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Molecular detection and characterization of rickettsia species in rodents and shrews from Kilombero district, Tanzania

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Abstract

Background: Rickettsia is a genus of obligatory intracellular bacteria transmitted by arthropods such as lice, ticks, and fleas. Rodents and shrews are well known for harboring a plethora of zoonotic pathogens including rickettsia. The diseases associated with Rickettsia are often misdiagnosed with other febrile infections such as Malaria and typhoid fever. This study aimed to detect and characterize the prevailing Rickettsia species in rodents and shrews from Kilombero district, Tanzania.

Methods: This study was conducted in Kilombero district between June 2022 and March 2023. The rodents and shrews were captured inside the households and outside households or in farms in the selected areas baited with maize bran and peanut butter. Prior to identification, rodents and shrews were anaesthetized by using Halothane was administered at the dose of 2mg/kg. DNA extraction from 204 tissue samples was done using DNeasy Blood and Tissue Kit from Qiagen. Amplification of the Citrate synthase (gltA) gene was done by Nested PCR using two sets of primer sequences in both rounds. PCR products were sequenced twice in the forward and reverse directions, consensus sequences were created and BLAST analysis was done. A phylogenetic tree was constructed using the Neighbor-Joining Method and 1000 bootstrap replications using the same software. The relationship between rodents' rickettsiosis status to different factors were analyzed using chi-squared test.

Results: The overall molecular prevalence of Rickettsia species in rodents and shrews was: 10.20% (95% CI: 0.0634-0.153; n/N=20/196) and 12.5% (95%: CI: 0.0032-0.5265; n/N=1/8) respectively. Upon sequencing, all sequences were identified as Rickettsia conorii subsp. raoultii with percentage identity ranging from 98% to 100%.

Conclusion: This study confirms that rodents and shrews are infected with Rickettsia conorii in the study area.

Keywords: Rodents, Shrews, Zoonoses, PCR, Reservoir, Ticks, Fleas, Tanzania

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Background

Rickettsia is a genus containing pleomorphic, Gram-negative, non–spore-forming, non-motile microorganisms that can take the shape of threads, bacilli or cocci [1]. The genus is composed of 27 species, and 17 of them are considered pathogenic to humans and animals [2]. Rickettsia species are known to cause zoonotic diseases which are categorized into typhus and spotted fever and the scrub typhus groups [3]. The mode of disease transmission in the spotted fever group is primarily through transovarial and transstadial transmission, while the typhus group is primarily associated with ticks (Rickettsia felis) and mites (Rickettsia akari), as well as fleas (Rickettsia typhi). The typhus group's mode of disease transmission is exclusively through transstadial transmission [4]. Rickettsia species are transmitted to mammalian hosts during blood feeding by infected ticks and mites or by contaminated feces of infected lice and fleas. Fleas and lice bite a mammal host, and the bite tears the skin, creating a wound. When fleas and lice feed, they excrete. The bacteria found in this excrement can be rubbed to bite wounds or other wounds to induce an infection [5]. Additionally, infected flea and lice filth can be rubbed into the eyes or inhaled. There is no human-to-human transmission of these bacteria [6]. Humans may exhibit clinical symptoms of rickettsioses six to fourteen days following exposure. The clinical signs include fever, skin rash, joint pain, headaches, and chills [7]. Similar clinical symptoms are associated with other illnesses, like malaria and typhoid, which makes diagnosing the disease clinically difficult [8]. Rickettsioses can cause organ damage, coma, and even death if left untreated. Doxycycline is an antimicrobial recommended

for the treatment of rickettsial diseases [9]. In Tanzania, studies have reported the occurrence of R. typhi in human, rodents and fleas. A study by[10] reported prevalence of 1.8% in Northen Tanzania in past, [11] reported the prevalence of 10% in rodent fleas from Lushoto district [9], reported prevalence of 24% among rodent fleas in Mbulu district and [12] reported prevalence of 18.75% among rodents in Ngorongoro district. The studies focused mainly on rodent fleas. However, only few studies reported the occurrence of Rickettsia species in rodent tissues taking into consideration that rodents and shrews are reservoir of R. typhi [13]. Kilombero is a wetland area favoring the existence of rodents and shrews and the climatic condition favors tropical diseases like rickettsioses. In the study area, eleven villages were purposefully chosen based on the number of rodents and agricultural activity close to human settlement. The objective of this study was to detect and characterize Rickettsia species in rodents and shrews in Kilombero District, Tanzania, in order to better understand their role as potential reservoirs of infection. Given the public health significance of rickettsial diseases in Tanzania where they are often underdiagnosed due to similar symptoms with common febrile illnesses such as malaria and typhoid, this study focuses on rodents and shrews as key hosts that may contribute to the maintenance and transmission of these pathogens. By generating data on the occurrence of Rickettsia in rodent and shrew populations, the study aims to inform improved surveillance and control strategies for rickettsioses in endemic areas.

Methods

Study area

This study was conducted in Kilombero district in Morogoro Region, south-western Tanzania, from June 2022 to March 2023 (Figure 1). The Kilombero District contains the largest freshwater wetland at a low altitude (<300 m above sea level), and the floodplains of the valley form one of Africa's largest wetlands and are recognized by the International Union for Conservation of Nature (IUCN), as being of global importance [14]. The Kilombero Valley floodplain covers some 260 km by 52 km fed by many rivers and with huge seasonal variations in the water dynamics, embodying an exceptionally wide variety of wetland types [14]. The floodplains support agriculture and are one of Tanzania's largest inland fisheries, providing cash income for many inhabitants of Morogoro. Agriculture is the main activity undertaken in the Kilombero Valley by both small- and large-scale farmers (e.g., sugarcane plantations) [15].

