



Prevalence of *Campylobacter* and *Brucella* species isolated from a diversity of rodents in Kasulu District, Tanzania: A public health risk indicator

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Abstract

Rodents reserve and transmit zoonotic bacteria, including *Campylobacter* and *Brucella*, to susceptible species. However, the prevalence of bacterial species they reserve remains unclear in Kigoma. This study aims to assess the prevalence of *Campylobacter* and *Brucella* species with reference to rodents as their reservoir species in Kasulu, Kigoma, Tanzania. A cross-sectional study was carried out in three villages of Kasulu district in Kigoma region. Rodents were captured from households, cultivated, fallow land, and wild areas using baited traps, then counted and identified to species level with a morphological key. Rectal swabs were collected for the isolation of *Campylobacter* and *Brucella* species. Conventional microbiological methods were employed for the initial isolation of bacterial species. Further identification was done by using conventional PCR. Sanger sequencing was also employed to generate sequences whose phylogenetic reconstructions were obtained with the aid of MEGA X software. A one-way ANOVA test was employed for statistical inference. A total of 182 rodents from 11 species were captured in the study. *Mastomys natalensis* was the most abundant species (54/182), while *Lophuromys* and *Arvicanthis* species were the least abundant. Rodent species diversity was highest in wild areas ($H' = 1.83$) and lowest in households ($H' = 0.28$). No statistical significance was observed between diversity and habitats ($p\text{-value} > 0.05$). The prevalence of *Campylobacter* and *Brucella* species was 1% (2/182) and 2% (4/182), respectively. *Campylobacter jejuni* and *Brucella pseudogrignone* strains were identified. These results revealed that *Mastomys natalensis* and *Rattus rattus* are species harboring the pathogens of interest in this study. These two species are distinguished from others by their closer proximity and interactions with human habitats, where they are

most abundant. This close association between a reservoir and a susceptible host positively influences the chain of transmission between the two.

Keywords: abundance, habitat, zoonosis, pathogens, PCR

Introduction

Zoonotic pathogens usually originate from wild animals and contribute to approximately 60-70 % of all emerging human infections (Azimi et al., 2021). Campylobacteriosis and Brucellosis are some of the most common zoonotic diseases reported to affect humans and domestic animals worldwide (Seleem et al., 2010; Nkogwe et al., 2011). *Campylobacter* is a genus of gram-negative bacteria consisting of 39 species and 16 sub-species responsible for cases of gastroenteritis disease reported in humans and some animals (Liu, 2020). *Campylobacter jejuni* and *Campylobacter coli* are more clinically significant *Campylobacter* species as they appear to be responsible for about 98% of all human gastroenteritis cases (Gharbi et al., 2021). Infection from its causative pathogens affects primarily children under five years as well as immune-compromised people and therefore should be considered to be an opportunistic zoonotic disease of public health significance in high, middle, and low-income countries (Roshanjo et al., 2019; Sher et al., 2021). Antibiotic intervention may be necessary in most cases of severe infection, although the infection is normally self-limiting (Caffrey et al., 2021). The prevalence of *Campylobacter* infections in the human population, particularly in Tanzania's asymptomatic children under five years, is approximately 94% (Komba, 2017). Poor hygiene, sanitation and proximity between humans and animals accelerating humans and animals accelerate the increasing prevalence of *Campylobacter* infections in humans (Deogratias et al., 2014). On the other hand, Brucellosis is another zoonotic bacterial disease caused by pathogens belonging to the genus *Brucella* (Tiller et al., 2010). The disease remains one of the most common zoonoses with higher prevalence, particularly in Middle Eastern and North African countries (Wareth et al., 2022). *Brucella abortus*, *Brucella melitensis*, *Brucella canis*, and *Brucella biovar* 1 and 3 are the most commonly identified *Brucella* species of public health significance (Tiller et al., 2010). A new *Brucella* species (*Brucella pseudogrignensis*) was recognized, reported to cause human infection (Li et al., 2021). Human brucellosis is characterized by relapsing fever and flu-like symptoms, usually occurring within 2-3 weeks of inoculation (Young, 1995; Mugizi et al., 2015; Wareth et al., 2022). Rodents are free-living small mammals belonging to the order Rodentia with 2277 known species, encompassing approximately 42% of all mammalian species, making them the largest group of small mammals (Pimsai et al., 2014). Rodents potentially preserve many zoonotic pathogens of public health importance (Dahmana

