Sokoine University of Agriculture

MSc Dissertation

Metagenomics Analysis of Pathogens in Rodents in Selected Human-Wildlife Interfaces in Tanzania

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METAGENOMICS ANALYSIS OF PATHOGENS IN RODENTS IN SELECTED HUMAN-WILDLIFE INTERFACES IN TANZANIA

Dissertation Submitted to Sokione University of Agriculture in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Public Health Pest Management

By

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EXTENDED ABSTRACT

Rodents belongs to the order Rodentia, which comprise the largest proportions of all of the terrestrial mammals in the world. In Africa, rodents occur in 14 families, 89 genera and 290 species, East African region contains 14 families, 62 genera and 161 species of rodents. Over decades, rodents have been reported to harbor more than 60% of emerging zoonotic pathogens including viruses, bacteria and a number of parasites. Several human diseases including the most devastating in the history of mankind “Plague” have been reported to have originated from rodents. Rodent borne diseases are transmitted either directly (Hantavirus, Lassa fever, Lymphocytic choriomeningitis) or indirectly through consumption of foods and water contaminated with rodent feces and urine (Leptospirosis, tularemia) or through ectoparasites carried by rodents (leishmaniasis, Lyme disease). Risks of human infection is linked to the possibility of contact between rodents, humans and animals, along with the closeness between rodent and human lives. Since there is constant interaction between humans, animals and rodents; it is therefore, essential to understand patterns of pathogen diversity and focusing surveillance in rodents in order to identify zoonotic potential pathogens prior to spill over to humans. Rodents were captured in Kibondo, Kyerwa and Uvinza in 2018. Trapping of rodents was done in peri-domestic areas by using Sherman box and Tomahawk traps added with peanut butter mixed with maize bran as baits. They were anaesthetized by using Isoflurane, and species identification was done by using morphological identification keys. In metagenomics sequencing a total of 116 rodents archived oral-pharyngeal and rectal swabs were subjected to RNA extraction and the products were pooled into twelve pools based on species of rodents, locations and swab types. There were eleven pools of oral-pharyngeal swabs and a single pool of rectal swabs. Pooled samples were sequenced on oxford Nanopore MinIon sequencing platform. This study employed 16S rRNA metagenomics sequencing analysis. Sequences were analyzed by using Kraken 2 classification

and Kaiju software. A total of 5263 small mammals were trapped from Kibondo, Uvinza and Kyerwa during the three years of data collection. Majority (87.6%, n=4613) were rodents and 650 (12.4%) were shrews. Among the rodents, rats were the majority (70.0%; n=3683) while mice were 873 (16.6%) and squirrels were only 44 (0.8%). The dominant species in the group of rodents were *Mastomys natalensis* which constituted 63.6% (n=3346). Among the mice, *Arvicanthis* spp. were the majority as they constituted 7.1% (n=373) of all small mammals trapped. A total of 13 (0.3%) small mammals identified as rodents their genus was not established. In a metagenomics analysis, 44 bacteria species of public health, veterinary and environmental health importance were identified. They were detected in eight pools, while there was no detection in four pools. A total of 10 (22.7%) bacteria species including *H. pylori* were detected in the rectal swabs pool and the remaining 34 (77.3%) were distributed among the pools of oral-pharyngeal swabs. A total of 15 potentially pathogenic, zoonotic and bacteria of unknown zoonotic potential including *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Haemophilus influenzae*, *Klebsiella pneumonae*, *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli*, *Chlamydia psittaci*, *Campylobacter sputorum*, *Acinetobacter baumannii* and *Acinetobacter pitti* were identified in this study. The findings of this study are in line with several previous studies that have reported a number of rodent species in western Tanzania; also, citing rodents as reservoirs of disease-causing agents; and has the potential to spread and maintain transmission cycles of human and livestock diseases. This study presents the first reports of natural infection of rodents with *Helicobacter pylori* and *Mycobacterium tuberculosis*. These findings provide baseline information to inform surveillance systems and the public in general on potential health risks that are associated with interaction between rodents, humans and livestock. Furthermore, it gives insight on the potential of using a unified approach “One Health” between sectors in order to achieve better health and a safer community. However, since the findings of this

study do not explain pathogen transmission from rodents to humans and vice versa, we recommend further studies to characterize them and understand their transmission dynamics.

Keywords: Rodents, Zoonotic, Metagenomics, human-wildlife interfaces, bacteria

IKISIRI KUU

Panya ni miongoni mwa viumbe katika kundi la Rodentia, ambalo linajumuisha idadi kubwa zaidi ya mamalia wote wa duniani. Katika Afrika, panya hutokea katika familia 14, genera 89 na aina 290, eneo la Afrika Mashariki lina familia 14, genera 62 na aina 161 za panya. Kwa miongo kadhaa, panya wameripotiwa kuwa na zaidi ya 60% ya vimelea vya magonjwa vinavyoibuka ikiwa ni pamoja na virusi, bakteria na minyoo. Magonjwa kadhaa ya wanadamu yakiwemo mabaya zaidi katika historia ya wanadamu "Tauni" yameripotiwa kuwa yametokana na panya. Magonjwa yanayoenezwa na panya huambukizwa ama moja kwa moja (Hantavirus, Lassa fever, Lymphocytic choriomeningitis) au kwa njia isiyo ya moja kwa moja kupitia ulaji wa vyakula na maji yaliyochafuliwa na kinyesi na mkojo wa panya (Leptospirosis, tularemia) au kupitia wadudu wanaobebwa na panya (leishmaniasis, ugonjwa wa Lyme). Hatari za kuambukizwa kwa binadamu zinahusishwa na muingiliano kati ya panya, wanadamu na wanyama, pamoja na ukaribu kati ya maisha ya panya na wanadamu. Kwa kuwa kuna mwingiliano wa mara kwa mara kati ya wanadamu, wanyama na panya; kwa hivyo, ni muhimu kuelewa mifumo ya aina mbalimbali za vimelea na kulenga ufuatiliaji katika panya ili kutambua viini vinavyoweza kusababisha magonjwa yaambukizwayo baina ya biadamu na wanyama kabla ya kutokea kwa binadamu. Katika utafiti huu panya walikusanywa Kibondo, Kyerwa na Uvinza mwaka 2018. Utegaji wa panya ulifanyika katika maeneo ya mashambani kwa kutumia mitego ya Sherman box na Tomahawk ambayo iliwekewa siagi ya karanga iliyochanganywa na pumba za mahindi kama chambo. Walipigwa ganzi kwa kutumia Isoflurane, na utambuzi wa spishi ulifanywa kwa kutumia vitufe vya utambuzi wa kimofolojia. Katika metagenomics jumla ya panya sampuli 116 zilizohifadhiwa zilitumika katika kupata vinasaba aina ya RNA na baadae ziliwekwa Pamoja katika makundi kumi na mawili kulingana na spishi za panya, mahali zilipokusanywa na aina za sampuli. Kulikuwa na makundi kumi na moja ya swabu ya mdomo-

koromeo na kundi moja la swabs za kinyesi. Sampuli hizo zilichakatwa katika katika mfumo wa oxford Nanopore MinIon. Utafiti huu ulitumia uchanganuzi wa mpangilio wa 16S rRNA. Ugunduzii wa aina ya vimelea ulifanywa na mfumo wa uainishaji wa Kraken 2 na programu ya Kaiju. Jumla ya mamalia wadogo 5263 walikusanywa kutoka Kibondo, Uvinza na Kyerwa katika kipindi cha miaka mitatu ya ukusanyaji wa takwimu. Asilimia kubwa (87.6%, n=4613) walikuwa panya na 650 (12.4%) walikuwa ndezi. Miongoni mwa kundi linalojumuisha panya, panya walikuwa wengi (70.0%; n=3683) wakati panya wadogo (maisi) walikuwa 873 (16.6%) na chindi walikuwa 44 (0.8%) tu. Spishi kubwa katika kundi la panya walikuwa *Mastomys natalensis* ambayo ilijumuisha 63.6% (n=3346). Miongoni mwa panya wadogo, Arvicanthis spp. walikuwa wengi kwani walikuwa 7.1% (n=373) ya mamalia wadogo walionaswa. Jumla ya mamalia wadogo 13 (0.3%) waliotambuliwa katika kundi la panya jenasi yao haikutambulika. Katika uchambuzi wa metagenomics, aina 44 za bakteria wenye umuhimu katika afya ya binadamu, mifugo na mazingira zilitambuliwa. Waligunduliwa katika makundi nane ya sampuli, wakati hakukuwa na kugunduliwa katika makundi manne. Jumla ya spishi 10 (22.7%) za bakteria zikiwemo *H. pylori* ziligunduliwa kwenye kundi lililokuwa na swabu za kinyesi na 34 zilizobaki (77.3%) ziligawanyika kati ya makundi ya swabuza mdomo na koromeo. Jumla ya aina 15 za bakteria wanaoweza kusababisha magonjwa, ikiwa ni pamoja na *Mycobacterium tuberculosis*, *Helicobacter pylori*, *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Haemophilus influenzae*, *Klebsiella pneumonae*, *Staphylococcus aureus*, *Streptococcus sptrotum*, *Acinetobacter baumannii* na *Acinetobacter pitti* zilitambuliwa katika utafiti huu. Matokeo ya utafiti huu yanawiana na tafiti kadhaa za awali ambazo zimeripoti idadi kadhaa ya spishi za panya magharibi mwa Tanzania; pia, zikitaja panya kama hifadhi ya vimelea vinavyosababisha magonjwa; na kuwa na uwezo wa kueneza na kudumisha mzunguko wa maambukizi ya magonjwa ya binadamu na mifugo. Utafiti huu unatoa ripoti za kwanza za maambukizi ya asili ya panya na *Helicobacter pylori* na *Mycobacterium tuberculosis*. Matokeo haya

yanatoa maelezo ya kimsingi ili kufahamisha mifumo ya uchunguzi na umma kwa ujumla kuhusu hatari zinazoweza kutokea za kiafya zinazohusishwa na mwingiliano kati ya panya, binadamu na wanyama. Zaidi ya hayo, inatoa ufahamu juu ya uwezekano wa kutumia mbinu ya umoja "Afya Moja" kati ya sekta mbali mbali ili kufikia afya bora na jamii salama. Hata hivyo, kwa kuwa matokeo ya utafiti huu hayaelezi maambukizi ya vimelea kutoka kwa panya hadi kwa binadamu na kinyume chake, tunapendekeza tafiti zaidi ili kuzibainisha na kuelewa mienendo yao ya maambukizi.