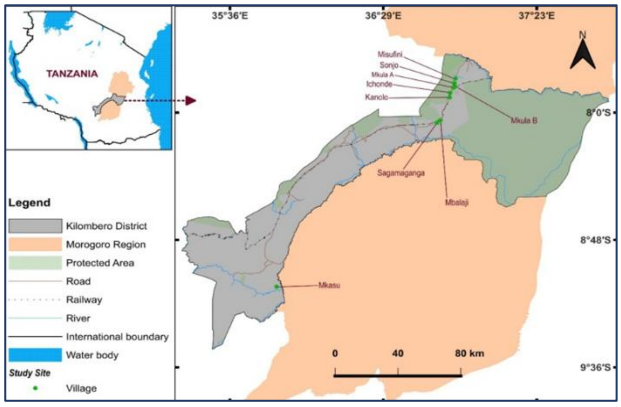

Figure 1: Map of Tanzania showing the location of the study area Map created by Qgis 3.34.1; Source: Prizren; EPSG: 4326-WGS 84

Study Design

This was a cross-sectional study. The target study population included rodents and shrews trapped in human residences and farms around human residences.

Sample Size

The sample size was calculated by using the following formula $n = \frac{Z^2PQ}{L^2}$ [16].

Where n = required sample size

P = known/ estimated prevalence

Q = (1-P)

L = Allowable error for estimation

Z = Point on standard normal distribution curve corresponding to a significance level of 5% (its value is 1.96)

P = Estimate prevalence 15.55% [17].

According to this formula, the minimum sample size (n) was 202. However, a total of 204 rodents and shrews were trapped from selected farms and human residences.

Selection of areas for setting of rodent's traps

The study employed a cross-sectional design which used random method of data collection. In the study area, eleven villages were purposefully chosen based on the number of rodents and agricultural activity close to human settlement. At least twenty houses were chosen to take part; each household was chosen based on its proximity to rice and sugarcane plantations, where there was a high level of rodent-human interaction. Six traps were placed in each sampling site: two in the crop vegetation, two in the peridomestic area, and two within the house [18].

Rodent and Shrew Trapping

Rodents and shrews were trapped by using Sherman and wire traps inside the households and outside households or in farms in the selected areas of the Kilombero district. Baits included a mixture of maize bran and peanut butter (ratio 4:1), ripe banana, avocado and green maize. The traps were set in the evening at locations then inspected and collected the following morning at 07:00 hours for three consecutive nights per trapping session [18].

Rodents and shrews' identification and sample collection

Prior to identification, rodents and shrews were anaesthetized by using Halothane at 2mg/kg dosage. Captured rodents and shrews were identified based on their morphological features such as body length, tail, fur, pes and ear. All these procedures used the morphological identification keys and illustrations developed by [19]. Both rodents and shrews were sacrificed using the procedure outlined by [20] was followed to dissect anesthetized rodents and shrews. Internal organs such as the liver, spleen, lungs and kidneys were collected and preserved in cryovials containing absolute ethanol. Tissue samples were transported in a cold chain to the Institute of Pest Management Laboratory, Sokoine University of Agriculture (SUA), for storage at –20°C until further analyses.

Bacterial Detection

DNA extraction

DNA extraction was done from spleen and liver tissue using DNeasy Blood and Tissue Kit (cat# 69506) from Qiagen (Hilden, Germany), according to manufacturer instructions [21].

Detection of Rickettsia species by nested-PCR amplification of citrate synthase (gltA) gene

The citrate synthase (gltA) gene was amplified using primer sequences shown in Table 1.1. Outer PCR was performed in a 25µL reaction volume containing 2 µL of extracted DNA, 12.5 µL of OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (from New England Biolabs, Inc) 0.5µL of forward primer (RpCS.877p), 0.5 µL of reverse primer (RpCS.1258n) and 9.5µL of nuclease-free water. Cycling conditions consisted of initial denaturation at 95◦C for 5 min followed by 7 cycles of 95 ºC for 15 s, 56 ºC for 15 s and 72 ºC for 30 s; 8 cycles of 95 ºC for 15 s, 55 ºC for 15 s and 72 ºC for 30 s and 20 cycles of 95 ºC for 15 s, 54 ºC for 15 s and 72 ºC for 30 s. A final extension at 72 ºC for 5 min was performed to complete the elongations. Nested PCR was performed with 2µL of 10-fold diluted outer PCR products, 12.5 µL of OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (from New England Biolabs, Inc) 0.5µL of forward primer (RpCS.896p), 0.5 µL of reverse primer (RpCS.1233n) and 9.5µL of nuclease-free water in a 25µl reaction volume. Nested PCR was performed under the cycling conditions which consisted initial denaturation at 95◦C for 5 min followed by 5 cycles of 95 ºC for 15 s, 56 ºC for 15 s and 72 ºC for 30 s; 5 cycles of 95 ºC for 15 s, 55 ºC for 15 s and 72 ºC for 30 s and 20 cycles of 95 ºC for 15 s, 54 ºC for 15 s and 72 ºC for 30 s. A final extension at 72 ºC for 5 min was performed to complete the elongations [22].

