on *Orthobunyaviruses* and *Orthohantaviruses* present in rodents and humans in the study area.

The current study spotted several *Retroviruses* in rodents and domestic dogs. The significant retroviruses screened in rodents were *Abelson murine leukemia virus*, *Avian myelocytomatosis*, *Simian retrovirus virus*, *Ovine enzootic nasal tumour virus* and *Mason-Pfizer monkey virus*. The *Mason-Pfizer monkey virus* is a retrovirus that predominantly targets monkeys and has been thoroughly investigated as a model for studying retroviral replication and the development of cancer (Ahmed *et al*., 1974). The *Ovine enzootic nasal tumour virus* is a retrovirus that principally causes nasal tumours in sheep (Ahmed *et al*., 1974). The *Simian retrovirus* is a type of retrovirus discovered in different primates' species, encompassing both monkeys and apes, and may induce infections in humans (Lerche *et al*., 2001; Lerche, 2010). The *Avian myelocytomatosis virus* is a group of retroviruses that mainly cause tumours in birds and there is no evidence of causing illness in humans (Schat and Erb, 2014). *Murine leukemia virus* has the potential to cause Lymphosarcoma in mice and can be transmitted to other animal species, including humans (Goff, 1985). Furthermore, the research identified *Baboon endogenous virus* in dogs, which is the virus naturally occurring within Baboons (Van der Kuyl *et al*., 1995). Because endogenous viruses are inherited and passed down through generations (Van der Kuyl *et al*., 1995), it's likely that dogs acquired them by consuming Baboons. These results highlight the importance of conducting thorough studies on the genetic evolution of retroviruses in various animals and understanding how they are transmitted.

This study also found *Herpesviruses* in rodents, dogs and humans, pointing to their widespread occurrence among different animal hosts. Several herpesviruses were spotted in rodents, comprising *Felid alphaherpesvirus*, *Bovine gammaherpesvirus*, *Tupaiid betaherpesvirus*, *Equid gammaherpesvirus* and *Elephant endotheliotropic herpesvirus*. The *Equid gammaherpesvirus* primarily affects members of the equid family, which includes horses, donkeys and Zebras and has been linked with infections of reproductive, respiratory and neurological systems (Nolte *et al*., 2020). The *Equid gammaherpesvirus* was also previously found in bats, which indicates the likelihood of cross-transmission among animal species (Shabman *et al*., 2016). The *Elephant endotheliotropic herpesvirus* (EEHV) has been linked to the occurrence of deadly hemorrhagic disease in young elephants in Asia and Africa (Lee *et al*., 2021). The *Tupaiid betaherpesvirus* is a type of herpesvirus that primarily affects Tupaiid (tree shrews), and its effect on tree shrew health and possible zoonotic threat remain unidentified (Bovo *et al*., 2022). The *Bovine gammaherpesvirus* causes bovine gammaherpesvirus encephalitis and reproductive disorders in cattle (Headley *et al*., 2022). Additionally, some gammaherpesviruses can cross species barriers and potentially infect other animals even humans in rare cases (Headley *et al*., 2022). The *Felid alphaherpesvirus* primarily affects cats and can cause various health problems such as Feline Viral Rhinotrachetis (FVR), Feline Keratoconjunctivitis and systemic infections (Slaviero *et al*., 2022). Domestic dogs were found to harbour *Ateline alphaherpesvirus* in this study. This virus primarily affects monkeys and cause oral and genital ulcers in affected non-human primate (Eberle and Black, 2017). In the human part, *Murine roseolovirus* and *Panine betaherpesvirus* were detected. The *Murine roseolovirus* is genetically closely related to *Human roseolovirus,* and it mainly infects mice (Patel *et al*., 2017). The *Panine betaherpesvirus* was initially found in Apes, chimpanzees and Gorillas, and its public health importance is still under investigation (Cagliani *et al*., 2020). The findings of this study mirror the research done by Ntumvi et al. (2019) identified a significant variety of herpesviruses in rodents and shrew species in Central Africa. Therefore, these results underscore the significance of closely observing and comprehending how herpesviruses spread among various animal groups to avert inter-species transmission.

Moreover, the study found unclassified RNA viruses Shim 16 in dogs and humans. The RNA viruses detected in dogs were *Sanxia water strider virus 19*, *Beihai Charybdis crab virus*, *Wuhan house centipede virus*, *Wenzhou tombus-like virus 15*, and *Hubei picorna-like virus*, while *Wuhan centipede virus* was found in humans. These species of viruses were primarily discovered in the invertebrates collected around human settings (Cholleti *et al*., 2018).