Maneno muhimu: Panya, Zoonotic, Metagenomics, miingiliano ya binadamu na wanyamapori, bakteria

DECLARATION

I, **Agnes Abel**, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

Agnes Abel Date
(MSc. Candidate)

The declaration is hereby confirmed by;

Dr. Coletha Mathew Date
(Supervisor)

LIST OF MANUSCRIPTS

Manuscript One

Title: Survey of rodent species in humans-wildlife interface areas of western Tanzania

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Manuscript two

Title: Rodent Reservoirs: Unraveling the Spectrum of Zoonotic and Pathogenic Bacteria

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Lastly, I express my deep appreciation to all those who have participated in different stages of this work. To those who participated in sample collection and to everyone who provided technical assistance, my friends, my classmates, and all the laboratory staffs; thank you.

DEDICATION

To my proud Mother.

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LIST OF ABBREVIATIONS AND ACRONYMS

ATP	Adenosine Triphosphate
COSTECH	Tanzania Commission for Science and Technology
DNA	Deoxyribonucleic acid
E-PAP	*E. coli* Poly (A) Polymerase
FAO	Food and Agriculture Organization
FUO	Fever of unknown origin
GR	Game Reserve
HALi	Health for Animals and Livelihood Improvement
KCRI	Kilimanjaro Christian Research Institute
MAGs	Metagenome-Assembled Genomes
mNGS	Metagenomics Next-Generation Sequencing
NCBI	National Center for Biotechnology Information
NGS	Next Generation Sequencing
NP	National Park
ONT	Oxford Nanopore Technology
PA	Protected Area
PCR	Polymerase Chain Reaction
QGIS	Quantum Geographic Information System
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
Spp	Species
SQK	Sequencing Kit
SUA	Sokoine University of Agriculture
TAWIRI	Tanzania Wildlife Research Institute
TB	Tuberculosis
VTM	Viral Transport Media
WHO	World Health Organization

CHAPTER ONE: GENERAL INTRODUCTION

Rodents (order Rodentia) are the most abundant and globally distributed mammalian group. According to the Mammal Diversity Database maintained by the American Society of Mammologists, there are 2590 extant species within the order Rodentia. In Africa, rodents occur in 14 families, 89 genera and 290 species. The East African region contains diverse population of rodents in 14 families, 62 genera and 161 species. The rodent fauna is diverse, ranging from larger porcupines (*Hystrix*sp.) weighing 20 kg to small African Pygmy mice (*Mus minutoides*) weighing only 5 - 7g (Fiedler, 1990).
Rodents are known reservoirs of several evolving zoonotic pathogens (Luis *et al*., 2013). Being the most abundant, diverse and geographically distributed rodents come into close contact with livestock in the agricultural background and humans in urban and rural areas (Akhtar *et al*., 2023). The likelihood of humans and livestock getting infected is closely related to the probability of their contact with infectious particles that are aerosolized from rodent excrement; in fact, a higher density of infected rodents in an area increases the chances of disease transmission (Tersago *et al*., 2009). Therefore, the risk of infection of human infection is connected to three routes: a sylvatic route, linked to activities in the natural habitat of wild rodents; a rural route, where contact with rodent excrement happens in and around human and livestock habitats near wooded areas; and an urban route, where rodents coexist with humans in residential areas as pests or companion animals. Therefore, place of residence also plays a role in determining risk, with individuals residing near wooded areas, particularly those with farm animals and woodsheds nearby face an increased risk. Some professions, such as forestry and farming, might be categorized as 'higher risk, but certain activities can also raise the risk, like spending leisure time in forests and handling firewood.

1.1 Rodents

Rodents belong to the mammalian order Rodentia, Families in order Rodentia are family *Muridae* (including rats and mice), *Sciuridae* (squirrels), *Echimyidae* (spiny rats), *Heteromyidae* (pocket mice and kangaroo rats), and *Dipodidae* (jerboas and jumping mice) (Aplin *et al*., 2003). Animals belonging to this group are distinguished by a diastema, which is a gap between their incisor and pre-molar teeth, facilitating chewing and gnawing. They also possess a pair of incisors that continuously grow without roots (Aplin *et al*., 2003). This mammalian orders have been noted for harboring a relatively high diversity of pathogens and this may be directly related to the high diversity in this mammalian taxa. They have higher reproduction rates and ability to survive in various habitats including terrestrial, fossorial, aquatic and arboreal areas; Rodents inhabit in all continents of the world except in the Antarctica (Singleton *et al*., 2021). The population dynamics of rodents fluctuate within and between years and are significantly influenced by factors like climate variations, food supply, habitat fragmentation, social interactions, and predation (Hayes *et al*., 2017).

Rodents have mixed diets, some are herbivorous which feed on grasses (graminivorous), seeds (granivorous), or fruits (frugivorous). They can also be omnivorous capable of consuming a variety of foods and a few are insectivorous feeding mostly on insects (Fiedler, 1990). Similar to other parts of the world, the distribution of rodent species varies across different geographical zones in Tanzania. The North-eastern Tanzania, has a diverse species composition which includes *Mastomys natalensis*, *Lophuromys flavopunctatus*, *Grammomys dolichurus*, *Arvicanthis nairobae*, *Praomys delectorum* and *Mus* sp. (Makundi *et al*., 2005).

Rat infestations in human settlements lead to several effects including destruction of properties, distraction of stored grains and the more serious increased risks to over 20 animal and human disease (Singleton *et al*., 2021). In certain Indian traditions, black

rats are involved in cultural and religious rituals; where rats are permitted to consume some foods before human do, symbolizing good fortune for the community members (Singla *et al*., 2008). Similarly, in Tanzania there are tribes that consume black rats as foods. These practices are risky to humans as they increase the chances that humans may be exposed to some potential pathogens.

1.2 Rodent Borne Diseases

Emerging infectious diseases that pose public health and economic threats are mainly zoonoses, with over 70% estimated to originate in wildlife (Jones *et al*., 2008). As a minimum 61% of human infectious diseases are caused by pathogens shared with wild or domestic animals (Woolhouse *et al*., 2001). Zoonotic diseases originating from rodents encompass a wide spectrum; from relatively mild conditions like cowpox, which result in isolated skin lesions at the point of infection, to severe and often fatal illnesses like bubonic plague. These diseases can be transmitted directly from rodents to humans, as seen with hantaviruses, or indirectly through various means, such as arthropods like fleas in the case of plague, ticks in the case of Lyme disease, flies in the case of leishmaniasis, or even through respiratory droplets (Dahmana *et al*., 2020). The first pathway involves a direct method in which rodents can transmit pathogens to humans, either through bites or through the consumption of food or water contaminated with rodent excrement (Meerburg *et al*., 2009). Additionally, humans may be exposed to surface water contaminated with rodent urine, leading to diseases like leptospirosis, or inhale pathogens present in rodent excrement, as in the case of Hantaviruses. Rodent-borne diseases can also be transmitted indirectly to humans, as rodents act as amplifying hosts for these pathogens and can facilitate their transmission to humans through arthropod vectors like ticks, mites, and fleas (Akhtar *et al*., 2023). On the other hand, if livestock accidentally or intentionally consume rodents, they can acquire and transmit pathogens. If these pathogens are not effectively eliminated through proper cooking of food products, it can lead to human illness and morbidity,

Furthermore, rodents play a role in sustaining the transmission cycles of pathogens across a range of environments, including densely populated urban areas, rural regions, and wilderness settings (Meerburg *et al*., 2009).

Several bacterial diseases with rodent reservoirs in Africa pose significant health risks, causing considerable morbidity and mortality. Regarding public health, the transmission of zoonotic diseases through rodents encompasses various illnesses, including salmonellosis, plague, leptospirosis, leishmaniasis, toxoplasmosis, rat-bite fever, taeniasis-like infections, and zoonotic babesiosis (Dahmana *et al*., 2020). Rodents are also good reservoirs of various disease-causing pathogens including agents of tularemia, tick-born relapsing fever, Lyme disease, ehrlichiosis, bartonellosis, listeriosis, and Q fever (Rabiee *et al*., 2018). They may also harbor different complex bacteria like *Mycobacterium tuberculosis*, *Mycobacterium microti*, and *Escherichia coli* (van Soolingen *et al*., 1998).

Rodent borne zoonotic viruses on the other hand, poses a huge threat to public health especially due to the abundance of rodent species and their increasing interaction with humans. Important rodent borne hemorrhagic viruses include those in the genera Arenavirus and Hantavirus. Some of the Flaviviruses that are pathogenic to humans that have also been detected in rodents includes tick-borne encephalitis virus (Mansfield *et al.,* 2009), Powassan virus (Ebel, 2010), Kyasanur Forest disease virus (Shah *et al*., 2018), Dengue virus (de Thoisy *et al*., 2009) and West Nile virus (WNV) (Platt *et al*., 2008). Majority of people become exposed to rodent borne viruses through agricultural activities (Riccò *et al*., 2021). The transmission of Korean hemorrhagic fever, caused by hantaan virus have been reported to occur mainly during rice harvest period (Weber de Melo *et al*., 2015). Conversion of grassland to maize fields have also been directly correlated with human infection with Argentine hemorrhagic fever (AHF) caused by junin virus (Castillo *et al*., 2003). The patterns of transmission of

rodent borne viruses to humans can also be explained by environmental change, forest fragmentation and other forms of land use changes consequent to rapid population growth (White & Razgour, 2020). Therefore, human social economic status, occupation, frequency of interaction with infected rodents and access to health care significantly affect the transmission dynamics of rodent borne viruses (Fulhorst *et al*., 2007).

1.3 Metagenomics

The recent approach, metagenomics, is increasingly providing an alternative for analyzing mixtures of nucleic acids recovered directly from a variety of sources and diverse environments without requiring prior knowledge of the target sequence. Metagenomics RNA sequencing allows for identification of multiple pathogens within a sample in a non-targeted and unbiased manner. Several methods for pathogen identification are based on comparisons with known pathogens, these methods have limitations in virus because most viruses cannot be propagated in cell lines. This limitation is overcomed by the application of next generation sequencing (NGS) in combination with sequence independent amplification (mNGS). Metagenomics have enabled detection of all genomes present in a given sample without previous knowledge of their nucleotide sequence thus, provides an excellent tool for analyzing samples collected from species that are known to harbor a diverse of known and unknown pathogens including rodents (Phan *et al*., 2011; Sachsenröder *et al*., 2014). Metagenomics has led to novel virus discovery such as a rhabdovirus causing hemorrhagic fever in central Africa (Grard *et al*., 2012) and identified causative agents in outbreaks, e.g., Lujo virus in South Africa (Paweska et al., 2009), Bundibugyo ebolavirus in Uganda (Towner *et al*., 2008); It also provides genomic information for typing and surveillance.