Gel-electrophoresis of PCR products

Agarose gel of 1.0% strength was prepared by dissolving 1.0 g agarose powder into 100 mL of 1X Sodium borate buffer in a conical flask and heated on a hot plate until dissolved completely and stained by 80 µL of GreenStar™ Nucleic Acid Staining Solution I (Bioneer Corporation). Each well of the gel was loaded with 3 µL of each sample and 3µL of 100 bp DNA ladder was loaded to one well in order to indicate the size of any fragments. The voltage of 100V was used and electrophoresis was allowed to run for 60 minutes. The gel was transferred to the gel documentation machine (Gel Doc™ EZ Imager from Bio-Rad Laboratories) for visualization. The DNA fragments were observed as grey bands against a black background.

Sequencing

After purification using the QIAquick PCR purification kit (Qiagen), gltA gene PCR products corresponding to the RpCS.896p and RpCS.1233n primer pairs were sequenced twice in the forward and reverse directions. A volume of 20 µL from 11 PCR products/samples was sent for Sanger dideoxy sequencing to the Macrogen Europe, Netherlands laboratory. The Applied Biosystem's 96 Capillary 3730xl DNA Analyzer (Thermo Fisher Scientific, Carlsbad, CA, USA) was used to sequence each sample in both forward and reverse directions preceded by purification of the PCR products [23] (Table 1).

Creating consensus sequences and BLAST analysis

A total of 22 raw nucleotide sequences (ab1 files) from 11 samples sent for sequencing were acquired. The raw sequences from forward and reverse primers were cleaned and trimmed using the Bioedit software. The same software was used to generate consensus sequences. Good consensus partial coding sequences of the citrate synthase (gltA) gene with about 338 base pairs were obtained in 8 of the 11 samples sent for sequencing. Three sequences had too much noise and were considered bad quality and, therefore, excluded from downstream analysis. The 8 consensus sequences were compared with the published sequences in the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST) to confirm each sample's taxonomic identity. The taxonomic identification was based on BLAST results, which had the highest percentage of identity, query cover and minimum E-value.

Multiple sequence alignment and phylogenetic analysis

The consensus sequences were aligned with the Molecular Evolutionary Genetics Analysis (MEGA) version 11 software using the ClustalW statistical algorithm. A phylogenetic tree was constructed by using the Neighbor-Joining Method and 1000 bootstrap replications using the same software in order to evaluate the relatedness of Rickettsia spp isolated from this study with those from other published studies [24]. About 14 sequences of 6 different Rickettsia species, some belonging to the Spotted fever group and some from the Typhus group, were retrieved from the NCBI nucleotide database for the downstream phylogenetic analysis. One Bartonella henselae gltA sequence was downloaded from GenBank (Accession L38987) and included in the analysis as an out-group for rooting the tree.

Results

Rodents and shrews captured in the study area

A total of 204 small mammals were captured from houses and peri-domestic and crop cultivation field areas in Kilombero district. Among them, 196 were rodents from three genera (M. natalensis, Lemniscomys spp, R. rattus) and eight were shrews. Rattus rattus was the predominant species, making 73.0 percent of the trapped animals, while the least captured species was Lemniscomys species (0.5%) (Table 2).

Rodents species and their habitat

Almost 81.87% of R. rattus was captured in houses, shrews were mostly caught in peridomestic areas (87.50%), Lemniscomys species was only found in the peridomestic areas, and a lower percentage of Mastomys were caught in houses (5.75%) as indicated in Table 3.

Molecular prevalence of Rickettsia species

A total of 196 rodents and 8 shrews were sampled in Kilombero District; the majority of the rodents, 73.04% (149/204), were R. rattus. A DNA sample from each rodent was detected for Rickettsia species by two nested PCR protocols followed by gel electrophoresis (Figure 2).

Prevalence of Rickettsia species in villages, rodents, habitats and seasons

The overall molecular prevalence of Rickettsia species in rodents and shrews was:10.20% (95% CI: 0.0634-0.153; n/N=20/196) and 12.5% (95%:CI:0.0032-0.5265; n/N=1/8), respectively. A higher prevalence (25%) was observed in Mbalaji village compared to other villages. Male rodents had a higher prevalence than females, while adult rodents had a lower prevalence than

young rodents. A higher prevalence was observed in rodents captured during the dry season, while a higher prevalence was observed in rodents captured in houses compared to those captured in peri domestic and crop vegetation. Thirty rodents were infested by ectoparasites, five of which were detected positive by Nested PCR. Furthermore, the Chi-square test showed that the prevalence of Rickettsia species was significantly higher in rodents captured from houses compared to other habitats (p= 0.03). However, insignificant differences were observed between villages, sexes, ages, and seasons; the results on the prevalence of Rickettsia species in rodents are summarized in Table 4.

Sequencing/BLAST results

A total of 8 good sequences were compared with the published sequences available in the National Centre for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) to confirm each sample's taxonomic identity. All sequences were identified as R. conorii subspecies raoultii with percentage identity ranging from 98% to 100%. The results are summarized in Table 5.

Phylogenetic Analysis Results

The phylogenetic tree was reconstructed by using 8 nucleotide sequences, which were identified as R. conorii subsp. raoultii confirmed a close linkage between isolates from this study with same species isolated from other studies in different mammals whose sequences clustered together in the same clade. The phylogenetic analysis also confirmed that the isolates from this study belong to the Spotted Fever Group Rickettsia (SFGR), clustering with other group members, namely, R. japonica, R. felis and R. conorii subsp. heilongjiangensis. The output of phylogenetic analysis (phylogenetic tree) is shown in Figure 3.