Additionally, the study pinpointed several pathogenic and zoonotic viruses exclusively in rodents like *Avian Influenza A virus*, *Sicinivirus A*, *Kunsagivirus A*, *Human orthorubulavirus*, *Tailam jeilongvirus*, *Middelburg virus*, *T'Ho virus*, *Hainan torovirus*, *Human* and *Bat masterdenovirus*, *Poxvirus*, and *Torque teno virus*. The *Avian Influenza A virus* was isolated for the first time in humans from Scotland in 1959, and it is a zoonotic virus (Msoffe *et al*., 2010). The *Sicinivirus A* was primarily found in commercial chickens showing serious illnesses in China (Zhou *et al*., 2015). The *Kunsagivirus A* was formerly isolated from the faeces of the migratory bird and later in blood samples collected from the wild yellow baboon (Boros *et al*., 2013; Buechler *et al*., 2017). The *Middelburg virus* was identified in *Aedes* spp in South Africa in 1957 (Kokernot *et al*., 1957). This virus has been linked to neurological ailments in domestic animals (cattle and shoats), wild animals and humans in South Africa (Kokernot *et al*., 1957; Fourie *et al*., 2022) and in horses in Zimbabwe (Attoui *et al*., 2007). The *T'Ho virus* is a newly discovered virus from *Culex quinquefasciatus* mosquitoes in the Yucatan Peninsula of Mexico (Briese *et al*., 2017). Although there was no published information on associated diseases, it's worth noting that diverse *Flaviviruses* can lead to zoonoses (Briese *et al*., 2017). The *Hainan hebius popei torovirus* was first found in freshwater snapping turtles in New South Wales along the coastal river (Zhang *et al*., 2018). This virus caused severe illnesses associated with the high fatality rate of the infected turtles in the river (Zhang *et al*., 2018). *Human mastadenovirus* C (HAdV-C) is associated with respiratory infection in children, and most *Mastadenoviruses* are zoonotic, capable of causing illnesses in various mammals (Medkour *et al*., 2020). These outcomes strongly suggest an in-depth examination of diverse viruses identified in rodents. Such an analysis is crucial to ascertain their genetic composition and fully understand their possibilities of causing zoonoses in the populations in the study area.

In this study, it was discovered that 8.52% of participants exhibited a strong understanding of rodent related diseases and their control.

This proportion can be attributed to individuals with a secondary education who demonstrated a significant (P=0.017) level of knowledge about rodent-borne diseases and their management. The knowledge at this level suggests that the communities in Ngorongoro district have a limited understanding of rodent-borne infections and the measures to control them. Comparable results were noticed in Trinidad, where fewer than 50% of the population had awareness regarding rodent-borne diseases and how they are spread (Hardgrove *et al*., 2021). Furthermore, Begon in 2003 and Suwannarong *et al*. (2022) found that a substantial portion of rural residents lacked awareness about rodent-borne diseases and effective control strategies. Additionally, Banda *et al*. (2022) noted that certain members of the community may possess general knowledge about zoonotic diseases but may lack specific information about rodent-borne zoonoses.

The results indicated that, with the exception of education, most demographic factors did not exert a significant (P>0.05) influence on the level of knowledge regarding rodent-borne diseases and their prevention. In contrast to individuals without formal education, those with a secondary education demonstrated a significantly higher (P=0.017) level of understanding regarding rodent-borne diseases and their control. This study found that there was a significant (P=0.02) connection between general knowledge and overall practice scores as well as attitudes and practice scores (P=0.000). Consequently, considering these results, it’s logical to suggest that enhanced knowledge would likewise have effect on how communities perceive the management of rodents.

In the current study, it was found that 64.5% of participants displayed a negative attitude towards rodent-borne diseases, with 65% of them expressing disbelief in the likelihood of contracting rodent-borne zoonotic infections. The current findings could be influenced by the communities’ limited understanding of rodent-borne diseases. These observations indicated a positive correlation (r=0.0609) between knowledge levels and attitudes towards rodent-borne diseases. Moreover, within the demographic characteristics of the respondents, sex, education level, and occupation exhibited a notable and positive influence on attitudes towards rodent-related diseases and their management. Individuals who had received formal education displayed a significantly (P<0.05) positive attitude towards prevention of rodent-related diseases and control of rodents compared to those without formal education. It has been noted that education tends to promote favourable perspectives on behaviours and actions (Kravitz and Klineberg, 2000).

Furthermore, this research discovered that 94.3% of participants demonstrated effective practices in preventing rodent-borne zoonotic diseases. Positive rodent management behaviours observed included abstaining from consuming rodents, avoiding direct contact with deceased rodents using bare hands, and employing cats and rodenticides. However, even though they scored well in overall practice, a staggering 99.43% of respondents confessed to not using protective gear when cleaning their surroundings. This practice puts the community at risk of rodent-borne diseases through direct exposure to rodent droppings or urine. Studies conducted in the United States and various parts of the world have indicated that rodents transmit pathogens to humans through their excrement (Diaz and Hardgrove *et al*., 2021). Therefore, there is a need for educational initiatives on rodent-borne diseases to be implemented not only in the Ngorongoro district but also in other regions of Tanzania.

Similarly, this study examined the levels knowledge, attitudes, and practices related to RVF among both agropastoral and pastoral communities. This research discovered that 36.1% of participants exhibited sound knowledge, 38.64% displayed a favorable attitude, and 16.19% adopted effective preventive measures against RVF infection. In broad terms, these findings suggest that the communities under examination possessed inadequate knowledge, unfavorable attitudes, and ineffective practices in preventing RVF virus infections. Comparable outcomes were observed in numerous studies conducted in Africa, which revealed that agropastoral and pastoral communities generally exhibited limited knowledge, attitudes, and practices when it came to RVF occurrences (Shabani *et al*., 2015; Ng'ang'a *et al*., 2015; Mutua *et al*., 2017; Hassan *et al*., 2017; Alhaji *et al*., 2018)). The lack of education may have influence on KAP of the communities' readiness to adopt interventions aimed at preventing the risks of RVF virus transmission and RVF epidemics.