Metagenomics has been extensively used as an efficient screening method worldwide; In Tanzania for instance it has been used in screening of microbiomes, where microbiomes derived from ocean

water, freshwater, soils, feces, and wastewater were screened and estimates of the most prevalent toxins and most pathogen-enriched environments were identified (Li, 2019). Another study used metagenomics to identify rare pathogens and antibiotic resistance in waters during a cholera outbreak in Tanzania (Baraka *et al*., 2023). These studies demonstrate the power of metagenomics in identifying pathogens in various environments and conditions, contributing significantly to the field of infectious disease research. Moreover, in a study that compared the performance of metagenomic next-generation sequencing (mNGS) and conventional culture in detecting pathogens in febrile patients with suspected infections it was found that mNGS is more sensitive and accurate than traditional culture, making it ideal for identifying pathogens and screening infectious diseases, especially for those with uncultivated or difficult-to-cultivate species (Yang *et al*., 2024).

Nanopore sequencing is a third-generation sequencing method with two significant advantages over second-generation technologies: it produces longer sequence reads and allows for real-time sequence analysis (Greninger *et al*., 2015). So far, the longer Nanopore reads have facilitated the assembly of prokaryotic and eukaryotic genomes and the sequencing of cultured bacterial and viral isolates (Ashton *et al*., 2015). Long-read sequencing has gained popularity in the analysis of metagenomics data due to its ability to provide more accurate, complete, and high-resolution data (Kim *et al*., 2024; Marić *et al*., 2024; Zhang *et al*., 2023). Long reads significantly improve the contiguity of metagenomic assemblies, this is because long reads can span repetitive regions in the genome that are often longer than the read length of short-read sequencing technologies; this allows for more accurate assembly of complex regions. The use of long reads can lead to the recovery of more than double the number of high-quality Metagenome-Assembled Genomes (MAGs) compared to short-read sequencing (Zhang *et al*., 2023). This is because long reads provide more information about the genomic context which can help in the assembly of novel and rare genomes which can be

missed in short read sequencing. Moreover, It also provide a higher resolution of microbial communities, allowing for more accurate taxonomic classification (Marić *et al*., 2024).

1.4 Problem Statement and Justification

Over the years, rodents are known to be reservoir hosts for at least 60 zoonotic diseases including bacterial and viral zoonoses; and are known to play an important role in their transmission and spread in different ways (Barbour, 2017; Dahmana *et al*., 2020). According to Nowak (1991), rat-borne diseases have claimed more lives in the past thousand years than wars. To date, research shows that, greater than half of all human infectious diseases are zoonotic, a majority of which originating from the cross-species transmission of pathogens from wildlife (including rodent) to humans (Olival *et al*., 2017). Globalization, environmental and anthropogenic changes provide sufficient chances for spillover and emergence of zoonotic diseases (Taylor *et al*., 2001). When examining potential sources and carriers of the primary zoonotic pathogens responsible for human diseases, a crucial and common factor is their association with rodent hosts. Among mammalian orders, rodents serve as hosts to a wider array of zoonotic pathogens than any other, and when combined with bats and other primates, they collectively carry the majority of zoonotic viruses (Akhtar *et al*., 2023; Ecke *et al*., 2022). Rodents are vectors, reservoirs and carry a diversity of pathogens including helminths, bacteria, viruses, and zooanthroponoses (Khaghani, 2007). Since there is constant interaction between humans and livestock and rodents together; it is therefore, essential to understand patterns of pathogen diversity and focusing surveillance in rodents in order to identify zoonotic potential pathogens prior to spill over to humans (Moussa *et al*., 2021). Hence, this study aimed at conducting metagenomics analysis to determine potentially zoonotic pathogens in rodents as an early warning surveillance, so as to monitor the infection situation in natural hosts which is important for control and prevention of

outbreaks of emerging and/or re-emerging diseases originating from rodents (He *et al.,* 2013).

1.5 Objectives

1.5.1 General Objective

Establishment microbial diversity of rodents from selected human-wildlife interfaces in Tanzania, by using metagenomics approach

1.5.2 Specific Objectives

i. To identify the species of rodents, present in the selected human-wildlife interfaces in Kagera and Kigoma regions.
ii. To identify microbial diversity of rodents by using metagenomics approach.

CHAPTER TWO: MANUSCRIPT ONE

Survey of Rodent Species in Humans - Wildlife Interface Areas of Western Tanzania

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*The material contained in this chapter is in preparation for submission to the Journal of Ideas in Health.

2.1 Abstract

Rodents and shrews are essential parts of ecosystems across the globe, contributing to ecological dynamics, pests to crops, vectors of pathogens and destructive to property. The aim of this study was to survey the rodent species available in humans - wildlife interface areas in Kibondo, Uvinza and Kyerwa. In the selected interface areas in districts, rodents capture was done during wet and dry seasons of 2016, 2017 and 2018. Trapping of rodents was done in peri-domestic areas by using Sherman box and Tomahawk traps added with peanut butter mixed with maize bran as baits. Captured rodents were anaesthetized by using isoflurane and species identification was done based on morphological features as per morphological identification keys. A total of 5263 small mammals were trapped during the three years of data collection. Among the rodents, rats were the majority (70.0%) while mice were 16.6% and squirrels were only 0.8%. *Mastomys natalensis* constituted the majority of the trapped rodents (63.6%) Of the mice, identified, *Arvicanthis* spp. were the majority (7.1%). It was also found that most of the rodents caught (39.4) were from Kyerwa interface areas. Male rodents were dominant (52.7%) and most of the catch (57.7%) were realized during wet season. Of interest, more than 60% of the rodents were caught from crop production areas suggesting the likely destruction of crops that were in the study districts. It is concluded that different types of small mammals are prevalent in humans - wildlife interface areas of western Tanzania that poses threats to public through raiding of crops, transmission of pathogens and destruction of household property. This calls for the concerted efforts integrated approaches are needed to control the rodents in order to alleviate the impacts caused by rodents to the public.

Keywords: rodents, humans – wildlife, interface, trap, Tanzania

2.2 Background

Rodents are members of the kingdom Animalia and the Chordata phylum, belonging to the class Mammalia, with over 2590 species worldwide. They are classified under the order Rodentia, with over 29 families and 468 genera. The most common families within Rodentia are *Muridae* (rats and mice), *Sciuridae* (squirrels), *Echimyidae* (spiny rats), *Heteromyidae* (pocket mice and kangaroo rats), and *Dipodidae* (jerboas and jumping mice), where the most prominent family is *Muridae*, which encompasses over 395 species and 98 genera (Aplin *et al*., 2003). Rodents are remarkably successful and abundant species, constituting around 42% of the total mammals (Aplin *et al*., 2003). Their success can be attributed to their prolific reproductive rates and their ability to thrive in various habitats including terrestrial, fossorial, aquatic, and arboreal environment (Ademola *et al*., 2021). Rodents are found naturally in a variety of habitats globally except Antarctica. However, forest habitats exhibit greater diversity of rodent species compared to lowland environments (Madden *et al*., 2019; Mulungu *et al*., 2008). Rodents are abundant in Sub-Saharan Africa's savanna forests, forest clearings, and cultivated areas (Witmer and Shiels, 2017). In Tanzania, the most common species include *Acomys spinosissimus*, *Aethomys chrysophilus*, *Arvicanthis neumanni*, various *Graphiurus* species, *Geebilliscus vicina*, *Lemniscomys zebra*, *Lemniscomys griselda*, *Mus minutoides*, and *Rattus rattus* (Massawe *et al*., 2011).

On the other hand, shrews are small mammals, insectivores, which have cylindrical body, short, narrow limbs, and clawed fingers. With the exception of short-tailed and water shrews, their ears are rounded and somewhat large, and their eyes are small but typically visible through the fur. The length of a species' tail varies; some are noticeably longer than their bodies, while others are considerably shorter (Schlitter *et al*., 2014; Wilson & Reeder, 2005). Most shrews live on the ground, but some tropical species, such as the forest musk shrews (genus *Sylvisorex*) and white-toothed shrews (genus *Crocidura*) forage and travel in bushes, vines, and small trees

beneath the forest canopy (Haring *et al*., 2023). They are differentiated from rodents by presence of diastema (a gap between premolar and incisor teeth), continuous gnawing and presence of ever-growing long incisor teeth in rodents. The 24 genera of “true” shrews are classified in three subfamilies (*Crocidurinae*, *Soricinae*, and *Myosoricinae*) within the family *Soricidae* (Gliwicz & Taylor, 2002; Haring *et al*., 2023).

Rodents play crucial roles in ecosystems worldwide, serving as both prey and predators and influencing ecological dynamics (Singleton *et al*., 2021). Moreover, they pose considerable threats to both human and animal health by compromising food security and serving as reservoirs and vectors for a wide range of pathogens, including bacteria, rickettsia, viruses, fungi, and parasites (protozoa, helminths), as well as fleas that transmit diseases (Eisen *et al*., 2018; Meerburg *et al*., 2009; Ribas *et al*., 2016). Thus, making their identification and monitoring essential for public health. Numerous studies have highlighted the direct and indirect consequences of uncontrolled rat populations in certain areas, leading to the transmission of zoonotic diseases to humans and domestic animals (Dahmana *et al*., 2020; Issae *et al*., 2023; Meerburg *et al*., 2009; Rabiee *et al*., 2018). Moreover, previous studies reported several rodent-borne zoonoses in Kagera and Kigoma including bartonellosis and leptospirosis (Mhamphi *et al*., 2023; Motto *et al*., 2021). However, there is little information about rodent’s species composition in human-wildlife interfaces in Kagera and Kigoma. Therefore, the purpose of this study was to establish the baseline data on rodent species available in humans - wildlife interface areas in Kibondo, Uvinza and Kyerwa.

2.3 Material and Methods

2.3.1 Study area

The study was conducted in Kibondo and Uvinza districts in Kigoma and Kyerwa district in Kagera (Fig. 2.1). The suitability of these areas as study sites for rodent species identification lies in their rich

biodiversity, varied habitats, agricultural importance, and conservation significance.