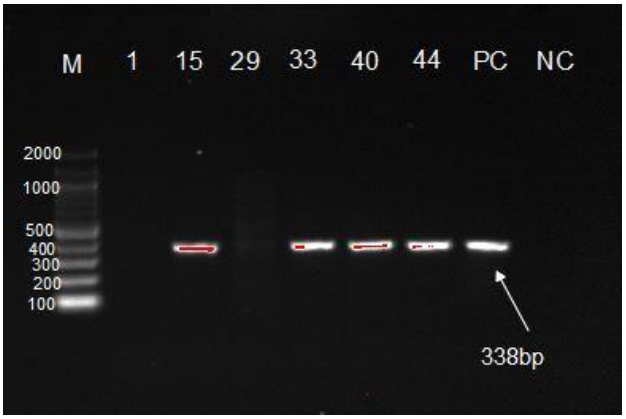

Figure 2: Agarose gel electrophoresis used to demonstrate the 338 bp citrate synthase gene (gltA) in rodent M stands for ladder marker; positive samples are 15, 33, 40, and 44. PC=Positive Control, bp = Base Pairs; NC = Negative Control

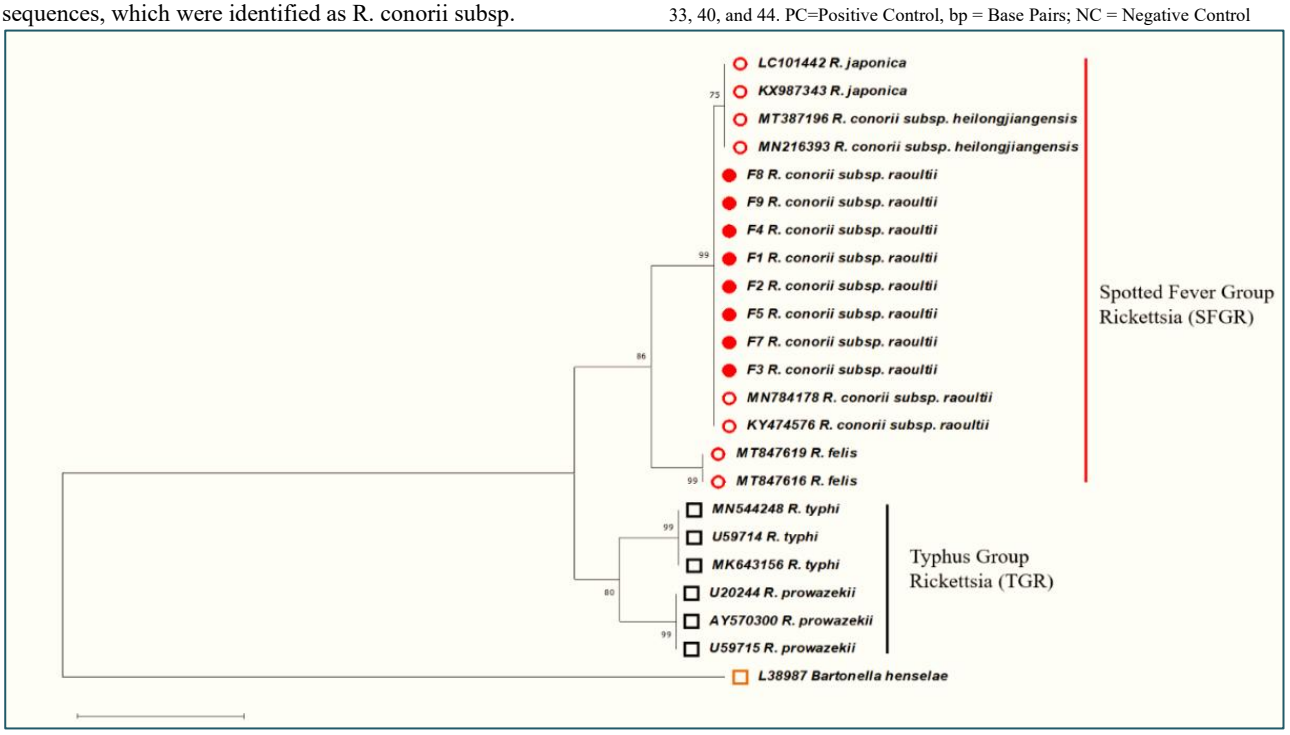

Figure 3: Phylogenetic tree illustrating Rickettsia connorii subsp raoultii's position in relation to other Rickettsia species. The tree was constructed by using the Neighbor-Joining Method and 1000 bootstrap replications using MEGA 11 software in order to evaluate the relatedness of Rickettsia spp isolated from this study (shaded circles) with those from other published studies (Tamura et al., 2021) Bartonella henselae was included in the analysis as an out-group for rooting the tree.

Table 1: Primer sequences used for the detection of Rickettsia species.