The latest study showed that overall knowledge of RVF was not linked to factors like age, marital status, or religion. However, it was notably associated with sex, education level, and geographical location. Males' participants demonstrated a higher likelihood of RVF awareness compared to females (P=0.04). This is possibly linked with the socio-cultural practices of the Masai community, where males are commonly involved in animals 'diseases management compared to females. The results of this research contrast with the findings of Abdi *et al.* (2015) in Kenya, as sex was not observed to be associated with knowledge. Additionally, this study revealed that individuals who had received formal education (including primary, secondary and college education) exhibited greater knowledge about RVF compared to those with informal education (P<0.05). these findings suggest that one's education level can impact their level of knowledge. This aligns with previous research by Owange *et al.* (2014) in Kenya, which observed that individuals with tertiary education showed higher knowledge about RVF. Hence, enhancing the quality of public education will result in a greater understanding, ultimately promoting better practices.

In general, the study conducted in the Ngorongoro district revealed the prevalence of zoonotic bacteria and viruses among rodents, domestic dogs, and humans, filling a gap in understanding their transmission dynamics. Airborne, arthropod-borne, waterborne, and foodborne bacteria were identified in all three populations, highlighting the need for control measures to reduce transmission risks. *Orthobunyaviruses* and *Orthohantaviruses* were confirmed in both rodents and humans for the first time, emphasizing their public health significance. Retroviruses and Herpesviruses were widespread among all hosts, indicating further research needs. Additionally, the study uncovered gaps in community knowledge, attitudes, and practices regarding rodent-related diseases and Rift Valley fever (RVF), suggesting targeted educational interventions are needed to improve preventive measures, particularly among vulnerable populations.

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CHAPTER SEVEN

7.0 CONCLUSIONS, IMPLICATIONS, CONTRIBUTIONS AND RECOMMENDATIONS

7.1 General Conclusions

In summary, this thesis has offered valuable insights into the pathogenic and zoonotic bacteria and viruses identified in rodents, domestic dogs, and humans at the human-animal interaction region. The presence of airborne/contagious, arthropod-borne, and food/waterborne bacteria in rodents, dogs and humans in the Ngorongoro district underscores the risk of disease transmission among the coexisting populations. The airborne/contagious bacteria, such as *Mycobacterium* spp, *Mycoplasma* spp, *Legionella* spp, and *Bordetella* spp, arthropod-borne bacteria included *Borrelia miyamotoi*, *Rickettsia* spp, *Bartonella* spp, *Streptobacillus moniliformis*, *Ehrlichia* spp and *Yersinia pestis*, and food/waterborne bacteria like *Leptospira* spp, *Brucella* spp, *Salmonella* spp, and *Campylobacter* spp pose important threat to animal and public health. In general, this study detected high proportion of bacteria in rodents, followed by domestic dogs, and lastly humans. This underscores the significance of rodents as crucial reservoirs for bacteria. The identification of several bacteria in humans highlights the need for improved hygiene, such as proper ventilation and use of personal protective equipment. Enhanced surveillance and control measures targeting vectors are crucial to minimize the risk of arthropod-borne diseases. Also, the detection of contagious and food/waterborne bacteria underscores the importance of public health interventions aimed at promoting hygiene, safe food handling, and control of animal reservoirs. Additionally, good husbandry practices of domestic dogs need to be executed to decrease the risks of spreading bacteria to humans and other animals.

Likewise, the detection of *Orthobunyavirus*, *Orthohantavirus*, *Alphavirus*, *Flavivirus*, *Torovirus*, *Coronavirus*, *Retroviruses*, *Herpesviruses*, *Adenovirus*, *Poxvirus*, and *Picornavirus* in rodents underscores their capacity to acts as reservoirs of zoonotic viruses. Moreover, the identification of *Orthobunyavirus*, *Orthohantavirus*, *Herpesviruses* and the *Wuhan centipede virus* in humans indicates the potential of zoonotic transmission from arthropod vectors or animal reservoirs to humans. Also, the identification of the *Lymphocystis disease virus*, *Retroviruses*, *Herpesviruses*, and unclassified RNA viruses of Shim 16 in dogs suggests the likelihood of cross-transmission of viruses. These results emphasize the importance of conducting thorough molecular analysis and surveillance to grasp their significance in animal and public health. Lastly, the communities should apply proper rodents control measures in their settlements in order to deter the risks of contracting zoonoses. The government and health professionals can effectively reduce the burden of bacterial and viral infections and protect the public and animal health by using suitable measures, and raising awareness.

Moreover, the study demonstrated that the communities have limited understanding of diseases transmitted by rodents, underscoring the necessity for educational and awareness initiatives. Most of the participants exhibited negative attitudes towards rodent-borne diseases, which may lead to misconceptions and insufficient preventive measures. Most respondents were unaware that rodents carry and transmit infections to humans and animals. Therefore, promoting limited contact with rodents, use protective gear during cleaning environment, emphasizing good hygiene, proper food storage, and improving houses designs to prevent rodent infestations should be integral components of public health education efforts in the study area. Though, it was encouraging to see that most respondents reported adopting good does, such as refraining from consuming rodents, use of cats or rodenticides, and avoiding direct contact with deceased rodents. Additionally, the evaluation of Knowledge, Attitudes, and Practices (KAP) regarding Rift Valley fever revealed insufficient knowledge, unfavorable attitudes and ineffective preventive practices among the survey participants. Therefore, poor KAP highlight the need for education campaigns to improve the community’s understanding, promote positive attitudes, and encourage application of proper preventive measures.