The Kigoma region lies in the northwest part, along the scenic shores of Lake Tanganyika. To the north it borders with Burundi, to the east borders with Kagera, Shinyanga and Tabora regions. Its borders are shared with the Rukwa region to the south and the Congo to the west. The vegetation in this area is mostly composed of a combination of open and closed woodland, making up around 70% of its total area. It also includes varying-sized marshes and bushy grasslands. The Moyowosi Game Reserve in Kibondo, spans more than 6 000 square kilometers. It is well-known for its wide variety of fauna including rodents among other species. Uvinza covers an area of 10 058 km², making it the largest district in the Kigoma region. Almost 95% of people in Uvinza District Council are involved in agriculture. They cultivate food crops such as maize, cassava, rice, and beans, which could facilitate rodent activities in the area. Moreover, there are diverse habitats in Uvinza that ranges from forests, savannahs and wetlands, support different rodent species.

Kigoma region is characteristically tropical with a distinct long rainy season beginning from late October to May with short dry spell in January and February followed by a dry season from June to September. Annual rainfall is variable ranging from 600 mm - 1500 mm being the heaviest in highlands, intermediate in the lower slopes and low in the valley bottom and lake- off shore areas. Mean daily temperatures range between 25°C in December, January to 28°C in September. Temperature varies inversely with altitude.

The Kagera region lies in the northwestern corner of Tanzania and covers an area of 40,838 square km. Administratively; Kagera region comprises of seven districts namely Bukoba, Muleba, Karagwe, Ngara, Biharamulo, Missenyi and Kyerwa. The region borders with Uganda, Rwanda and Burundi and Lake Victoria. Agriculture is an

important economic activity of people where they grow coffee, bananas, vanilla, tea, cotton, tobacco and maize.

The climate of Kagera is characterized by annual rainfall that range from 800 to 2 000 mms, with a bi-modal rainfall patterns from March to May as long rains and from October to December as early (short) rains. High amount of rains is realized along Lake Victoria's costs and decreasing inland and away from the lake as well as with height. Dry seasons are from June to September and a short and less dry spell during January to February. The temperature ranges from 16°C to 26°C.

Kyerwa is situated in the northwestern part of the Kagera region, close to the border with Uganda. The Murongo ward, where samples were collected is located at latitude 1^{o} 3’ 47” South, longitude 30^{o} 40’13” East in Kyerwa district. Murongo is bordered to the north by Uganda and to the west by Rwanda and within the Ibanda and Rumanyika Game reserves. This area has a diversity of species including rodents.

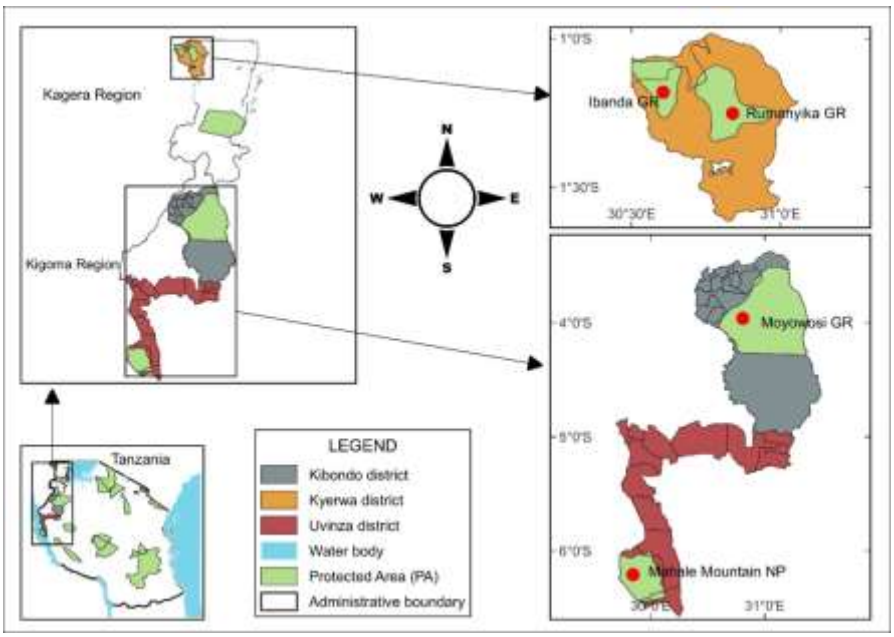

Figure 2.1: Map of the study area and surrounding wild life areas (game reserves and national park)

Sources: QGIS Version 3.24 "Tisler" retrieved on April 2024

2.3.2 Study design and sampling duration

This study employed a cross- sectional design. Trapping of small mammals was done in the selected study villages in specific districts twice per year; during wet season (October to May) and dry season (June to September). Trapping of small mammals was done for three years 2016, 2017 and 2018 consecutively through a PREDICT Project. The data of small mammals trapped was stored in the PREDICT Project database.

2.3.3 Selection of study villages

Study villages considered for setting of rodent traps were those bordering with wildlife protected areas. Information on villages were explored through informal interviews with village leaders and elders and thereafter potential sampling sites were identified. Village selection criteria of included:

a) Being in the human-wildlife interface areas of Burigi – Chato, Rumanyika, Ibanda-Kyerwa and Moyowosi game reserves and Ugalla – Moyowosi/Kigosi-Malagarasi ecosystem;
b) Conversion of land for agricultural activities and settlement degrading some of forests areas (changing dynamics of land use) and potential for wildlife-human contact through crops raiding interfaces;
c) Transboundary movement of people and animals between the villages and wildlife/forest protected areas;
d) Villages with biodiversity hotspot like rodents at in human dwellings, crops farms (Crops raiding interface), around human dwellings (Peri-domestic interfaces) and areas sharing of resources like food, water and habitats.
e) Reported high burden of fevers of unknown origin (FUO) at Health centers/dispensaries

A total of 13 villages were selected for this study that included six in Kibondo, four in Uvinza and three in Kyerwa. The names of villages in each study districts and the respective wildlife and forest protected areas are shown in Table 2.1.

Table 2.1: Sampling villages and their respective wildlife protected areas in Kibondo, Uvinza and Kyerwa

District	Names of study villages	Wildlife protected area
Kibondo (n=6)	Busunzu, Kigendeka, Kigina, Kisogwe and Nyankwi.	Moyowosi game reserves
Uvinza (n=4)	Basanza, Msebei, Chakulu, and Mwamila	Ugalla – Moyowosi/Kigosi-Malagarasi ecosystem
Kyerwa(n=3)	Murongo, Masheshe, Rwabikagati	Burigi – Chato, Ibanda-Kyerwa and Rumanyika

2.3.4 Characteristics of the selected areas for rodents trapping

Selected sampling fields in the study villages were classified based on human activities that were reported in a place as follows:

(i) Animal production area: These were fields used as grazing land

(ii) Animal and crop production: field that had grazing land and crop production
(iii) Crop production: The area had crop farms
(iv) Crop production and human settlements: The areas had crop farms and residential areas
(v) Crop production and industry for palm oil extraction: Areas with crop farms and palm oil extraction small factories

2.3.5 Trapping and handling of rodents

Rodent traps in each study village were set in strategic specific areas as were advised by village leaders and elders. The traps used were Sherman metal box traps 8×9×23 cm (H.B. Sherman Traps Inc., Tallahassee, USA) and Tomahawk traps (Machtinger and Williams, 2020). Setting of the traps was done in peri-domestic areas specifically around the households and farms. Peanut butter mixed with maize bran was used to bait the traps. Traps were set in the evening and checked for the catch in the following morning. Not less than 100 traps were set in each sampling site per night. An established grid of 100m x 100m containing 10 lines each containing 10 traps was set 5 meters apart. Areas where rodents were captured were geo-referenced.

2.3.6 Rodent species identification

Following capturing rodents were anesthetized by using isoflurane. About 0.4 ml of isoflurane was applied to a cotton ball and then later into a metal tea ball or plastic tube where the rodents were put. The dose was adjusted based on the size of the rodent. The rodents were put on a table and species identification was done based on morphological features (body length, tail, fur, hind-foot length-pes and ear) as described by (Wilson & Reeder, 2005) and Happold *et al*. (2013). After species identification biological samples were collected from each rodent and thereafter, they were monitored to fully recover from anesthesia and released back to their nature.

2.4 Results

2.4.1 Species groups and catch rates of rodent and shrews in study areas

A total of 5 263 small mammals were trapped from Kibondo, Uvinza and Kyerwa during the three years of data collection as shown in Table 2. Majority (87.6%, n=4613) were rodents and 650 (12.4%) were shrews. Among the rodents, rats were the majority (70.0%; n=3683) while mice were 873 (16.6%) and squirrels were only 44 (0.8%). The dominant species in the group of rodents were *Mastomys natalensis* which constituted 63.6% (n=3346). Among the mice, Arvicanthis spp. were the majority as they constituted 7.1% (n=373) of all small mammals trapped. A total of 13 (0.3%) small mammals were identified as rodents but their genus was not established. Details of the species trapped, their English name, groups and the number (percentage) of small mammals trapped in study areas are shown in Table 2.2.

Table 2.2: Species, names, groups and number (%) of rodents and shrews trapped in the study areas

Species	Common name	Group	Number (%)
Mastomys natalensis	Mice	Rodent	3346 (63.6)
Crocidura spp.	Shrews	Shrews	646 (12.3)
Arvicanthis spp.	Mice	Rodent	373 (7.1)
Lemniscomys spp.	Mice	Rodent	221 (4.2)
Lophuromys spp.	Rats	Rodent	114 (2.2)
Praomys tullbergi	Mice	Rodent	114 (2.2)
Mus spp.	Mice	Rodent	110 (2.1)
Rattus rattus	Rats	Rodent	79 (1.5)
Pelomys spp.	Rats	Rodent	58 (1.1)
Graphiurus murinus	Squirrel	Rodent	44 (0.8)
Mastomys spp.	Rats	Rodent	44 (0.8)
Lemniscomys rosalia	Mice	Rodent	32 (0.6)
Apodemus spp.	Rat	Rodent	21 (0.4)
Aethomys spp	Rat	Rodent	14 (0.3)
Myoxidae spp.	Mice	Rodent	16 (0.3)
Apodemus sylvaticus	Mice	Rodent	7 (0.1)
Praomys spp.	Rats	Rodent	7 (0.1)
Elephantulus spp.	Shrews	Shrews	4 (0.1)
Rodents	Unidentifed group	Rodent	13 (0.3)
Total			**5263**

2.4.2 Distribution of trapped rodents and shrews per year, season, district and type of interface area

Out of 5 263 small mammals, majority 2 673 (50.8%) were trapped in 2018. Kyerwa district that had three study villages namely Murongo, Masheshe, Rwabikagati had the highest number 2075 (39.4%) of small mammals trapped compared to the other districts.