Primer name	Orientation	Primer sequence (5' to 3')	Amplicon size (bp)
RpCS.877p	Forward	GGGGGCCTGCTCACGGCGG	382
RpCS.1258n	Reverse	ATTGCAAAAAGTACAGTGAACA	
RpCS.896p	Forward	GGCTAATGAAGCAGTGATAA	338
RpCS.1233n	Reverse	GCGACGGTATACCCATAGC	

Table 2: Composition of captured rodents and shrew species in the study area

Type	Genus/Species	Number	Percentage Composition
Rodent	Lemniscomys spp	1	0.5
Rodent	Mastomys natalensis	46	22.5
Rodent	Rattus rattus	149	73.0
Shrews	Shrews	8	3.9
TOTAL		204	100.0

Table 3: Rodent species and their habitat

Rodent and Shrew species	Habitat					
	House		Peridomestic		Crop Cultivation	
	No.	%	No.	%	No	%
Rattus rattus	122	81.87	15	10.06	12	8.05
Mastomys	8	5.75	19	41.30	19	41.30
Lemniscomys	0	0	1	100	0	0
Shrew	0	0	7	87.50	1	12.5

Table 4: Molecular prevalence of Rickettsia species

Variable	Categories	No. of Individual examined	No. of positive individuals	Prevalence (%)	P-Value
Overall	Overall	204	21	10.29	
Village	Kanolo	29	2	6.90	0.57
	Ichonde	28	3	10.71	
	Mbalaji	8	2	25.00	
	Misufini	41	3	7.32	
	Mkasu	8	0	0	
	Mkula A	12	1	8.33	
	Mkula B	26	3	11.54	
	Nyamwezi	13	0	0	
	Mlimani	24	5	20.83	
	Samarang	9	1	11.11	
	Sonjo	6	1	16.67	
Species	Lemniscomys	1	0	0	0.22
	Mastomys	46	1	2,17	
	Rattus	149	19	12.75	
	Shrew	8	1	12.50	
Sex	Female	102	10	9.80	1.00
	Male	102	11	10.78	
Age	Adult	169	16	9.47	0.58
	Young	35	5	14.29	
Season	Wet	109	10	9.17	0.73
	Dry	95	11	11.58	
Habitat	Crop veg.	32	0	0	0.05
	Houses	130	18	13.85	
	Peri domestic	42	3	7.14	
Presence of Ectoparasite	Absent	143	16	11.19	0.69
	Present	61	5	8.2	

Table 5: Summary of the BLAST results

S/N	Sample Id	Species Identified	Percentage Identity (%)	Query Cover Age (%)	E-Value
1	F1	Rickettsia conorii subsp. raoultii	99.68%	100%	0.0
2	F2	Rickettsia conorii subsp. raoultii	100%	100%	0.0
3	F3	Rickettsia conorii subsp. raoultii	99.67%	100%	0.0
4	F4	Rickettsia conorii subsp. raoultii	100%	100%	0.0
5	F5	Rickettsia conorii subsp. raoultii	99.39%	99%	0.0
6	F7	Rickettsia conorii subsp. raoultii	99.04%	100%	0.0
7	F8	Rickettsia conorii subsp. raoultii	98.41%	100%	0.0
8	F9	Rickettsia conorii subsp. raoultii	100%	100%	0.0

Discussion

This study reports the occurrence and characterization of Rickettsia species in rodents and shrews in Kilombero district. Rodents made up 10.20% of the overall prevalence, while 12.5% of shrews tested positive for Rickettsia species. This indicates that the Kilombero district is currently a home of rodent and shrew rickettsial infections, increasing the risk of human infection via an appropriate vector. The findings of this study show a lower prevalence than those of [17], who reported a prevalence of 15.5% in rodents in Namibia and South Africa. This is probably because the current study employed the organs (liver and spleen) of rodents and shrews, whereas the other study used fleas taken from rodents for laboratory diagnosis. However, the present findings are close to the study by [25], which revealed