The research findings on zoonotic diseases, particularly on rodents, dogs, and humans in the Ngorongoro district of Tanzania, carry significant implications for existing policies and interventions. The identification of numerous pathogenic and zoonotic bacteria and viruses in rodents, domestic dogs, and humans underscores the need for sustained surveillance systems. These systems should monitor the prevalence of these pathogens and their potential transmission routes. Such data can inform the development and implementation of targeted control measures, including vector control, sanitation practices, and vaccination programs, to mitigate the risk of disease transmission. The detection of arthropod-borne bacteria and viruses, such as *Borrelia miyamotoi*, *Rickettsia* spp, and *Bartonella* spp, highlights the importance of vector control strategies. This could involve initiatives aimed at controlling tick and flea populations, as well as educating communities on preventive measures to reduce exposure to vectors.

The study's findings reveal gaps in community knowledge, attitudes, and practices regarding rodent-borne diseases and Rift Valley fever (RVF). These gaps underscore the importance of implementing targeted public health awareness campaigns. Such campaigns should focus on educating communities about the risks associated with zoonotic diseases, promoting appropriate preventive measures, and encouraging early detection and reporting of suspected cases. The association between education level and knowledge about rodent-borne diseases and RVF suggests the importance of educational interventions. Policies aimed at improving access to formal education, particularly in rural areas, can contribute to enhancing community understanding of zoonotic diseases and their management. Given the interconnectedness of human, animal, and environmental health, the findings support the implementation of a One Health approach in disease surveillance and control efforts. This approach involves collaboration across disciplines and sectors to address health challenges at the human-animal-environment interface effectively. Engaging communities in disease surveillance, prevention, and control efforts is essential for sustainable outcomes. Policies should prioritize community participation and empowerment, fostering ownership of health initiatives and promoting behavior change at the grassroots level.

In summary, the thesis contributes significantly to our understanding of zoonotic disease dynamics in the Ngorongoro district and provides valuable insights for policymakers, public health officials, and wildlife management authorities. The findings underscore the importance of interdisciplinary approaches to address complex challenges at the interface of human, animal, and environmental health. Moreover, the research findings provided valuable insights that can inform policy development and implementation in Tanzania, with a focus on enhancing disease surveillance, vector control, public health awareness, education, gender sensitivity, and community engagement strategies. By incorporating these insights into existing policies and interventions, Tanzania can strengthen its capacity to prevent and control zoonotic diseases, ultimately improving public and animal health outcomes.

7.2 Implications for Wildlife Management and Conservation

This study has significant implications for wildlife management and conservation as follows: **Ecosystem health**: Rodents are an essential part of ecosystems and play crucial roles in nutrient cycling and seed dispersion. However, when they become reservoirs and vectors for zoonotic pathogens, they can negatively impact the health of wildlife populations. Understanding the prevalence and transmission dynamics of zoonotic pathogens can help inform wildlife management strategies aimed at maintaining ecosystem health.

Disease transmission at the wildlife-livestock-humans interface: In areas where humans, domestic animals, and wildlife interact, such as the Ngorongoro District, there is a high risk of zoonotic pathogens transmission among various species. Effective wildlife management practices need to consider the potential spillover of pathogens from wildlife to domestic animals and humans, as well as vice versa. This understanding can inform strategies to mitigate disease transmission while promoting coexistence between wildlife, livestock and humans.

Conservation of endangered species: Some zoonotic pathogens can pose significant threats to endangered wildlife species. By identifying the presence of these pathogens and understanding their transmission dynamics, wildlife managers can implement targeted conservation measures to protect vulnerable populations. This might include disease surveillance programs, habitat restoration efforts, and captive breeding initiatives aimed at maintaining genetic diversity.

Habitat management: Rodents survive in various habitats, including natural ecosystems and human-modified landscapes. Effective wildlife management and conservation policies should consider how land use practices, such as agriculture and urbanization, influence rodent populations and their interactions with other species. Implementing habitat management strategies that minimize human-wildlife conflicts and reduce opportunities for disease transmission can benefit both wildlife and human communities.

Community involvement in conservation: Raising awareness about the link between rodent-borne diseases and wildlife conservation can foster community engagement in conservation efforts. By empowering local communities to participate in disease monitoring, habitat restoration, and sustainable land management practices, policymakers can promote conservation initiatives that benefit both human health and biodiversity conservation.

In general, integrating the findings of this study into wildlife management and conservation policies can help protect biodiversity, maintain ecosystem integrity, and safeguard human and animal health in the Ngorongoro District and similar ecosystems around the world.

7.3 Contribution of my PhD Study

The study has made significant contributions to our understanding of public health and zoonotic diseases within the Ngorongoro district. Firstly, it established, for the first time, the remarkable diversity of pathogenic and zoonotic bacteria across various hosts, including wild rodents, domestic dogs and humans. Secondly, it expanded our knowledge by revealing the diversity of pathogenic and zoonotic viruses within the same populations, shedding light on potential disease reservoirs and transmission dynamics. Additionally, the research estimated the levels of knowledge, attitudes, and practices among human population concerning rodent-borne infections and Rift valley fever in Ngorongoro district, offering invaluable insights into community understandings. These results collectively may contribute to the development of strategies for diseases control and prevention in the region and other places in Tanzania.