Most of the small mammals trapped (52.7%; n=2773) were males and most of the catch (57.7%; n=3036) were realized during wet season. The type of interface area of small mammals trapping that had the highest catch (60%; n=3156) was crop production areas while animal production areas had the lowest catch (1.2%; n=64) for all the three years of study. More detailed information on trapped small mammals in the three years, seasonality of catch, districts and type of interface area is shown in Table 2.3.

Table 2.3:Trapped rodents and shrews grouped per year, sex, season, district and type of interface area

Parameter	Category	Number of small mammals	Proportion (%)
Year	2016	1183	22.5
	2017	1407	26.7
	2018	2673	50.8
District	Kyerwa	2075	39.4
	Kibondo	1923	36.5
	Uvinza	1265	24.1
Sex	Male	2773	52.7
	Female	2477	47.1
	Uknown	13	0.3
Season	Wet	3036	57.7
	Dry	2227	42.3
Type of interface area	Crop production	3156	60.0
	Crop production and industry for palm oil extraction	1210	23.0
	Crop production and human settlements	575	10.9
	Animal and crop production	258	4.9
	Animal production	64	1.2

2.5 Discussion

Rodents are prevalent in humans - wildlife interface areas of Kibondo, Uvinza and Kyerwa and poses a threat to crop field since they are known to be notorious pests in agriculture. They can cause significant damage to crop fields by feeding on crops or by digging burrows that disturb the soil and root systems. This poses a threat to local agricultural production and can potentially lead to food

insecurity for communities relying on agricultural crops such as the communities in the study area. Their prevalence is also associated to health risks as they are potential vectors of pathogens therefore the close proximity of rodents to human populations increases the risk of disease transmission, especially in areas with poor sanitation and hygiene practices. A recent study in Kigoma have reported rodents are carrier of Leptospira antibodies indicating potential public health threat. Nonetheless, rodents are also known to cause damage to property, particularly in residential areas. They may gnaw on wood, plastic, and electrical wiring, leading to structural damage and potential fire hazards in houses. This not only poses financial burdens on affected households but also increases the risk of accidents and injuries.

Kyerwa humans - wildlife interface areas were more infested with rodents' small mammals, especially at the Kaishunga humans - wildlife interface areas which is likely due to the result of a combination of factors, including climate and environmental conditions where changes in temperature and precipitation can affect the availability of food and shelter, which in turn can impact rodent population dynamics. The significant annual rainfall in Kyerwa could be contributing to the high rodent population. Also, the vegetation cover resulting from high rainfall provides ample food resources for rodents, while the wet conditions create favorable habitats. Moreover, together with favorable climate, human activities such as farming result in an abundance of food resources for rodents, leading to population increases. However, further research would be needed to determine the exact causes in this specific area. It's also important to note that while a high rodent population can pose challenges, rodents also play important roles in ecosystems, such as seed dispersal and as a food source for predators.

On the other hand, majority of the small mammals captured were rats, in particular *Mastomys natalensis*; this is linked to the species' ability to produce large litters, averaging approximately 12-24 young

per litter. However, presence of lowland cultivated areas might have contributed to the population growth due to the availability of food and suitable habitats for nesting and breeding in the study area. Similarly, abundance of this species might be attributed to the fact that *Mastomys natalensis* has a broad habitat tolerance and is known to consume a variety of food in response to the availability of food items. Previous studies have reported that *Mastomys natalensis* are spatially distributed and are found in a range of habitats including agricultural fields, fallow lands, woodlands, forest clearances and savannahs, which aligns with the findings of this study where rodents were collected in peri-domestic areas particularly in crop-raiding areas. However, abundance of *Mastomys natalensis* reported in this study is relatively high as compared to studies in other areas (Agbonlahor *et al.,* 2017; Massawe et al., 2012), which is probably attributed to spatial-temporal variations due to factors such as seasonal changes, food availability, breeding cycles and lack of predators.

A big number of mice were also captured but those of *Arvicanthis* spp. were the majority, these are commonly known as African rats or grass rats. They are typically found in grassland habitats across sub-Saharan Africa. These rodents are known for their adaptability to various environments and their ability to thrive in agricultural landscapes. Their dominance could be influenced by factors such as habitat suitability, availability of food resources in the study area. Considering their herbivores nature, feeding primarily on grasses, grains, and other vegetation; their abundance in agricultural areas can pose a significant threat to crop production, as they may consume and damage crops, leading to economic losses for local farmers; and an increased risk of diseases transmission to humans and animals due to the nature of their interaction.

Most of the small mammals trapped were males which could be attributed to the predominance of males among small mammals in the study site. It also might relate to the behavior and physiology of

these animals; where in some species, males tend to have larger home ranges and may be more exploratory, increasing their likelihood of encountering traps. Additionally, mating behaviors could lead males to be more active and therefore more likely to come into contact with traps.

Wet season had most of the catch (57.7%) compared to dry season in all the study districts. High abundance during wet season reported in this study is most likely due to the fact that rodents often breed more prolifically in moist environments, as the wet conditions provide suitable habitat and resources for reproduction. With more breeding pairs, rodent populations can quickly expand. Human activities related to farming, construction, or land development may disrupt rodent habitats during the wet season, forcing them to relocate and increasing the chances of capture. Moreover, moisture availability brings about lush vegetation and abundant crops, providing rodents with ample food sources and shelter. The findings of this study are concurrent with other reports in Tanzania that have reported seasonal variation as a factor contributing to abundance of rodents (Massawe *et al.,* 2012; Mulungu *et al.,* 2008; Yihune & Bekele, 2012). However, the findings of this study is contrary to the reports of some of studies of seasonality in other areas that have reported high abundance during dry season (Shilereyo *et al.,* 2023). Therefore, this necessitates the need to fully understand the complex interplay of ecological, environmental, and anthropogenic factors that influence their abundance and distribution.

Furthermore, crop production interface areas had up to 60% of the total catches while animal production areas had the lowest catch (1.2%). This is due to the fact that in crop productions there is food resources and the availability of suitable vegetation provide good-quality shelter which influence their survival and reproduction as compared to the animal production area. Moreover, this is attributed to the dominance of *Arvicanthis* spp. and *Mastomys natalensis* which are two most common species in crop production areas.

2.6 Conclusion and Recommendations

In conclusion, the prevalence of rodents in Kibondo, Uvinza, and Kyerwa poses significant threats to both the agricultural sector and public health. It is crucial to implement effective rodent control measures to mitigate these risks and protect the local communities. Nonetheless, addressing these challenges requires a multi-faceted approach that includes implementing measures for pest control, improving sanitation and waste management practices and promoting community awareness about the risks associated with rodents. Additionally, research and monitoring efforts are crucial for understanding the dynamics of rodent populations and their impact on both human and animal health and the environment. Therefore, further research is needed to better understand the behavior of these rodents and develop more effective strategies for their management.

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CHAPTER THREE: MANUSCRIPT TWO

Rodent Reservoirs: Unraveling Spectrum of Zoonotic and Pathogenic Bacteria.

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3.1 Abstract

Zoonotic diseases are the major public health threat worldwide. Almost 70% are estimated to have originated from wildlife. Despite performing several beneficial roles in the environment, rodents are known to carry and transmit several evolving zoonotic diseases to human. Rapid increase in agriculture, industrial change and land use change have been associated with an increase in rodent borne zoonoses. Some of these diseases includes hemorrhagic fevers, Plague, tularemia and leptospirosis; which are either transmitted directly or indirectly. The fact that diseases have been transmitted and undoubtedly will continue to be transmitted from rodents, necessitate the need to understand diversity of disease-causing agents in rodents in order to understand the necessity of intervention and how, when and where to direct those interventions. Therefore, this study aimed at screening and identification of various pathogenic and zoonotic bacteria harbored by rodents. A total of 116 rodents achieved samples (101 oral-pharyngeal and 15 rectal swabs) collected from Kibondo, Uvinza and Kyerwa were used. Total RNA was extracted from each swab sample and then pooled according to rodent species, location and swab types to make twelve pools. A portion of pooled swabs were polyadenylated and used for metagenomics sequence libraries preparation. A 16S rRNA metagenomics sequencing was performed on 12 pools in order to identify microbial diversity. A total of 13 different microbial communities including bacteria were identified, 15 families of potentially pathogenic bacteria were also identified; these included *Mycobacteriacea*, *Helicobacteriacea*, *Enterobacteriacea*, *Vibrionacea*, *Staphylococcaceae*, *Nocardiaceae*, *Bacillaceae*, *Pasteurellaceae*, *Streptococcaceae*, *Campylobacteraceae*, *Leptospiraceae*, *Brachyspiraceae*, *Moraxellaceae*, *Enterococcaea*, *Flavobacteriacea*. Potentially pathogenic, zoonotic and bacteria of unknown zoonotic potential including *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Helicobacter pylori* and *Vibrio parahaemolyticus* are reported in this study. Reporting of several bacteria of public and veterinary importance in this study indicates the chances of cross-

transmission, and an increased risk to human and animal infection, given the nature of proximity between rodents, humans and animals. This study is important first step toward understanding the risk of zoonotic disease transmission posed by rodents. Although this study was unable to track a tangible evidence of disease transmission by rodents, we hypothesize that rodents are potential source of human infection especially in resource poor environment where there is close contact between rodents and humans.