a 13% prevalence of Rickettsia in rodents originating from Kisangani, Democratic Republic of the Congo. This study reports the first genetic identification of R. conorii subsp. raoultii in rodents and shrews in Tanzania using sequencing technology. Sophisticated genetic techniques are used in the molecular characterization of R. conorii subsp. raoultii to clarify the species' diversity, mode of transmission, and clinical importance. However, when other techniques, such as PCR, are used for genetic identification, they cannot distinguish between Rickettsia species and only identify them at the genus level [26]. The prevalence of Rickettsia species with respect to different habitats was significantly higher in rodents captured from houses compared to peri-domestic and farms habitats. The large number of rodents found in houses likely sharing the ectoparasites could explain the difference. Another reason could be the house rodents moving from one house to another and interacting with other species in the peri-domestic areas. This could suggest a serious risk of infection spreading to humans in the area. More research is required to substantiate these assumptions. There was no significant difference in the prevalence of Rickettsia species between villages, indicating that the Rickettsial infection is similarly dispersed throughout the district since each village's ecological and geographical factors were the same. Even though there was no statistically significant difference, Nyamwezi had a higher prevalence (38.46%) compared to other villages. This can be attributed to the fact that houses in Nyamwezi were very close to each other, suggesting an increased rodent's interaction between houses. The lack of statistical significance difference in the incidence of Rickettsia among rodent species suggests that any species is prone to infection. The discovery of identical ectoparasites in shrews and various rodent species can explain this. This finding contradicts the findings of [9], who did his study in Mbulu District -Tanzania; he found that M. natalensis had a significantly higher prevalence than the other species, including Rattus, Arvicanthis spp., Lophuromys spp., Lemniscomys spp., and Grammomys spp. In comparison to shrews, shrews had a higher prevalence than rodents this might be attributed to the lower sample size in shrews. This result demonstrates no correlation between rickettsial infection and sex. This indicated that both sexes are potentially infectious. Despite not being statistically different, male rodents still had more prevalence than female rodents. This might be attributed to the fact that sex steroids in males, specifically androgens, modulate several aspects of host sex steroid hormones and alter genes and behaviors that influence susceptibility and resistance to infection. Thus, males may be more susceptible to infection than females because androgens reduce immunocompetence and because sex steroid hormones affect disease-resistance genes and behaviors that make males more susceptible to infection [27]. These findings are dissimilar to that of [28], which reported prevalence to be higher in males compared to females in Madagascar and showed a nearly significant difference in the seroprevalence of Spotted Fever Group Rickettsia in males and females (males 44% (n = 39), female (19%; n = 23; P = 0.052). These findings are dissimilar to that of [28], which reported prevalence to be higher in males compared to females in Madagascar and showed a nearly significant difference in the seroprevalence of Spotted Fever Group Rickettsia in males and females (males 44% (n = 39), female (19%; n = 23; P = 0.052). Regarding to age of small mammals, though not significant, young rodents had a higher prevalence [25], found similar results, suggesting that adult rodents were less likely to be infected and that this may be due to acquired immunity from constant lifelong exposure to the agent. Rodents and shrews caught during the dry season had a higher prevalence of Rickettsia species than those caught during the rainy season. The findings are consistent with those of, who found that rodents caught during the wet season, the prevalences of R. conorrii (32.3%), Thai tick typhi (31.7%) and Rickettsia typhi (6.7%) were lower than those of Rickettsia species, which were found in rodents captured during the dry season (46%), 48.5%, and 22.7%, respectively. This can be attributed to the higher ectoparasite activity in the dry season than in the wet season. The prevalence of rodents without ectoparasites was found to be higher (12.59%) than that of rodents with ectoparasites (8.92%), indicating that rodents act as reservoirs independent of the presence or absence of ectoparasites. Rickettsia conorii subsp. raoultii is a subspecies of the R. conorii bacterium and is transmitted by ticks, which act as its primary vectors. It has been associated with various tick-borne infections in humans. Recent studies have shown that this subspecies of Rickettsia conorii causes Mediterranean spotted fever (MSF) in humans in North Africa and South India. The molecular evidence indicates its role as a potential pathogen in these areas, highlighting its significance in public health (29,30). This study has shown the characterization of R. conorii subsp. raoultii in rodents and shrews. The recovery of R. conorii subsp. raoultii in rodents and shrews can be attributed to the infestation of ticks in rodents and shrews; this suggests the potential reservoir role of these animals. Our results are contrary to various studies, such as [31], which characterized R. felis, R. Helvetica, R. felis and R. slovaca in rodent fleas [32]. Occurrence of Rickettsia conorii subsp. raoultii in rodents suggests that, if this species completes its life cycle, it could contribute to the emergence of Mediterranean spotted fever in humans in Tanzania, thereby posing a potential public health risk in endemic areas. This study employed Sanger sequencing technology, which is a mature technology with high accuracy (99.99%) and can produce relatively long sequence reads, typically between 500 to 1000 base pairs [33]. This study had limitations that, unlike other studies [34], this study employed only one gene (gltA) to identify and characterize Rickettsia species. The gltA gene alone, however, has the potential to be a useful tool because multiple studies have demonstrated that it is more specific and capable of displaying significant variation (23,35). In addition, this study reported only the characterization of Rickettsia species in the small mammal population. However, we did not perform Rickettsial characterization in ectoparasites (mites, lice, ticks, and fleas) from captured small mammal hosts. Therefore, the rickettsial investigation of humans and other arthropod vectors should be performed to add to the epidemiological information on rickettsioses in the Kilombero district.

Conclusion

The overall results of molecular detection showed 10.2% of rodents and shrews were infected with Rickettsia species. Genetic characterization confirmed that rodents and shrews are infected with R. conorii subsp. raoultii in the study area. Given that the R. conorii subsp. raoultii are known to be zoonotic,

future studies should be done to understand the epidemiology of these rickettsia species.

Abbreviation

ACE	African centre of Excellence
BTD	Biosensor Technology Development
CI	Confidence Interval
IRPM	Innovative Rodent pest management
MS	Microsoft
PCR	Polymerase Chain Reaction
QGIS	Quantum Geographic Information System
SIPM	Sua Institute of Pest Management
SUA	Sokoine University of Agriculture
TZ	Tanzania
WHO	World Health Organization
M	Micro [106]
$\chi 2$	Chi-square

Declaration

Acknowledgment

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Availability of data and materials

Data will be available by emailing: fatuma.kindoro@sua.ac.tz

Authors' contributions

Fatuma Kindoro (FK) is the principal investigator (PI) who contributed to the conceptualization, data curation, formal analysis, and writing of the original draft of the manuscript. Alexanda Mzula (AM) is the core supervisor, and Abdul Katakweba (AK) is the main supervisor. AM and AK contributed to the methodology, supervision, review, editing, and re-writing of the manuscript. Ernatus Mkupasi (EM) supervised the fieldwork and reviewed the draft of the manuscript. All authors have read and accepted the final version of the manuscript for submission.

Ethics approval and consent to participate

This study was ethically approved followed appropriate research clearance and ethical protocols to ensure the protection of participants' rights and compliance with regulations. SUA approved the research clearance under reference number SUA/DPRTC/R/186/27.

Consent for publication

Not applicable

Competing interest

The author declares that he has no competing interests.

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References

1. Kovalenko N. Morphology of Bacteria, Viruses and Protozoa. Learn Guid 2nd 3rd year English media students Fac Med Fac Dent (Microbiology, Virol Immunol. 2017;1–76.