7.4 Recommendations

Based on the finding of this study, the following recommendations are suggested:

i. Implementing comprehensive education campaigns to raise awareness about existing zoonotic diseases, emphasizing the potential risks, mode of transmission, and preventive and control measures. The education program should target both the general population and specific high-risk groups, such as pastoral and agropastoral residing in areas of human-animal interfaces.

ii. Adopting a One Health approach that integrates human, animal, and environmental health sectors to address zoonotic disease transmission holistically. Facilitating multidisciplinary collaboration among health professionals, veterinarians, environmental scientists, and policymakers to develop and implement coordinated strategies for zoonotic disease prevention and control. This multidisciplinary approach is important for active surveillance, early detection, and management of zoonoses.

iii. Investing in training healthcare professionals, veterinarians, and other relevant stakeholders on zoonoses, with a focus on diagnostic techniques, and management options. This will improve the overall performance and enhance the ability to provide accurate information to the communities.

iv. Foster community engagement and participation in zoonotic disease prevention and control efforts through community-based organizations, local leaders, and healthcare providers. Encourage active involvement of communities in surveillance activities, including reporting sick or dead wildlife, domestic animals, or unusual human illnesses. This can be achieved through community outreach programs, workshops, and campaigns that empower individuals to take ownership of their health and the health of their animals. The workshops and seminars should focus on safe handling practices for wildlife, domestic animals, and their products to reduce the risk of exposure to zoonotic pathogens.

v. Allot resources for the improvement of healthcare infrastructure, laboratory facilities, and veterinary services to enhance disease detection, diagnosis, and response capabilities. Ensure adequate funding for research initiatives aimed at understanding the ecology, epidemiology, and transmission dynamics of zoonotic pathogens in the region.

vi. Allocate funds for research initiatives aimed at understanding the ecology, epidemiology, and transmission dynamics of zoonotic pathogens in the Ngorongoro district. Support policies that promote interdisciplinary collaboration among researchers and facilitate the translation of research findings into actionable interventions.

By implementing these recommendations, stakeholders can work together to strengthen zoonotic disease surveillance, prevention, and control measures in the Ngorongoro district, ultimately reducing the burden of disease on human and animal populations and safeguarding public health.

APPENDICES

Appendix 1: Various photos from field and lab works

Plate 1: Indoor trapping of rodent

Plate 2: *Ratus* captured from the store

Plate 3: Outdoor trapping of rodent

Plate 4: Rodent runway around home

Plate 5: Blood collection from dog

Plate 6: MinION sequencing

Appendix 2: Questionnaire tool

QUESTIONNAIRE TOOL

Questionnaire No…………… Date …………………

SOKOINE UNIVERSITY OF AGRICULTURE

COLLEGE OF FORESTRY, WILDLIFE AND TOURISM

Knowledge, attitude and practices of community on Rift Valley fever management and Rodent borne diseases in Ngorongoro district, Tanzania

Questionnaire for household heads

This questionnaire should be completed by household Heads

Good morning/afternoon/evening we are researchers from SUA conducting a study on knowledge**, attitude and practices of community on Rift Valley fever management and Rodent borne diseases.** We guarantee that any information you share will remain undisclosed to anyone. This interview will take 30 minutes only and your time will be highly appreciated.

I certify to the best of my understanding I have conducted the interview according to the study brief I was given.

Interviewer ID……………… Supervisor’s signature ……………

No	**Questions**	**Responses**	
SECTION 1: DEMOGRAPHIC OF RESPONDENT			
1.	Name		
2.	Respondent age	1. 18-24 2. 25-34 3. 35-44	4. 45-54 5. 55-64 6. >65 7. I don’t know
3.	Gender	1. Male 2. Female 3. Hermaphrodite	
4.	What is your highest level of education?	1. Not attended school 2. Did not completed primary school 3. Informal 4. Primary 5. Secondary	6. College 7. University 8. I don’t know
5.	What is your religion?	1. Muslim 2. Christian 3. Traditional 4. Others Mention………………………	5. I don’t know
6.	What is your marital status?	1. Single never married 2. Married 3. Widow/widower 4. Separated	5. Divorced 6. I don’t know
7.	Who is the head of this household?	1. Husband 2. Wife 3. Others mention………………..	4. I don’t know

8.	What is the main source of income in this household?	1. Livestock keeping 2. Crop farming 3. Employment 4. Business	5. Mixed sources 6. Other mention ………………………. 7. I don’ t know
SECTION 2: RESPONDENT’ S HOUSEHOLD CHARACTERISTIC			
9.	What is the size of your household?	1. 1-5 2. 6-10 3. >10	4. Other mention…… ………… 5. I don’ t know
10.	Walls of the house.	1. Mad 2. Blocks 3. Blocks with concrete 4. Grass	5. Animal feces 6. Others mention… …………………….
11.	Types of windows of the house.	1. Nets windows 2. Windows with shutters 3. Open windows	4. Other mention… …………………. 5. I don’ t know
12.	Type of roof of the house	1. Iron sheet 2. Grass 3. Mad	4. Other mention…… ……………...
13.	Do you use electricity in your house?	1. Yes 2. No	If yes 1. TANESCO 2. Solar
14.	Which type of toilet available in this household?	1. Pit latrine 2. Flush Sink	3. No toilet 4. I don’ t know
15.	How do you get news?	1. Radio 2. TV 3. Phone	4. News paper 5. Gatherings 6. Health/veterinary servants 7. Other mention… ……………...