Keywords: Zoonoses, Rodents, 16S rRNA metagenomics, Families, Bacteria

3.2 Background

Emerging infectious diseases that pose public health and economic threats are mainly zoonoses, with over 70% estimated to originate in wildlife (Akhtar *et al*., 2023; Jones *et al*., 2008). Zoonotic diseases impose a major morbidity and/or mortality burden worldwide (Cascio *et al*., 2011). Rodents are the most specious and diverse group of mammals and they perform several beneficial roles in environment (Rabiee *et al*., 2018; Zhang *et al*., 2021). Despite their benefits, they are also sources of zoonotic diseases. Over decades, wild and commensal rodents have been cited as a major reservoir of evolving zoonotic pathogens which cause diseases in humans (Woolhouse *et al*., 2001). Rodents are considered reservoir of several diseases including leptospirosis (Boey *et al*., 2019; Issae *et al*., 2023), plague (Makundi *et al*., 2005; Wobeser *et al*., 2009), toxoplasmosis (Islam *et al*., 2021) and hemorrhagic fevers (Borremans *et al*., 2011; Fulhorst *et al*., 2007; Phan *et al*., 2011). They have also been reported to harbor several complex bacteria like *Mycobacterium tuberculosis*, *Mycobacterium microti*, and *Escherichia coli* (van Soolingen *et al*., 1998). Agricultural intensification, urbanization, and industrialization throughout the globe, has contributed to a significant increase in rodent borne zoonotic diseases (Ecke *et al*., 2022; García-Peña *et al*., 2021). Rodents can transmit pathogenic agents

to humans and animals via direct contact, or through contamination of human food and water with rodents' stool and/or urine. Ectoparasites carried on the skin of most rodents are also able to transmit zoonotic pathogens. Occupations associated with rodent population handling, animal trade and large-scale traveling are among the risk factors associated with rodent-human pathogens transfer (Kurpiers *et al*., 2015). The fact that diseases can be transmitted between rodents and humans highlights the risks associated with the close contact between humans and commensal or peri-domestic rodents in Tanzania. Rodents have caused human disease outbreaks in the past, and they will certainly continue to do so in the future. However, it has been widely acknowledged that dealing with the problem of zoonotic infections is a task that is beyond medical and public health specialists alone, but rather it should include veterinary and environmental parameters, together with understanding of human social behavior. Information about the prevalence of various infections in rodents is essential in estimation of the risk for humans. This information is crucial in establishing the need for interventions along with how, when and where to intervene. Therefore, this study focused on screening and identification of various pathogenic and zoonotic bacteria circulating in rodents so as to provide baseline information on the potential health risks associated with the interaction between human, animals and rodents. Previous studies in rodents have been able to provide useful information through identification of several agents of public health importance, however most of these studies have used traditional/conventional methods of identification. In this study high throughput Next generation metagenomics sequencing was employed to identify the microbial diversity in rodents. Metagenomics is able to analyze multiple genomes of bacterial species (Miller *et al*., 2013); it also allows the identification of bacteria genomes directly from samples without culture and can reveal information related to the diversity of microbes that circulate in hosts.

3.3 Materials and Methods

3.3.1 Study area

The study employed rodent samples that were collected in 2018 in connection to another project and archived in SUA laboratory. Samples were collected in human-wildlife interfaces around three districts in two regions: Kyerwa district in the Kagera region as well as Kibondo and Uvinza districts in the Kigoma region as indicated in (Fig. 3.1). In Kyerwa samples were collected from Murongo ward, which is located at latitude 1° 3' 47" South, longitude 30° 40'13" East in Kyerwa district. Murongo is bordered to the north by Uganda and to the west by Rwanda and within the Ibanda and Rumanyika Game reserves. This is a high-risk interface characterized by the high transboundary movement of both humans and livestock. There is also land use change due to agricultural intensification and mining resulting in close contact between humans and wildlife including rodents. Kibondo district is located at latitude 3° 35' 11" South, longitude 30° 43' 13" East in the western part of Tanzania. The district is bordered to the North-West by Burundi which facilitates cross-border trade at Kumsenga and Mkarazi markets near the Burundi-Tanzania border. High demands for charcoal and firewood as well as illegal hunting in the Moyowosi game reserve pose risk for infection spillover and spread. Uvinza district is located at latitude 5° 6' 7.80" South, longitude 30° 23' 16.79" East in Kigoma region, where Illegal wildlife hunting and consumption in the Uvinza open area could facilitate zoonotic pathogen transmission.

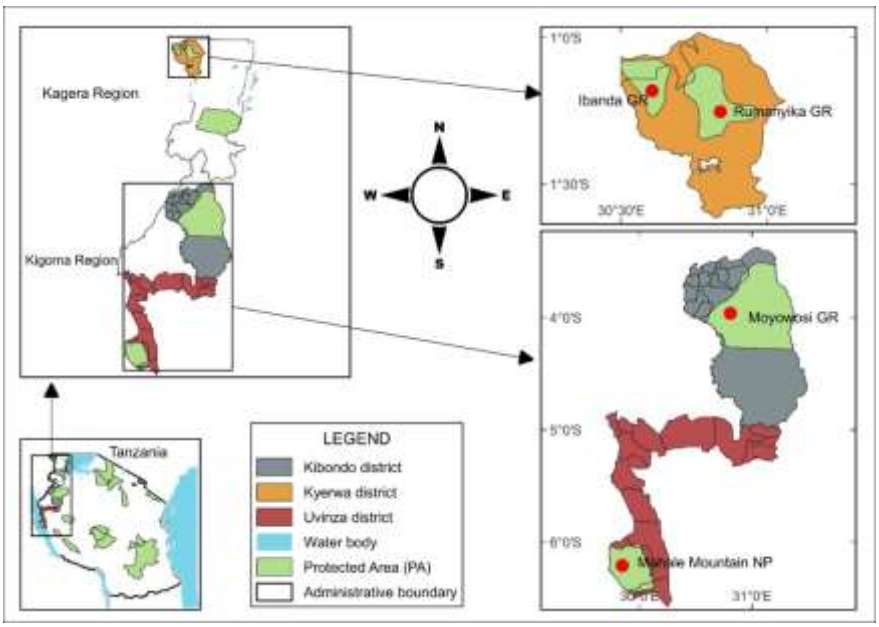

Figure 3.1: Map of the description of study areas where rodents and shrews were trapped in human-wildlife interfaces in Kibondo, Uvinza and Kyerwa districts

Sources: QGIS Version 3.24 "Tisler" retrieved on April 2024

3.3.2 RNA Extraction

A total of 116 swab samples (101 oropharyngeal swabs and 15 rectal swabs) collected from 101 rodents were used in this study. Total RNA was extracted by using Direct-zol RNA MiniPrep Kit (Zymo Research, Irvine, CA, USA) as per the manufacturer's instructions. In short, the extraction processes involved a series of centrifugation and filtration by using pre-wash and wash buffers. Elution of RNA from the silica membrane was done by using 50ul nuclease-free water. Extracted RNA was aliquoted and stored in a -80^{0}C freezer for a few days at the Sokoine University of Agriculture HALi Laboratory, and then dry shipped to the Kilimanjaro Clinical Research Institute-Biotechnology Laboratory (KCRI-BL) for metagenomics Next generation sequencing.

In the laboratory RNA samples were pooled according to the type of swabs (Oral-pharyngeal or rectal swabs) rodent species (*Crocidura* spp., *Lemniscomys* spp., *Mastomys natalensis* and *Rattus rattus* and *Mus muscullus* combined as domestic rats) and location (Kibondo, Kyerwa, Uvinza) to make twelve pools (Table 3.1). Oral-pharyngeal swabs made eleven pools (1-11) and rectal swabs made a single pool (the twelfth). Each district had four pools, with each pool comprising one of the rodent species i.e., a pool of *Mastomys natalensis*, *Crocidura* spp., *Lemniscomys* spp., and domestic rats (*Mus muscullus* and *Rattus rattus*) from Kibondo, Uvinza, and Kyerwa (with exception of the twelfth pool that comprised rectal swabs from all of the collected rodent species from Kyerwa). Each pool carried a different number of samples (a minimum of 1 and a maximum of 34) based on sample proportions; and hence different final volumes in the pools. Rodent species from Kibondo including, domestic rats (*Rattus rattus*+ *Mus muscullus*), *Mastomys natalensis*, *Lemniscomys* spp. and *Crocidura* spp. were included in pools 1, 2, 3, and 4 respectively. On the other hand, pools 5, 6, 7, and 8 included domestic rats (*Rattus rattus*+ *Mus muscullus*), *Mastomys natalensis*, *Lemniscomys* spp., and *Crocidura* spp. respectively from Uvinza. Pool 9, 10, and 11 were domestic rats (*Rattus rattus*+ *Mus muscullus*), *Mastomys natalensis*, and *Crocidura* spp. from Kyerwa respectively. Pool 12 were the rectal swabs from all of the species (domestic rats (*Rattus rattus*+ *Mus muscullus*), *Mastomys natalensis*, and *Crocidura* spp.) collected in Kyerwa. Pooling was done by taking 10µl of RNA (where samples were more than one), and 20µl (where there was only a single sample) from each species across three locations/districts, ending up with four pools from each location.

Table 3.1: Description of the pooling procedure for Metagenomics sequencing

Pool ID	Number of Rodent swab samples	Pooling Volume (ul) per Sample	Total Volume (ul) per Pool	Swab type
1	3	10	30	Oral-pharyngeal
2	34	10	340	Oral-pharyngeal
3	4	10	40	Oral-pharyngeal
4	9	10	90	Oral-pharyngeal
5	2	10	20	Oral-pharyngeal
6	30	10	300	Oral-pharyngeal
7	2	10	20	Oral-pharyngeal
8	2	10	20	Oral-pharyngeal
9	1	20	20	Oral-pharyngeal
10	10	10	100	Oral-pharyngeal
11	4	10	40	Oral-pharyngeal
12	15	10	150	Rectal swab

3.3.3 Poly (A) Tailing

A total of 20µl of the pooled RNA samples from each of the final pool was taken for Polyadenylation (a process of adding ≥150 bases poly (A) tail to RNA transcripts), by using enzyme E. coli Poly (A) Polymerase (E-PAP) and ATP at 370C, 60 minutes incubation.

3.3.4 Reverse transcription and strand switching

To obtain ≥1µg of metagenomics complementary DNA (cDNA) for the library required for the Nanopore sequencing protocol, randomly amplified cDNA was generated using a primer-extension pre-amplification method according to the Protocol (ONT) provided with PCR-cDNA Barcoding kit (SQK-PCB 109); as previously described by (Greninger *et al.,* 2015). A single-cycle PCR at 42°C for 90 minutes was performed for reverse transcription and strand switching. Polyadenylated RNA were reverse transcribed by using Maxima H Minus Reverse Transcriptase (Thermo Fisher Scientific), and amplified by random primers (VNP Primers). Strand switching was done by using Strand-Switching primer (Oxford Nanopore Technologies). Labeling of the samples was done by adding

barcodes to the reverse transcribed RNA (cDNA) sample in a Barcoding PCR that used barcode Primers (Oxford Nanopore Technologies) and LongAmp Taq 2x Master Mix (New England BioLab).

3.3.5 Preparation of Nanopore sequencing libraries

Barcoded cDNA from the PCR reaction mixture was purified using AMPure XP beads (Beckman Coulter, Brea, CA). 1µg cDNA was used as input into Oxford Nanopore SQK-PCB 109 kit for generation of MinION Oxford Nanopore-compatible libraries following manufactures protocol as described by (Joyon, 2021). Briefly, to each reaction tube, 1µl 20 Exonuclease 1 (New England BioLabs) was added and incubated on HulaMixer and eluted in 12 µl elution buffer (EB) on a magnet as per instructions of the kit. After elution, quantity measurement was carried out on Qubit 4 Fluorometer (Invitrogen) using Qubit™ dsDNA HS Assay Kit (Thermofisher Scientific). Subsequently, 1µl from each sample was pooled together in one reaction tube making the final volume of the pooled cDNA library 12 µl. Ligation to the protein-linked adapter was done by adding 1µl of Rapid Adapter (RAP) to the cDNA library. Again, libraries were quantified by using qubit fluorimeters and quality assessment was done by using gel electrophoresis, where observation of smear was an indication of good quality libraries.