et al., 2020; Jahan et al., 2021). This study focused on *Campylobacter* and *Brucella* species as pathogens of public health importance. Direct deposition of urine and faecal pellets from rodents onto food and water designated for human consumption can play a great role in transmitting these pathogens (Jahan et al., 2021). The diversity and abundance of the available species of rodents capable of transmitting zoonotic pathogens in Kasulu district, especially in the selected villages, remained unclear. This study aimed to assess the prevalence of *Campylobacter* and *Brucella* species with reference to rodents as their reservoir species. The findings have elucidated the precise distribution of these two species concerning particular rodent species that host them. This information will be useful in planning for rodent control and zoonosis management in the area.

Materials and Methods

Study site

The study was conducted in Kigoma region in the northwest of Tanzania, which is located between longitudes 29.5° and 31.5° East and Latitudes 3.5° and 6.5° South of the equator (Fig.1). Kigoma region shares boundaries with Burundi and Kagera region to the North, Shinyanga and Tabora regions to the East, Democratic Republic of Congo to the West and Rukwa region to the South. The region has a long history of hosting refugees from Burundi and Democratic Republic of Congo. The study sites where samples were collected were three villages (Makere, Nyamidaho, and Nyarugusu) that lie near the Nyarugusu refugee camp located in Kasulu district.

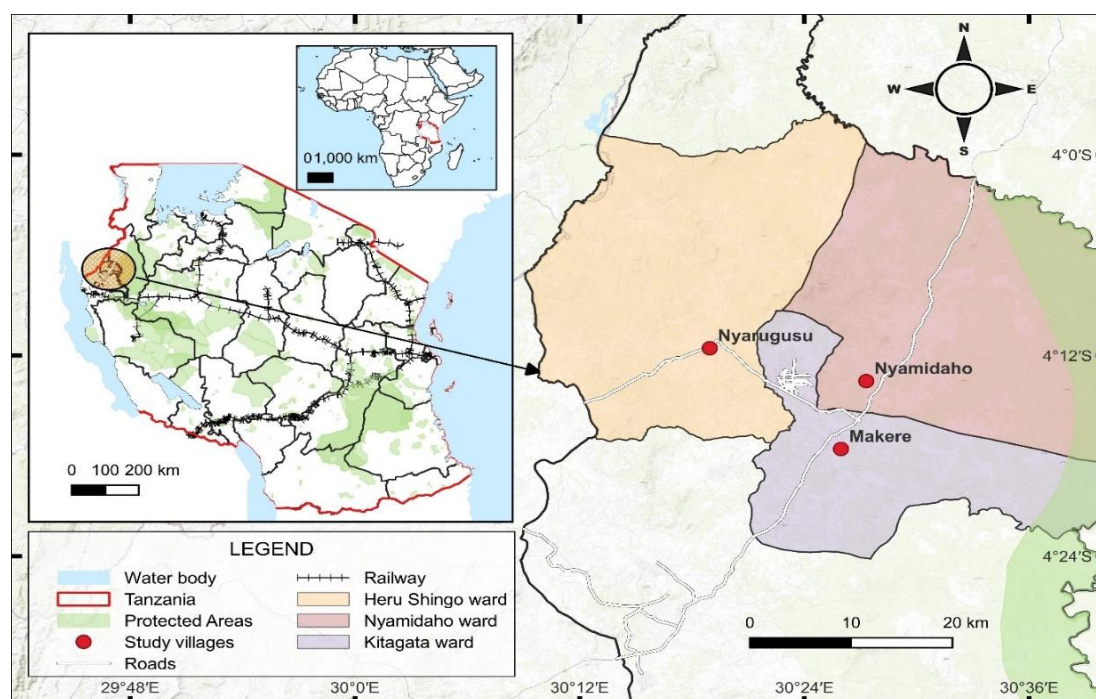


Figure 1. A Map Shows Study Sites

Study design

A cross-sectional study design was employed from February to March 2022. The target study population was all available rodents, and the sampling units were households, farmland, cultivated areas, and wild areas.