SECTION 3: KNOWLEDGE OF COMMUNITIES ON MOSQUITOES, RVF AND RODENTS			
16.	Have you heard about or seen mosquitoes?	1.Yes 2. No	
17.	How often do you get bitten by mosquitoes?	1. Everyday 2. Rarely	3. I don’t know
18	Which effect do mosquitoes have on your quality of life?	1. Transmit pathogens 2. Cause nuisance	3. No effect 4. I don’t know
18.	What are the diseases that are transmitted through mosquitoes ‘ bites?	1. Malaria 2. RVF 3. Dengue	4. Other mention ··· ……………………… ………... 5. I don’t know
19.	Which species of mosquitoes do you know?	1. Anopheles 2. Culex 3. Aedes	4. Other mention··· ……………………… … 5. I don’t know
20.	Which measures do you use to reduce mosquito population around home compound?	1. Kill as noticed 2. Use of insecticides sprays 3. Eliminate stagnant water	4. Bush clearance 5. Other mention··· …………. 6. I don’t know 7. I do nothing
21.	Which control measure do you take to protect yourself and your family from being bitten by mosquitoes?	1. Mosquito netting 2. Repellents 3. Stay indoor 4. Insect screens	5. Insect zapper 6. Other mention··· ………… 7. I don’t know
22.	If repellents used, which type?	1. Mosquito coils 2. Mosquito repellent liquid	3. Other………… ………

			4. I don’t know
23.	Which season of the year mosquito population is high?	1. Rain season 2. Dry season	3. Other···························. 4. I don’t know
24.	Have you heard about RVF disease?	1. Yes 2. No	
25.	Is the disease zoonotic?	1. Yes 2. No	
26.	If Yes in the above question which types of animals can be infected?	1. Humans 2. Ruminants 3. Rodents/wildlife 4. Dogs	5. Other························. 6. I don’t know
27.	What are the common clinical signs of RVF in humans?	1. Hemorrhagic fever 2. Febrile fever 3. Muscle/joint pain 4. Backache 5. Convulsion 6. Lethargy	7. Abortion 8. Headache 9. Blurred vision 10. Others mention··················. 11. I don’t know
28.	What are the general clinical signs of RVF in infected animals?	1. Hemorrhagic fever 2. Fever 3. Fetid diarrhea 4. Lameness 5. Lacrimation	6. Mucopurulent discharges 7. In coordination 8. Abortion 9. High mortality of new borne 10. Other mention····················· 11. I don’t know.

29.	What are the routes of transmission of RVFV to humans?	1. Direct contact with Animal fluid 2. Drinking raw milk 3. Consumption of raw or undercooked meat 4. Contact with contaminated surfaces 5. Mosquitoes' bites	6. Aerosol 7. Other mention…………………. 8. I don't know
30.	What are the routes of transmission of RVFV to animals?	1. Mosquitoes' bites 2. Blood feeding flies 3. Sheltering animals in houses 4. Other mention…………………...	5. I don't know
31.	Do you know rodents?	1. Yes 2. No	
32.	Mention species of rodents you know.	1. ………………………….. 2. …………………………… 3. …………………………..	4. ……………………… 5. I don't know
33.	Where have you seen them?	1. Inside the house 2. Around home compound 3. In the forest/bushes	4. In the farm 5. Other mention…………………. 6. I don't know

34.	Why rodents get inside the house?	1. ……………… ………….. 2. ……………… …………….. 3. ……………… ………………	4. I don’t know
35	What are the adverse effects of rodents in the communities?	1. ……………… ………… 2. ……………… ……………. 3. ……………… ……………..	4.……………… ……………. 5……………… ……………... 6 I don’t know
36.	Do you know that rodents harbor zoonotic diseases?	1. Yes 2. No	
37.	If yes in above question, what are the zoonotic diseases transmissible from rodents to humans?	1. ……………… …………… 2. ……………… …………… 3. ……………… ……………	4. ……………… …… 5. ……………… …… 6. ……………… ……
38.	How do you control rodents?	1. Use of cats 2. Rodenticides 3. Set traps	4. Other 5. I don’t know
39.	Which form of rodenticides do you use?	1. Rat poison liquid 2. Rat poison pellets	3. Rat poison powder 4. I don’t know
40.	What are health risks of using rodenticides in your house?	1. ……………… …………… 2. ……………… …………….	3. ……………… …….. 4. I don’t know

SECTION 4: RESPONDENTS ATTITUDE TOWARD RFV MANAGEMENT, MOSQUITOES AND RODENTS						
Tick the appropriate number (1= Completely agree, 2= Agree, 3= Neutral, 4= Disagree and 5= Completely disagree)						
	STATEMENT	**RESPONSES**				
		1	**2**	**3**	**4**	**5**
	Attitude toward RVF					
1.	Rift Valley Fever is a hazardous disease of public health importance					
2.	Humans are at high risk of being infected with RVF virus in this district					
3.	Spread of RVF virus infection to both humans and animals can be prevented					
4.	You believe that vaccinating animals against RFV is important.					
5.	You trust that wearing protective gears while cleaning the environment prevents RVF transmission.					
6.	You believe that interaction with rodents/dogs can spread RVF to humans.					
7.	Proper vectors (Mosquitoes) control can prevent RVF outbreak in this area					
8.	The interaction between human, domestic and wildlife animals can facilitate spreads of RVF virus.					
9.	Health workers can solve the problem of RVF outbreaks in this district.					
10.	You always provide information regarding sick or deceased animals					
	Attitude toward mosquitoes					
11.	You believe that mosquitoes do have impact on your life quality					
12.	You believe that you are at risk of acquiring mosquito borne diseases					
13.	You believe that mosquitoes' bites can transmit					

	diseases					
14.	You are always concern with mosquitoes' control and prevention					
	Attitude toward rodents					
15.	Rodents have adverse effects on public health					
16.	You are at risk of catching rodent borne zoonoses					
17.	You trust that rodents can harbor infectious agents					
18.	You are always concern with rodent controls and prevention					