3.3.6 Nanopore sequencing

Sequencing of the cDNA on the MinION device was performed in the R9.4.1 Flow cell (FAO17147). Before loading, the flow cell was washed with Flow Cell Wash Kit according to the protocol (ONT). Priming and loading the MinION Spot on the flow cell was done following the instructions of the PCR-cDNA Barcoding kit (SQK-PCB 109). The standard MinKNOW protocol script was used for the sequencing. The run time of the MinION device was set to 36 hours in 190 voltages without base-calling and the quality score cut-off was set to 7.

3.3.7 Bioinformatics analysis

MinION reads were basecalled with ONT Guppy version 6.4.2, using the 9.4.1_450bps_SUP model. Basecalls were demultiplexed with ONT Guppy barcorder 6.4.2. Reads were screened for human and vector contaminants by using FastQscreen v0.14.1 with GRCh38 and UniVec_Core. Basic QC metrics (read count, base count, Q score, N counts) were obtained using fastq-stats from fastq-utils 1.3.0 Taxonomic classification was performed with Kraken2 v2.1.2 using the Kraken2 “standard” databases, constructed from NCBI RefSeq data retrieved between 15-18 November 2022. Moreover, the sequencing reads were processed by trimming the sequencing adapters using Porechop version 0.2.4. The quality of the trimmed sequencing reads was checked using nanoplot version 1.41.0. Afterwards, the data were uploaded and run in Kaiju (Menzel *et al.,* 2016) for metagenomics classification overview.

3.3.8 Ethical statement

Capturing of rodents was done following ethical approval by the COSTECH and TAWIRI ethical review committee, along with the research approval from the Sokoine University of Agriculture.

3.4 Results

A total of 116 (101 oral-pharyngeal and 15 rectal swabs) samples were used in the present study. In a metagenomics analysis 44 species of bacteria were detected. Whereby, 10 (22.7%) of the bacteria species including *H*. *pylori* and other *Helicobacter* species of were also detected in the rectal swab pool which included (domestic rats (*Rattus rattus*+ *Mus muscullus*), *Mastomys natalensis*, and *Crocidura* spp.) from Kyerwa district; the remaining 34 (77.3%) of the detected bacteria species was distributed among oral-pharyngeal swabs pools (Fig. 3.2).

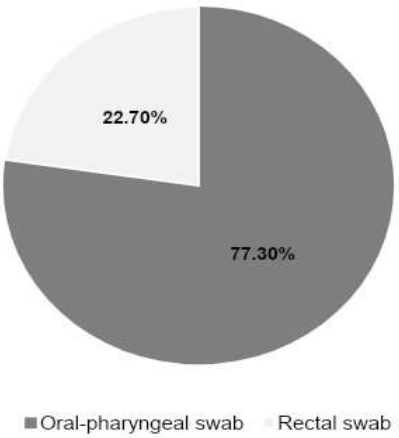

Figure 3.2: Proportion of bacteria detected across swab types collected from rodent species in Kagera and Kigoma regions, Tanzania

Bacteria species were detected in eight pools (1, 2, 4, 5, 6, 10, 11 and 12). Whereas, in pools 3, 7, 8 and 9 there was no any detected bacteria species (Fig. 3.3)

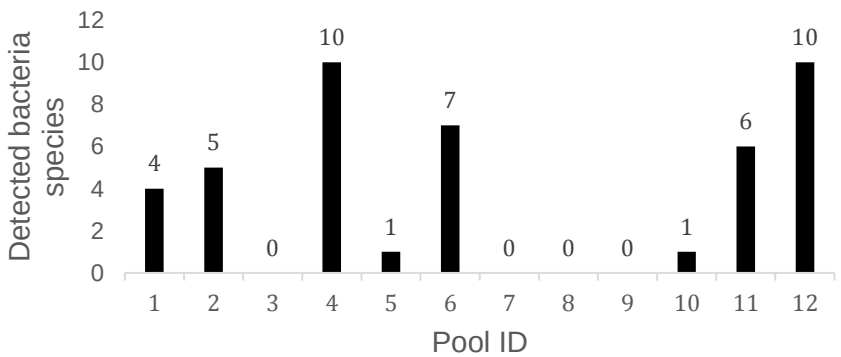

Figure 3.3: Number of bacteria specie detected in different pools of rectal and oropharyngeal swabs from rodent species obtained from Kagera and Kigoma regions, Tanzania

A total of 13 Microbial families with diverse characteristics, which play essential roles in various ecosystems were identifies in a metagenomics analysis (Fig. 3.4).

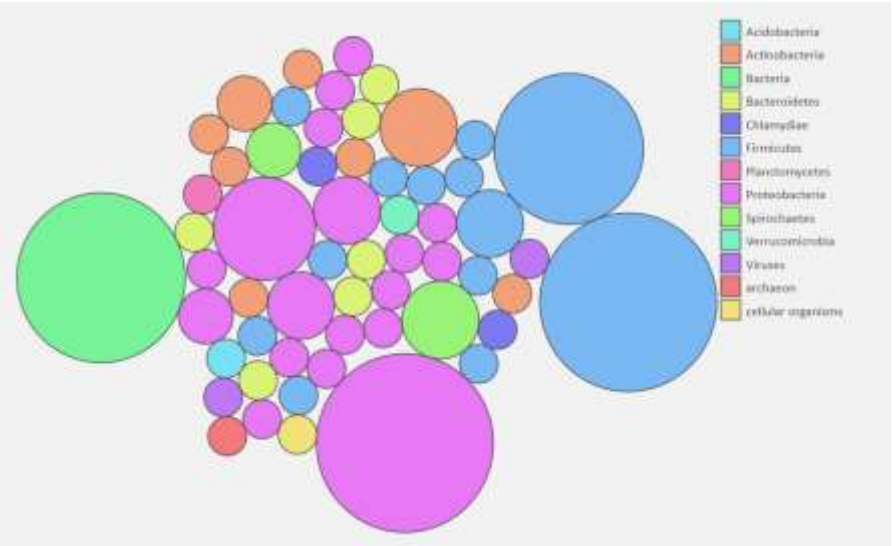

Figure 3.4: Metagenome overview of oropharyngeal and rectal swabs collected from rodents in human-wildlife interfaces in Kigoma and Kagera regions, Tanzania.

A total of 15 bacterial families including groups of pathogenic, zoonotic and bacteria of unknown zoonotic potential were identified in a metagenomics analysis as indicated in Figure 3.5, below.

Figure 3.5: Tree diagram showing families and species of bacteria detected in a metagenomics analysis of oropharyngeal and rectal swabs collected from rodent species from Kigoma and Kagera region, Tanzania

3.5 Discussion

A total of 15 bacterial families and numerous potentially pathogenic, zoonotic and bacteria of unknown zoonotic potential including *Mycobacterium tuberculosis*; *Vibrio cholerae*; *Helicobacter pylori* and *Vibrio parahaemolyticus* among others, are reported in this study (Fig. 3.5). Bacteria of public health and animal health importance were identified in a metagenomics analysis. These detected bacteria have various transmission methods including, airborne, direct contact, through contaminated food products and other fomites; indicating the possibility of cross-transmission of diseases between human, animals and rodents. In rural settings, rodents exist in large population where they live and feed in close proximity to humans than many other mammal species. As urbanization continue most successful synanthropic species are likely to assume significance role in zoonotic disease transmission as the complexity of the human-animal-rodents interface increase. This study is an important first step toward understanding the risk of zoonotic disease transmission posed by rodents.

This study was unable to track tangible evidence of tuberculosis transmission by rodents. However, it was found that rodents are potential reservoir of *Mycobacterium tuberculosis*; suggesting the circulation of the pathogen between humans and animals in the area. Although rodents have been extensively used as animal models for tuberculosis (Li, 2023; Singh & Gupta, 2018) and have been used in sniffing studies to detect tuberculosis (Weetjens *et al*., 2009); there is scarce of information regarding natural infection of rodent with *M. tuberculosis*. To the best of our knowledge this is the first report of natural infection of rodents with *M. tuberculosis* which indicates the possibilities of cross-transmission. The life styles of the people in these communities, close contact between animals, humans and rodents and/or the habit of consuming raw animal products, are the possible factors for transmission of *M. tuberculosis* between human, animal and rodents; consequently, impacting on the tuberculosis (TB) control programs in human. In Tanzania,

human TB control program have been widely implemented, however, the role of rodents in the transmission of the causative agent has been neglected which could be one of the challenges for an effective control program. The findings of this study together with previous studies that suggest rodents as a reservoir of *Mycobacterium microti* - a member of the *M. tuberculosis* complex (Behr & Gagneux, 2011; Cavanagh *et al*., 2002; Wells, 1937); necessitates the need for integrating animal TB control as an effective element of TB control for both human and animal using One Health approach.

Detection of *Helicobacter pylori* (*H. pylori*) in rodents may be an indication of environmental contamination and circulation of the bacteria in an ecosystem. One possibility is that, rodents acquire the bacteria from the environment but also rodents can be reservoirs host with the potential of transmitting infections to human and other animals. Moreover, because humans live in close association with rodents and over the decades human have been cited as the only natural host of *H. pylori* there are possibilities that rodents have acquired these bacteria from humans. Weather and how rodents naturally harbor or have acquired the bacterium from humans and surrounding environment is still a subject to research. To date, there is still no any reported case of clinical condition caused by *H. pylori* in rodents; however, this is the first report of natural infection of rodents with *H. pylori* in Tanzania. Therefore, the presence of this bacteria in rodents is of more concern to human health as it indicates circulation of the bacteria and the possible increase in the chances of human infection; especially in rural settings as in the study area and many other resources poor environments, where there is normally a constant close association between humans, animals and rodents. Assuming the fecal-oral mode of transmission and the proximity of rodents to human settlement in a way that rodent even defecates in human foods, presence of this bacteria in rodent not only indicate increased risks to human health but also the

increase in antibiotic resistance of *H. pylori* which is mentioned among the list of WHO antibiotic resistance priority pathogen.