Rodent trapping strategy**Household**

A total of 15 houses were purposely selected in each of the three villages (Makere, Nyamidaho, and Nyarugusu) by asking the members of the house if there were rodents in their houses. A total of 25 locally made live wire traps were used per night, where a maximum of two traps were set per house based on the size of the house and details implying the presence of the rodent in responses from questioned house members. Each trap was baited with tomatoes and a mixture of peanut butter, maize bran, and small fish. The traps were set at 1800 hours and inspected at 0700 hours at strategic points for three consecutive nights to increase the capture rate (Mulungu et al., 2008).

Cultivated, fallow land, and wild areas

Rodent trapping was conducted using Sherman LFA live traps with a mixture of peanut butter and maize bran, banana, and avocados used as bait (Mulungu et al., 2008). A total of 100 traps were set in each sampling site per night. An established grid of 100m x 100m containing 10 lines, each containing ten traps, is set 10 meters apart. This orientation was, however, allowed to vary in correspondence with the variation of the landscape in different habitats (Katakweba et al., 2012). The traps were set at 1600 hours and inspected at 0700 hours for three consecutive nights to increase the capture rate.

Identification of rodents captured in the field

Captured rodents were anaesthetized with ether and identified at the species level using a morphological key (Katakweba et al., 2012). The morphometric data such as body weight to the nearest gram, length of the head and body, tail, hind feet and ear, as well as the state of the vagina or position of the testes, were also measured and recorded (Mulungu et al., 2008).

Laboratory specimen collection, processing, and transportation

The abdominal cavity of each rodent anaesthetized using ether was dissected using a sterile surgical blade and pair of forceps to open the gastrointestinal tract from which a rectal content was taken using sterile microbiology swabs (Nkogwe et al., 2011). Each rectal swab collected was kept in sterile special tubes, each containing 5mls of maximum recovery transportation media, and then stored at 4°C for preservation as Richard-Greenblatt et al. (2020) described. All collected samples were stored at Makere dispensary for one week and

then transported to Sokoine University of Agriculture microbiology laboratory at 4°C for isolation of *Campylobacter* and *Brucella* species.

Isolation and identification of *Campylobacter* and *Brucella* species

***Campylobacter* species**

A total of 5mls of each sample in the transportation media was well shaken and poured into a sterile Eppendorf tube containing a mixture of Bolton broth (Oxoid, Basingstoke, UK) enrichment media with antibiotics as a supplement and 5% lysed horse blood. The mixture was incubated at 42°C for 48 hours in microaerophilic conditions. Another subculture was conducted after that incubation period, whereby a loopful of the incubated sample was inoculated and streaked on *Campylobacter* blood-free agar containing mCCDA (modified Charcoal Cefoperazone Deoxycholate Agar) supplement (Oxoid, L.t.d, UK) and incubated for 48 hours at 42°C in microaerophilic conditions. Observation of bacterial growth was then made, and the results were recorded. Suspected bacterial colonies were then subcultured into a BA plate (Blood agar) from which pure bacterial colonies were preserved in Muller Hinton Broth at - 8°C for further bacterial confirmation tests. Pure suspected colonies were biochemically identified using catalase and oxidase tests. The catalase test was performed by pouring 2 mL of hydrogen peroxide solution on a sterile microscope glass slide, followed by a pure suspected bacterial colony loop. The enzymatic bacterial reaction was observed and recorded. An oxidase test was done using a wet filter paper on which 1% of oxidase reagent was poured; then, suspected colonies were rubbed on using a wire loop. After 1 min, the results were then observed and recorded. Bacterial cell shape, size, and arrangement were studied using a Gram staining technique, and slides were observed in a light microscope using 100 magnifications. All processes were done as described by Nkogwe et al. (2011).

***Brucella* species**

A loopful of a sample from maximum recovery enrichment broth was taken and inoculated on Blood and MacConkey agar, followed by aerobic incubation at 35°C for 24 hours. After that, the micromorphological and macromorphological study was carried out during this process and subsequent subculturing. Biochemical tests, including catalase and oxidase, to identify the bacterium and resistance tests to some β -lactam antimicrobials, were also conducted to identify the bacterium initially.