SECTION 5: RESPONDENTS PRACTICES ON RIFT VALLEY FEVER AND OTHER RODENTS BORNE ZOONOSES PREVENTION

	STATEMENT	**RESPONSES**	
1.	Do you avoid contact with blood/fluids from sick animals?		
2.	Do you avoid handling of aborted fetuses on bare hands?		
3.	Do you eat well cooked meat products from domestic and wildlife animals?		
4.	Do you drink properly boiled milk?		
5.	Do you eat wild animals?		
6.	Do you drink fresh blood collected from animals?		
7.	Do you wear protective gears during handling sick or dead animals?		
8.	Do you avoid keeping animals inside your house?		
9.	Do you use Insecticides treated nets in your house?		
	Total score RVF practices		
10	Do you use mosquito repellents?		
11	Do you treat ponds/stagnant water with		

	insecticides?		
12	Do you avoid staying outdoors at night?		
	Total score Mosquitoes practices		
13	Do you consume rodents?		
14	Do you control rodents in your house?		
15	Do you handle dead rodent with your bare hands?		
16	Do you wear protective gears during environmental cleanliness?		
	Total score Rodents practices		

THANK YOU FOR YOUR VALUABLE TIME AND Responses

Appendix 3: Guideline for focus group discussion

QUESTIONS FOR FOCUS GROUPS DISCUSSION		
1		
	How do you understand RVF?	
	What are the adverse effects of RVF in this district?	
	What are risk factors which contribute to RVF occurrence in this district?	
	What can be done to avoid RVF episodes in this district?	
2	**Questions on rodents**	
	Do you know rodents	
	Do you think rodents have adverse effects to this community?	
	Mention rodent-borne diseases	
	How do this community control rodents?	

THANK YOU FOR YOUR VALUABLE TIME AND RESPONS

Appendix 4: Guideline for key informants' interviews

Awareness and socioeconomic importance of RVF in the district		
No	**Questions**	
1.	What do you understand about RVF? Does this district experienced RVF?	
2.	What are risk factors which contribute to RVF occurrence in this district?	
3.	Do you think that the lifestyle of this community predisposes them to RVFV infection?	
	What were the major effects of RVF in this community?	
4.	Briefly explain how the previous RVF outbreaks managed in this district?	
Community knowledge on RVF		
5.	Do you think that the community is aware of the risk factors which contribute to RVF epidemic?	
6	Does the community know the clinical signs of RVF in both humans and animals?	
7	What is the attitude of the community toward RVF?	
8.	What are the challenges due to health services provided in this district?	
9.	Further views on RVF if any.	
Knowledge about rodent-borne diseases		
10.	Do you know rodents?	
11.	What are adverse effects of rodents in the community?	
12.	Do you think rodent can transmit diseases?	
13.	How do this community control rodent population?	

Appendix 5: Assessment of rodents’ inhabitation in houses and around houses in Ngorongoro district

Household Identity	Presence of rodent runways (Yes/No)	Rodents’ droppings (Yes/No)	Presence of burrows (Yes/No)	Rodent trapping (Yes/No)

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NIMR/HQ/R.8a/Vol. IX/3676

19th May 2021

Amina Ramadhani Issae
Assistant Research Fellow
Sokoine University of Agriculture
P O Box 3110
Morogoro

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled: **Prevalence, characterization, community awareness and risk factors of Rift Valley fever virus in Ngorongoro district, Tanzania (Ramadhan A. et al.)**, has been granted ethical clearance to be conducted in Tanzania.

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

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

Approval is valid for one year: 19th May 2021 to 18th May 2022.

Name: Prof. Yunus Daud Mgaya

Signature
CHAIR PERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Dr. Aifello Wedson Sichalwe

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

CC: Director, Health Services-TAMISEMI, Dodoma.
RMO of Arusha region.
DMO/DED of Ngorongoro district.

UNITED REPUBLIC OF TANZANIA
PRESIDENT'S OFFICE
REGIONAL ADMINISTRATION AND LOCAL GOVERNMENT

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REGIONAL COMMSSIONER'S OFFICE,
P.O. Box 3050,
ARUSHA.

In reply please quote:
Ref. No. FA.132/95/01/38

12/02/2021

District Administrative Secretary,
Ngorongoro District,
P.O.Box 10.
ARUSHA

RE: RESEARCH PERMIT

Reference is hereby made to the letter dated 3th February, 2021 from The Sokoine University of Agriculture Office of the Vice - Chancellor concerning the above underline subject.