Previous studies have also revealed several other environments where *H. pylori* have been detected. For instance, Grubel *et al*. (1997) demonstrated that housefly has the potential to transmit *H. pylori* mechanically, and thus fly excreta might also contaminate human foods. In Chile, consumption of uncooked vegetables that had been irrigated with water contaminated with untreated sewage was associated with *H. pylori* seropositivity (van Duynhoven & de Jonge, 2001). These hypotheses may be of the most significant in areas of the world with poor sanitation and close association between rodents and humans which is the case in our study area. In this study, although the dose of *H. pylori,* required to cause infection in humans is unknown, and we don't know if the amount released by rodents is enough to cause infection in humans; due to their diversity and the proximity to human settlements, rodents cannot be ruled out as a potential reservoir and vector of *H. Pylori* pathogen found in the present study. The reports of *H. pylori* detection in various biotic and abiotic environments such as in surface water, waste water and drinking water, in flies and now in rodents calls for ONE HEALTH effort to strengthen surveillance and detection.

Moreover, in this study rodents appear to harbor *Vibrio cholerae*; a causative agent of Cholera. Cholera affects both children and adults and can kill within hours if untreated, it is a global threat (WHO) (2022). Therefore, detection of *V. cholerae* in rodents in the study area, sadly where the communities are poor, with lack of social improvements and poor sanitation, suggest increased risks of human infections. The presence of *V. cholera* cDNA in rodents indicates the possibility that bacteria are shed into the environment through feces and potentially infecting people. Nonetheless, recently in Tanzania (April 2022) there was a report of Cholera outbreak in Tanganyika and Uvinza districts, the latter is one of the study sites. As a regard to the nature of the areas, Uvinza for instance, where

there are challenges to attain access to safe and clean drinking water, along with inadequate sanitation and a close proximity between human and rodents as a result of environment fragmentation and agriculture intensification rodents cannot be ruled out as the possible sources of human infection. Despite success in control and containment of the outbreak, in order to strengthen surveillance and preparedness it is essential to look on the other potential reservoir of *V. cholerae* including rodents. Moreover, the detection of the none-routinely investigated *Vibrio parahaemolyticus*; a causative agent of cholera-like diarrhea associated with consumption of seafoods (Gavilan *et al*., 2013) should be considered as a serious public health concern as it can lead to an unpredictable impact on populations. Therefore, this calls for a unified efforts between public, animal and environmental health which will help in preventing and controlling outbreaks and incidences of diarrhea.

Furthermore, several other bacteria of zoonotic and unknown zoonotic potential were detected. These includes, *Streptococcus mutans* a pathogen for dental caries, and a known cause of bacteremia and infective endocarditis (Zhang, 2013). *Chlamydia psittaci* responsible for avian chlamydiosis (psittacosis) in birds. In human psittacosis can cause mild illness or pneumonia (Chu *et al*., 2023), *Campylobacter sputorum* which causes gastrointestinal infections through consumption of contaminated food or contact with infected animals (Igwaran & Okoh, 2019). *Acinetobacter* species (*Acinetobacter baumannii, Acinetobacter pitti*) the most common causes of bacteremia and nosocomial pneumonia. *Haemophilus influenzae* which is reported to cause pneumonia, bacteremia, meningitis, epiglottitis, cellulitis and infectious arthritis (Brueggemann *et al*., 2021). Moreover, *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* were also identified in a metagenomics analysis. *Escherichia coli*, *Staphylococcus aureus* and *Haemophilus influenzae* were detected at least in two pools each, indicating a relatively higher recovery rate of these bacteria in

rodents, mainly because they are mostly normal flora; nonetheless, although very few strains of these bacteria are known to cause diseases in animals and humans it is essential to follow up and identify the specific strains harbored in rodents so as to identify risks in humans. On the other hand, some genera including *Streptococcus*, *Campylobacter*, *Acinetobacter* and *Helicobacter* reported in this study their role in causing diseases should not be overlooked and this should alarm the scientific community; as the possible dissemination and amplification in the environment may continue the transmission cycle and exacerbate antibiotic resistance problem in both humans and animals. Therefore, the findings of this study are in line with several other studies that have cited rodents as potential reservoir of zoonotic pathogens(Akhtar *et al*., 2023; Issae *et al.*, 2023; Jones *et al.,* 2008; Rabiee *et al.*, 2018).

3.6 Conclusion and Recommendations

We hypothesize that rodent carriers can facilitate pathogen spread and maintain disease transmission cycles, especially in regions lacking adequate sanitation infrastructure. Rodents can be potential source of human infection as we have seen in the present study that they carry a number of potentially pathogenic and zoonotic bacteria. However, despite the high potential for zoonotic transmission, the interactions among humans, animals, and rodents are still somewhat understudied. Since no molecular characterization has been done in the present study, therefore this study is not conclusive of the several pathogenic bacteria being present in rodents; hence, it is recommended to perform follow up studies to further characterize these bacteria identified as being either pathogenic or non-pathogenic strains. Moreover, to further understand the occurrence, transmission dynamics and characterize risks so as to develop effective prevention and control plans. Yet, the public has to ensure proper hygiene and food safety practices are improved in order to minimize the risk of zoonotic infections.

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CHAPTER FOUR: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

4.1 General Discussion

In this study we accomplished to report eighteen species of small mammals including rodents and shrews that are found in the study area (Manuscript One, Table 2.1). These includes *Mastomys natalensis*, *Crocidura* spp., *Arvicanthis* spp., *Lemniscomys* spp., *Lophuromys* spp., *Praomys tullbergi*, *Mus* spp., *Rattus rattus*, *Pelomys* spp., *Graphiurus murinus*, *Mastomys* spp., *Lemniscomys rosalia*, *Apodemus* spp., *Aethomys* spp, *Myoxidae* spp., *Apodemus sylvaticus*, *Praomys* spp., *Elephantulus* spp. In a metagenomics analysis which included four species of rodents (*Rattus rattus*, *Mus muscullus*, *Mastomys natalensis*, *Lemniscomys* spp.) and one species of shrews (*Crocidura* spp); at least each species from the three districts has shown a potential to carry disease causing agents (exception is for the *Lemniscomys* spp. from Kibondo, *Lemniscomys* spp. and *Crocidura* spp. from Uvinza and a group of domestic rats (*Rattus rattus* + *Mus muscullus*) from Kyerwa where there was no detection of any microorganism possibly due to sample storage limitations). The information provided from this study is important for understanding the burden of rodent pests and species diversity in the study area, developing rodent control strategies and understanding potential health risks.

The findings of this study inform and update the public on the potential health risks associated with rodents through reporting several pathogenic, potentially zoonotic bacteria and bacteria of unknown zoonotic potential as indicated in Manuscript 2, (Fig. 3.5)**Error! Reference source not found.**. This study reports a diversity of bacterial species, which encompasses bacteria with public health, veterinary and environmental importance; which highlights the importance of surveillance in rodent population. The use of metagenomics approach in this study has contributed to understanding microbial diversity since it is a potent and efficient tool

in microbial surveillance. It has enabled to determine and indicate the presence of pathogens before they inflict harm on humans. It's advantages over the conventional traditional methods has significantly increased understanding of microbial diversity. It is efficient in identifying microbial composition in rodent sample adds up to several other studies where it has led to the discovery of new microorganisms, genes, and biochemical pathways (Datta *et al*., 2020; Nam *et al*., 2023). The success of metagenomic as a suitable tool in surveillance in this study lies in its ability to bypass individual sample culture and identifying not only microorganisms but also their functions.

Emergence and re-emergence of these pathogens in human populations is on the face of it linked to increasing urbanization and low socio-economic status in developing countries and the ecology of zoonotic pathogens in rat populations (Hassell *et al*., 2017). The current global change context (e.g., land-use change, urbanization) is particularly suitable for the expansion of several rodent species beyond their natural distribution areas, particularly due to their synanthropic affinities (Dahmana *et al*., 2020). Rodent zoonoses exemplify the interconnectedness of human, animal, and environmental health. As regards human health, most recent attention has been directed at emerging infections, which includes some rodent-reservoir zoonoses which are sleeping giants that may awake at any time. Many human infections are never assigned an etiological agent, and the sources of many human pathogens remain unknown. Rodent-reservoir zoonoses may be important in both cases as multiple pathogens (including the less studied) are reported to originate from rodents. Therefore, recovery of bacteria that are human potential pathogens and opportunistic pathogens in rodents it is of serious concern because of the level of proximity between human life and rodents. In Public health, the fact that rodents carry various infectious diseases that can be transmitted to humans dictate the need to understand the transmission dynamics, identifying risk factors, and developing effective prevention and

control measures. By understanding the pathogens carried by rodents, public health officials will be able to implement targeted interventions to reduce the risk of disease transmission and protect human populations. Moreover, Rodents have been implicated in the emergence of several significant infectious diseases in the past, such as Hantavirus pulmonary syndrome and Lassa fever. By studying rodent zoonoses, scientists can better understand the factors that contribute to the emergence and spread of these diseases. This knowledge is crucial for early detection, surveillance, and timely response to potential outbreaks, in that way prevent or minimize their impact on public health.

Rodent-borne diseases are also a similar concern to the veterinary and environmental health sectors as a number of potentially zoonotic bacteria have been identified. This necessitates the need to adopt One Health approach in order to be able to gain insights into the complex interactions between rodents, humans, and the environment, and scale-up more effective disease control strategies. Furthermore, Rodents play important roles in ecosystems as prey, seed dispersers, and as soil engineers. When rodent populations are affected by disease outbreaks, it can have cascading effects on ecosystems.

On the other hand, rodents have been reported to be carriers of various pathogenic viruses for centuries. However, in this study which employed archived swab samples collected in rodents in 2018 there were very few viral sequences in a metagenomics analysis. This is probably because of the long storage of samples, life cycle of viruses, and the viral load at time of collection.

4.2 Conclusion

It is evident that rodents carry zoonotic pathogens of veterinary and public health importance. This study reports presence of some highly pathogenic and several opportunistic and commensal bacteria which were detected in both rectal and oral-pharyngeal rodent’s

swabs. Presence of pathogens in rodents indicates cross transmission between animals and humans in interfaces; and a possibility of an increased risk of human infection along with escalating antibiotic resistance due to continuous circulation of pathogens in the environment.

4.3 Recommendations

- Need for a One Health approach to brings together expertise from various fields, including epidemiology, veterinary medicine, ecology, environmental health and public health in addressing the problem
- Development of effective preventive measures and control strategies; including improving sanitation practices, implementing effective rodent control measures, developing diagnostic tools, and advancing the development of vaccines and therapeutic
- Prompt surveillance prior to disease spillovers
- Further studies to characterize the reported bacteria and understand transmission dynamics

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