Molecular identification of *Campylobacter* and *Brucella* species

DNA extraction

A boiling DNA extraction method was used whereby 100 μ l of nuclease-free water and three pure bacterial colonies were added and mixed well into the 1.5 mL Eppendorf tubes. This

solution was boiled in a water bath (95°C) for 5 minutes. After 5 minutes, the solution was rapidly cooled in the refrigerator at – 20 °C and left for 10 minutes. This boiling and rapid cooling was repeated twice, then the solution was allowed to cool at room temperature for 2 minutes, then centrifuged at 1200 rpm for 2 minutes. Finally, 80 µl of supernatant was transferred into a new 1.5 ml Eppendorf tube, and the extracted DNA was stored at -20 °C for further analysis (Gharajalar et al., 2020).

Polymerase Chain reaction amplification for *Campylobacter* species

Identification of bacterial species using the conventional PCR method was performed using where 16S rRNA gene using genus-specific primers, including a 19bp-forward primer (C412F) and an 18bp-reverse primer (C1228R), and their respective amplification conditions were employed as previously described by Linton et al. (1996).

Polymerase Chain Reaction Amplification for *Brucella* species

A PCR amplification targeting the 16S rRNA gene with 1500bp from a genomic DNA sample of *Brucella* spp was conducted (Gharajalar et al., 2020). A 25µl of the reaction mixture was used. The mixture contained 12.5 µl of premix, 0.5 µl of each reverse and forward primer, 8.5 µl of nuclease-free water, and 3 µl of targeted DNA from each sample. Then, PCR amplification reactions were performed using a thermal cycler. A 2-hour PCR amplification was achieved. The reaction cycles started with the denaturation process at 95°C initial temperature for 5 minutes and 94°C final temperature for 30 seconds, followed by annealing at 58°C for 30 minutes, whereby a total of 30 cycles were made. Finally, the elongation process was done at 72°C for 2 minutes, and the reaction was allowed to cool at 4°C. PCR products for both bacterial genera were subjected to a 1.5 % agarose gel stained with 6 µl of Gel Red for 30 minutes at 80 volts. A gel documentation system machine was used for visualization of results (Gharajalar et al., 2020).

Gene sequencing

For identifying *Campylobacter* and *Brucella* species, the positive PCR products were submitted for sequencing using Sanger sequencing technology (ABI sequencer) at Macrogen Europe, Netherlands.

Data analysis

The raw data obtained were recorded and organized using Microsoft Office Excel 2019. The abundance of rodents captured from both sites was determined by using the total counting method, while the species diversity of rodents was determined by using the standard Shannon-Wiener index (Mulungu et al., 2008) outlined below;

Where H = species diversity index, s = total number of species, and p_i = proportion of total sample within species. One-way ANOVA test was performed using Epi Info software to test a statistically significant difference in species diversity from different habitats and understand the effect of habitat structure on species diversity (Nkwabi et al., 2018).

Prevalence of *Campylobacter* and *Brucella* species was calculated using the formula below;

$$P = N_o / N_T \times 100 \%$$

Where P is prevalence, N_o is the number of positive samples, and N_T is the number of total samples tested.

Molecular analysis was performed to allow identification to the species level by assembling the sequences from PCR products and comparing them to those in the database using BLAST. All sequences (from this study and references) were aligned, and the phylogenetic tree was constructed using MEGA X software.

Ethical clearance

Sokoine University of Agriculture, Tanzania, approved research clearance and ethical permission under the Research Ethics Board (ref. SUA/ADM/R.1/8/793).

Results

The abundance of rodents captured in three villages in Kigoma

A total of 182 rodents with 11 different species were captured from all selected habitats (Fig.2). *Mastomys natalensis* (55/182) was the most abundant species, while *Lophuromys spp.* (1/182) and *Arvicanthis spp.* (1/182) They were the least abundant species in both villages. Four types of rodent habitats were classified in each village (household, farmland, fallow land, and wild area). In relation to these, *Mastomys natalensis* (29/77) was also the most abundant species in fallow land and farm (16/26) compared to other captured species. On the other hand, *Rattus rattus* (44/51) was more abundant in household habitats. *Mus spp.* (7/28) was more abundant in wild areas than other rodent species captured (Table 1).