2. Bermúdez CSE, Troyo A. A review of the genus Rickettsia in Central America. Res Rep Trop Med. 2018 Jun 29; 9:103-112. doi: 10.2147/RRTM.S160951.

3. Mahajan SK. Rickettsial diseases. J Assoc Physicians India. 2012 Jul;60:37-44.

4. Minichová L, Hamšíková Z, Mahríková L, Slovák M, Kocianová E, Kazimírová M, et al. Molecular evidence of Rickettsia spp. in ixodid ticks and rodents in suburban, natural and rural habitats in Slovakia. Parasit Vectors. 2017 Mar 24;10(1):158. doi: 10.1186/s13071-017-2094-8.

5.Valdez KR, Mendell NL, Escárcega-Ávila AM, de la Mora-Covarrubias A, Jiménez-Vega F, Waldrup KA, et al.. Survey of Fleas and Ticks for Rickettsia rickettsii and Rickettsia typhi in the El Paso Community and Other Areas in Texas, New Mexico, and Ciudad Juarez, Mexico. Am J Trop Med Hyg. 2025 Jun 17;113(3):659-665. doi: 10.4269/ajtmh.24-0709.

6. Noden BH, Davidson S, Smith JL, Williams F. First detection of rickettsia typhi and rickettsia felis in fleas collected from client-owned companion animals in the southern great plains. J Med Entomol. 2017 Jul 1;54(4):1093-1097. doi: 10.1093/jme/tjx069.

7. Zahoor A, Abbas RZ, Khurram A, Aslam A, Mateen A, Aslam A, et al. Vector-borne zoonotic diseases. In: Abbas RZ, Hassan MF, Khan A and Mohsin M (eds), Zoonosis, Unique Scientific Publishers, Faisalabad, Pakistan, 2023.Vol. 2: 12-36. https://doi.org/10.47278/book.zoon/2023.49

8. Kumar K, Patnaik R, Kumari H, Sharma N. Acute febrile illness: a systematic review of infectious aetiologies among patients. Journal of Pharmaceutical Negative Results 2022;13 (Special Issue): 5080-5093. DOI:10.47750/pnr.2022.13.S08.667

9. Thomas CA, katakweba AAS, Massawe AW, Makundi RH, Machang RS, Kessy ST. Prevalence of Rickettsia typhi in rodent fleas from areas with and without previous history of plague in Mbulu district, Tanzania. African Journal of Microbiology Research 2020;14(2):65–70. https://doi.org/10.5897/AJMR2019.9198

10. Moorthy GS, Rubach MP, Maze MJ, Refuerzo RP, Shirima GM, Lukambagire AS, et al. Prevalence and risk factors for Q fever, spotted fever group rickettsioses, and typhus group rickettsioses in a pastoralist community of northern Tanzania, 2016-2017. Trop Med Int Health. 2024 May;29(5):365-376. doi: 10.1111/tmi.13980.

11. Leulmi H, Socolovschi C, Laudisoit A, Houemenou G, Davoust B, Bitam I, Raoult D, Parola P. Detection of Rickettsia felis, Rickettsia typhi, Bartonella Species and Yersinia pestis in Fleas (Siphonaptera) from Africa. PLoS Negl Trop Dis. 2014 Oct 9;8(10):e3152. doi: 10.1371/journal.pntd.0003152.

12. Issae AR, Katakweba AS, Kicheleri RP, Chengula AA, van Zwetselaar M, Kasanga CJ. Exploring Pathogenic and Zoonotic Bacteria from Wild Rodents, Dogs, and Humans of the Ngorongoro District in Tanzania Using Metagenomics Next-Generation Sequencing. Zoonotic Diseases. 2023; 3(3):226-242. https://doi.org/10.3390/zoonoticdis3030019

13. Moonga LC, Hayashida K, Nakao R, Lisulo M, Kaneko C, Nakamura I, et al. Molecular detection of Rickettsia felis in dogs, rodents and cat fleas in Zambia. Parasit Vectors. 2019 Apr 11;12(1):168. doi: 10.1186/s13071-019-3435-6.

14. Rwela AG. Socio-economic and institutional determinants of membership in Agricultural Marketing Co-operative Societies in Mvomero and Kilombero districts, Tanzania. Development Studies Research, 2023; 10(1): 5095. https://doi.org/10.1080/21665095.2022.2163679

15. Advisory R, Report M. United Republic of Tanzania Ramsar Advisory Mission Report. 2017; 1173.

16. Martin W, Meek AH, Willeberg P, Wayne Martin by S, Meek A. ©1987 S Part 1: Basic Principles Chapter 3: Measurement of Disease Frequency and Production. Vet Epidemiol Princ Methods. 1987.

17. Essbauer S, Hofmann M, Kleinemeier C, Wölfel S, Matthee S. Rickettsia diversity in southern Africa: A small mammal perspective. Ticks Tick Borne Dis. 2018 Feb;9(2):288-301. doi: 10.1016/j.ttbdis.2017.11.002.

18. Mwamengele G, Sabuni C, Mathew C. Seroprevalence and risk factors for brucellosis in cattle and rodents in Kilosa district, Morogoro, Tanzania. J Ideas Heal. 2024;7 (4):1123–30. https://doi.org/10.47108/jidhealth.Vol7.Iss4.363

19. Schlitter DA, Ferguson AW, McDonough MM, Mammals of Africa (Vol. I-VI.), Journal of Mammalogy, Volume 95, Issue 6, December 2014, Pages 1299–1303, https://doi.org/10.1644/14-MAMM-R-122.