2. I hereby taking this opportunity to introduce to you **Ms. Amina Ramadahani Issae** from The Sokoine University of Agriculture Office of the Vice - Chancellor. At the moment he is conducting a research focusing on **"Prevalence, Characterization,Community Awareness and Risk Factors of Rift Valley Fever Virus in Ngorongoro District, Tanzania".**

3. Permission is granted to conduct her research at **Ngorongoro District** from **March,2021 to February,2022.**

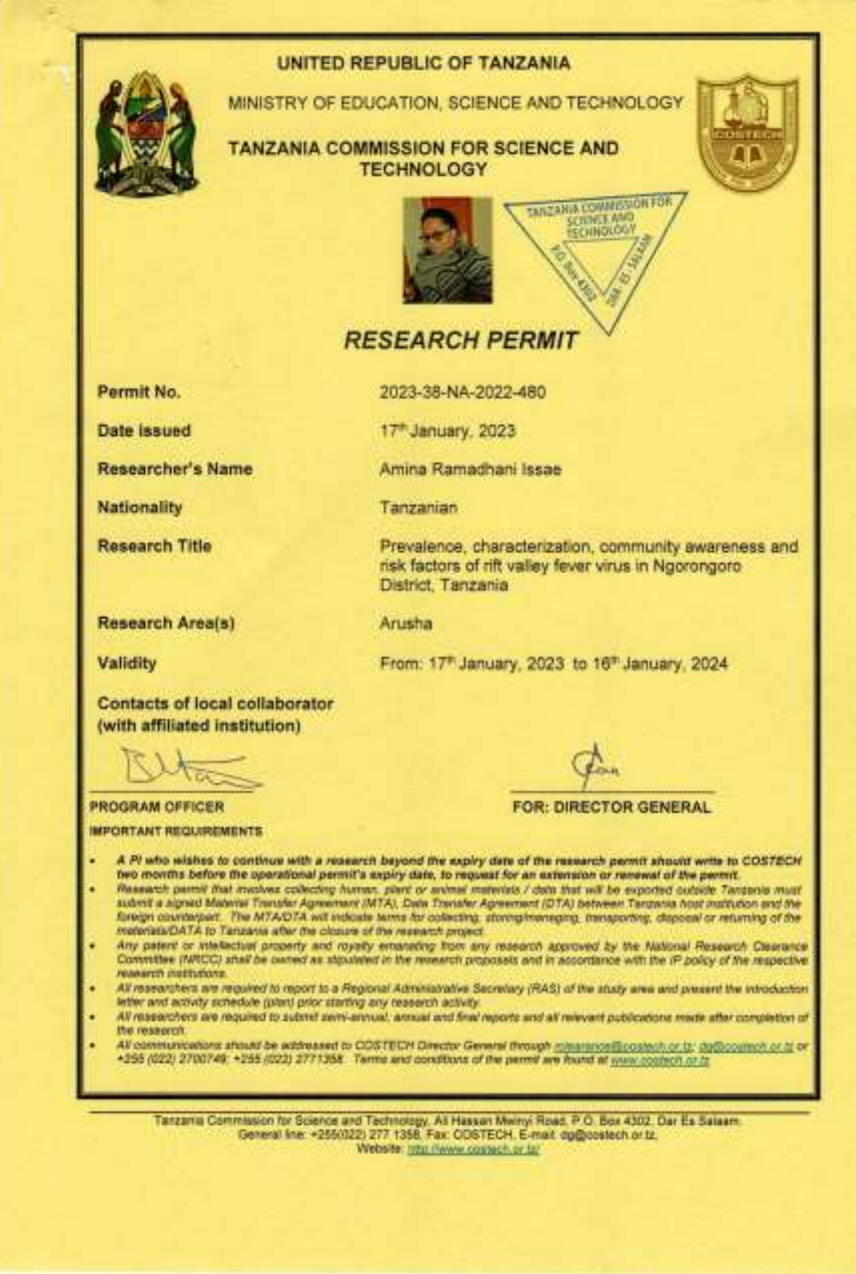
4. Please give her any necessary administrative assistance.

Thank you for your cooperation.

KATIBU TAWALA WA MKOA
ARUSHA.

M.Kassim
FOR: REGIONAL ADMINISTRATIVE SECRETARY
ARUSHA.

Copy: Ms. Amina Ramadahani Issae



UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

TANZANIA COMMISSION FOR SCIENCE AND TECHNOLOGY

RESEARCH PERMIT

Permit No.	2023-38-NA-2022-480
Date Issued	17th January, 2023
Researcher's Name	Amina Ramadhani Issae
Nationality	Tanzanian
Research Title	Prevalence, characterization, community awareness and risk factors of rift valley fever virus in Ngorongoro District, Tanzania
Research Area(s)	Arusha
Validity	From: 17th January, 2023 to 16th January, 2024

Contacts of local collaborator (with affiliated institution)

PROGRAM OFFICER

FOR: DIRECTOR GENERAL

IMPORTANT REQUIREMENTS

- ***A PI who wishes to continue with a research beyond the expiry date of the research permit should write to COSTECH two months before the operational permit's expiry date, to request for an extension or renewal of the permit.***
- *Research permit that involves collecting human, plant or animal materials / data that will be exported outside Tanzania must submit a signed Material Transfer Agreement (MTA), Data Transfer Agreement (DTA) between Tanzania host institution and the foreign counterpart. The MTA/DTA will indicate terms for collecting, storing/managing, transporting, disposal or returning of the materials/DATA to Tanzania after the closure of the research project.*
- *Any patent or intellectual property and royalty emanating from any research approved by the National Research Clearance Committee (NRCC) shall be owned as stipulated in the research proposals and in accordance with the IP policy of the respective research institutions.*
- *All researchers are required to report to a Regional Administrative Secretary (RAS) of the study area and present the introduction letter and activity schedule (plan) prior starting any research activity.*
- *All researchers are required to submit semi-annual, annual and final reports and all relevant publications made after completion of the research.*
- *All communications should be addressed to COSTECH Director General through rclearance@costech.or.tz; dg@costech.or.tz or +255 (022) 2700749; +255 (022) 2771358. Terms and conditions of the permit are found at www.costech.or.tz*

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Website: http://www.costech.or.tz/