Table 1. An abundance of rodent species captured from different habitats in three different villages in the Kasulu district

Rodent species	HABITATS				Grand Total
	Fallow land	Farm	Household	Wild area	
<i>Arvicanthis spp.</i>	0	0	0	1	1
<i>Dasymys spp.</i>	11	0	0	3	14
<i>Gerbilliscus spp.</i>	4	0	1	3	8
<i>Grammomys spp.</i>	2	0	0	0	2
<i>Graphiurus spp.</i>	7	0	0	0	7
<i>Lemniscomys rosalia</i>	0	0	0	4	4
<i>Lemniscomys striatus</i>	12	2	1	3	18
<i>Lophuromys spp.</i>	0	0	0	1	1
<i>Mastomys natalensis</i>	29	16	5	5	55

<i>Mus spp.</i>	11	7	0	7	25
<i>Rattus rattus</i>	1	1	44	1	47
Grand Total	77	26	51	28	182



Figure 2. Diverse rodent species captured in different habitats in Makere, Nyamidaho, and Nyarugusu villages; **(A)** Rodent captured in Fallow land and wild areas, **(B)** Rodent species captured in household habitat

Diversity of rodent species in different habitats

Variation in species diversity was observed in different habitats from both villages, whereby the highest diversity was observed in Wild areas ($H' = 1.83$) followed by the fallow land ($H' = 1.72$), and no diversity ($H' = 0$) in the household and farm habitats in Nyamidaho village. In Makere village, high species diversity was observed in fallow lands ($H' = 1.24$) and low in the wild habitat ($H' = 0.5$). On the other hand, in Nyarugusu village, the highest diversity was observed in wild areas ($H' = 1.52$) and the lowest ($H' = 0.28$) in households (Table 2). Despite the ecological difference in species diversity observed between habitats in each village, this variation was not statistically significant ($p = 0.123$).

Table 2. Species diversity in different habitats in each village

Habitats	Makere	Nyamidaho	Nyarugusu
Fallow land	1.24	1.72	0.90
Farm	0.53	0	1.14
Household	0.73	0	0.28
Wild area	0.50	1.83	1.52

Diversity index ($H' \approx$ to 2 decimal places) in each village

*No statically significant difference in rodent diversity between habitats: P-value = 0.12384

Isolation and identification of *Campylobacter* and *Brucella* species

The morphological characteristics of *Campylobacter* suspected colonies (2/182 samples) were small and medium, dewdrop-like, transparent, and mucoid on mCCDA media. Suspected *Brucella* spp (4/182) demonstrated beige, non-hemolytic, distinct small circular colonies on blood and MacConkey agar plates within 24 hours of incubation at 35°C. Microscopic findings revealed gram-negative bacteria, with both genera having small, slightly curved rod cell shapes and others with straight cell shapes. All six isolates were catalase and oxidase-positive.

Molecular characterisation of *Campylobacter* and *Brucella* strains

Both genera had sequence homologues ranging from 97–99 % with several reference sequences of *Campylobacter jejuni* and *Brucella pseudogrignoneis* from the Gene Bank. The phylogeny grouped the isolates from this study into the clusters of *Campylobacter jejuni* and *Brucella pseudogrignoneis* in relation to reference sequences from the gene bank. The tree was generated using the Neighbor-Joining method (p-distance model), with bootstrap values expressed as percentages of 1,000 replications. *Aeromonas hydrophila* (MK41656) and *Escherichia coli* (NR024570) serve as an out-group for *Campylobacter* and *Brucella* strains, respectively (Mzula et al., 2019). The results showed that *Campylobacter jejuni* strains TZ 173K and TZ 83K were grouped in the same clade and more closely related to the *Campylobacter jejuni* (NR 041834) and *Campylobacter jejuni* subspecies *jejuni* (NR 118520). The three strains of *Brucella pseudogrignoneis* (TZ 167K, TZ87K and TZ 67K) are grouped in the same clade (Fig. 3).

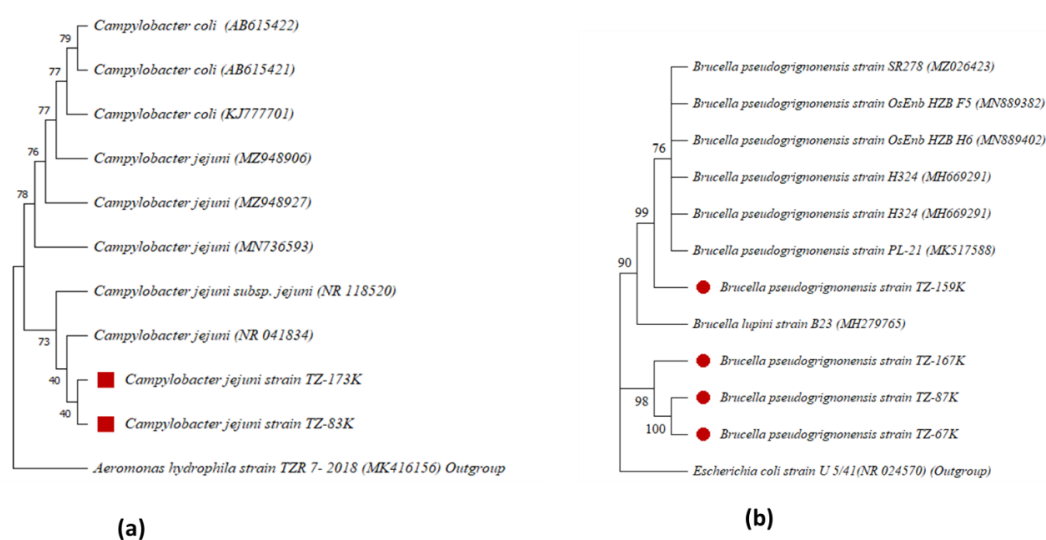


Figure 3. Phylogenetic tree for *Campylobacter* spp and *Brucella* spp from this study (red rectangle and circle, respectively) and closely related taxa from the gene bank. The tree was generated using the Neighbor-Joining method (p-distance model), with bootstrap values

expressed as percentages of 1,000 replications. *Aeromonas hydrophila* (MK41656) and *Escherichia coli* (NR024570) serve as an out-group, respectively

Prevalence of *Campylobacter* and *Brucella* species isolated from a diversity of rodents

After molecular identification and characterisation of specific *Campylobacter* and *Brucella* species, the results showed that 2/182 were *Campylobacter jejuni* and 4/182 were *Brucella pseudogrignonensis*, which is equivalent to 1% and 2%, respectively. Based on rodent species captured in both villages, *Campylobacter jejuni* was isolated from *Rattus rattus* (1/47) and *Lemniscomys striatus* (1/18). In contrast, *Brucella pseudogrignonensis* was isolated from *Mastomys natalensis* (2/54), *Rattus rattus* (1/47) and *Gerbilliscus species* (1/9) (Table 3).

Table 3. Molecular results show the prevalence of specific *Campylobacter* and *Brucella* species isolated from a diversity of rodents

Captured group	Species captured	No. individuals	Positive samples from molecular identification	
			<i>Campylobacter jejuni</i> .	<i>Brucella pseudogrignonensis</i>
Rodent	<i>Rattus rattus</i>	47	1	1
	<i>Mus species</i>	25	0	0
	<i>Mastomys natalensis</i>	54	0	2
	<i>Gerbilliscus species</i>	9	0	1
	<i>Lemniscomys rosalia</i>	4	0	0
	<i>Lemniscomys striatus</i>	18	1	0
	<i>Grammomys species</i>	2	0	0
	<i>Dasymys species</i>	14	0	0
	<i>Graphiurus species</i>	7	0	0
	<i>Lophuromys species</i>	1	0	0
	<i>Arvicanthis species</i>	1	0	0
	TOTAL	182	2	4
	Prevalence (%)	100	1	2

Discussion

This study's results found that 182 rodents from 11 different species were captured from the selected habitats in all three villages. The rodent species captured in the study included *Rattus rattus*, *Mastomys natalensis*, *Lemniscomys striatus*, *Lemniscomys rosalia*, *Arvicanthis spp.*

Mus spp, *Gerbilliscus spp*, *Lophuromys spp*, *Grammomys spp*, *Graphiurus spp*, and *Dasymys spp*. Most rodent species identified in this study were habitat-specific, with a few generalist species. This implies that the diversity and abundance of rodent species varied with respect to habitat structure, as Chidodo et al. (2020) reported. *Mastomys natalensis* and *Rattus rattus* were observed to be the most abundant species, displaying the ability to inhabit all four habitat types outlined in this study (Table 1). This finding is in line with studies conducted by Massawe et al. (2011), who similarly found a high abundance of *Mastomys natalensis* (>70%) compared to other rodent species captured. However, the number of habitat generalist species varied between habitats, where *Mastomys natalensis* was more abundant in fallow land and farmland, while *Rattus rattus* was more abundant in households than in other habitats. This finding is similar to that provided in a study conducted by Lema and Maggie (2018) as well as Nkwabi et al. (2018), who observed that *Mastomys natalensis* and *Rattus rattus* were the most abundant rodent species in human-exploited habitats compared to protected areas. On the other hand, rainfall patterns appeared to significantly affect the abundance of rodents in all habitats, as Makundi et al. (2010) described. During the rainy season, essential ecological needs for survival and reproduction, including vegetation cover and nutritional feeds, are highly available for rodents (Massawe et al., 2011). The availability of resources linked to rainfall patterns positively influences the breeding of rodent species, resulting in the high abundance of most species, particularly *Mastomys natalensis* (Makundi et al., 2010). Based on the diversity of rodent species captured among a diversity of habitats selected in this study, the findings displayed the highest diversity ($H' = 1.83$) in wild areas. This high diversity in such areas could be influenced by the absence of anthropogenic activity, as intensive grasses and trees characterize them. This result corresponds to that provided by Mulungu et al. (2008), who suggested that wild areas had higher species diversity than other disturbed habitats. However, the result is in contrast to that presented by Lema and Maggie (2018), who found that disturbed habitat (farmland) had higher species diversity than wild habitat. On the other hand, the lowest diversity ($H' = 0.28$) was observed in household habitats. Furthermore, *Rattus rattus* was observed to be the most dominant rodent species in household habitats compared to other rodent species captured in this study, as similarly reported by Katakweba et al. (2013). Furthermore, statistical analysis was used to recognize whether habitat structure significantly affected species diversity. However, the observed results in species diversity between habitats were not statistically significant at $p = 0.123$, which is not similar to the observation made by Nkwabi et al. (2018), who found that species diversity among habitat structures had statistical significance. The time limitation for

data collection and the low number of rodents captured in the field result in this difference. The isolation of *Campylobacter* and *Brucella* species from rodent samples has been achieved in other studies by Adhikari et al. (2002), Backhans and Fellström (2012) and Franco et al. (2007) similar to our study, with the aim of elucidating the role of rodent in reserving and transmitting these pathogens to humans and the animals they consume. The positive isolates of *Campylobacter* and *Brucella* spp. were obtained mostly from two rodent species, *Mastomys natalensis* and *Rattus rattus*, among 11 captured species. The two species observed to harbor the pathogens of interest are particularly distinguished by their noticeably closer proximity and interactions with human habitats and domestic animals compared to other rodent species (Backhans & Fellström, 2012; Katandukila et al., 2021). The close association between the reservoir and susceptible host (human and domestic animals) could positively influence the chain of transmission between the two. Furthermore, the molecular characterisation of *Campylobacter* and *Brucella* spp displays the genetic evolution of the two species isolated from different rodent species (Figure 3). I identified that *Campylobacter jejuni* was isolated from *Rattus rattus* and *Lemniscomys striatus* captured around human settlements. *C. jejuni* has been recognized as a species of public health significance. Subsequently, *Campylobacter jejuni* was the main species responsible for approximately 90% of human Campylobacteriosis cases reported worldwide (Haque et al., 2019; Roshanjo et al., 2019; Rossler et al., 2020; Gharajalar et al., 2020; Gharbi et al., 2021). On the other hand, *Brucella pseudogrignone* is also the newest species isolated from the human blood sample and has been considered an emerging zoonotic pathogen affecting immunodeficient and immunocompetent patients, whereby the first cases were reported in Taiwan (Li et al., 2021). Movement of people and goods from one country to another, due to the presence of refugee camp sites around the selected study villages, could influence the transportation of different bacterial species from one place to another. This study reveals a noticeably low prevalence (1%) of *Campylobacter jejuni* isolated from rodents compared to a higher prevalence (10.8%) of the same pathogen obtained by Adhikari et al. (2002) in rodents. However, the variation observed in the two studies could be resultantly influenced by the varying sample sizes employed in each study. This observation implies that the threat of zoonotic transmission of pathogens from rodents to humans is relatively low, as the pathogen appears more prevalent in other reservoir hosts like poultry (Jahan et al., 2020). On the other hand, this study observed a lower prevalence (2%) of *Brucella* spp from captured rodents, unlike other reservoir hosts, as observed by Li et al. (2021), who found a higher prevalence of the pathogen in humans.

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Conflict of Interest: The authors declare no conflict of interest.

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