20. Kaasalainen U, Kirika PM, Mollel NP, Hemp A, Rikkinen J. The Lichen Genus Sticta (Lobariaceae, Peltigerales) in East African Montane Ecosystems. Journal of Fungi. 2023; 9(2):246. https://doi.org/10.3390/jof9020246.

21. Perles L, Barreto WTG, de Macedo GC, Calchi AC, Bezerra-Santos M, Mendoza-Roldan JA, Otranto D, Herrera HM, Barros-Battesti DM, Machado RZ, André MR. Molecular detection of Babesia spp. and Rickettsia spp. in coatis (Nasua nasua) and associated ticks from midwestern Brazil. Parasitol Res. 2023 May;122(5):1151-1158. doi: 10.1007/s00436-023-07815-5.

22. Palacios-Santana R, Wei L, Fernandez-Santos NA, Rodriguez-Perez MA, Uriegas-Camargo S, Mendell NL, et al. Spotted Fever and Typhus Group Rickettsiae in Dogs and Humans, Mexico, 2022. Emerg Infect Dis. 2023 Jul;29(7):1443-1446. doi: 10.3201/eid2907.230333.

23. Tsui PY, Tsai KH, Weng MH, Hung YW, Liu YT, Hu KY, Lien JC, Lin PR, Shaio MF, Wang HC, Ji DD. Molecular detection and characterization of spotted fever group rickettsiae in Taiwan. Am J Trop Med Hyg. 2007 Nov;77(5):883-90. Erratum in: Am J Trop Med Hyg. 2008 Jul;79(1):140. Wang, Hsis-Chieh [corrected to Wang, Hsi-Chieh].

24. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol Biol Evol. 2021 Jun 25;38(7):3022-3027. doi: 10.1093/molbev/msab120.

25. Laudisoit A, Falay D, Amundala N, Akaibe D, de Bellocq JG, Van Houtte N, et al. High prevalence of Rickettsia typhi and Bartonella species in rats and fleas, Kisangani, Democratic Republic of the Congo. Am J Trop Med Hyg. 2014 Mar;90(3):463-8. doi: 10.4269/ajtmh.13-0216.

26. Chitanga S, Chibesa K, Sichibalo K, Mubemba B, Nalubamba KS, Muleya W, et al. Molecular Detection and Characterization of Rickettsia Species in Ixodid Ticks Collected From Cattle in Southern Zambia. Front Vet Sci. 2021 Jun 7;8:684487. doi: 10.3389/fvets.2021.684487.

27. Rio P, Caldarelli M, Miccoli E, Guazzarotti G, Gasbarrini A, Gambassi G, Cianci R. Sex Differences in Immune Responses to Infectious Diseases: The Role of Genetics, Hormones, and Aging. Diseases. 2025 Jun 7;13(6):179. doi: 10.3390/diseases13060179.

28. Rakotonanahary RJ, Harrison A, Maina AN, Jiang J, Richards AL, Rajerison M, Telfer S. Molecular and serological evidence of flea-associated typhus group and spotted fever group rickettsial infections in Madagascar. Parasit Vectors. 2017 Mar 4;10(1):125. doi: 10.1186/s13071-017-2061-4.

29. Nallan K, Ayyavu V, Ayyanar E, Thirupathi B, Gupta B, Devaraju P, et al. Molecular Evidence of Rickettsia conorii subsp. raoultii and Rickettsia felis in Haemaphysalis intermedia Ticks in Sirumalai, Eastern Ghats, Tamil Nadu, South India. Microorganisms. 2023 Jun 30;11(7):1713. doi: 10.3390/microorganisms11071713.

30. Cossu CA, Cassini R, Bhoora RV, Menandro ML, Oosthuizen MC, Collins NE, Wentzel J, Quan M, Fagir DM, van Heerden H. Occurrence and molecular prevalence of Anaplasmataceae, Rickettsiaceae and Coxiellaceae in African wildlife: A systematic review and meta-analysis. Prev Vet Med. 2024 Sep;230:106257. doi: 10.1016/j.prevetmed.2024.106257.

31. Maina AN. Sero-epidemiology and molecular characterization of Rickettsiae infecting humans, selected animals and arthropod vectors in Asembo, western Kenya,. 2012;2007–10.

32. Heglasová I, Víchová B, Kraljik J, Mošanský L, Miklisová D, Stanko M. Molecular evidence and diversity of the spotted-fever group Rickettsia spp. in small mammals from natural, suburban and urban areas of Eastern Slovakia. Ticks Tick Borne Dis. 2018 Sep;9(6):1400-1406. doi: 10.1016/j.ttbdis.2018.06.011.

33. Chen L, Cai Y, Zhou G, Shi X, Su J, Chen G, Lin K. Rapid Sanger sequencing of the 16S rRNA gene for identification of some common pathogens. PLoS One. 2014 Feb 14;9(2):e88886. doi: 10.1371/journal.pone.0088886.

34. Zhu WJ, Ye RZ, Tian D, Wang N, Gao WY, Wang BH, et al. The first direct detection of spotted fever group Rickettsia spp. diversity in ticks from Ningxia, northwestern China. PLoS Negl Trop Dis. 2025 Jan 2;19(1):e0012729. doi: 10.1371/journal.pntd.0012729.

35. Rungrojn A, Chaisiri K, Paladsing Y, Morand S, Junjhon J, Blacksell SD, Ekchariyawat P. Prevalence and Molecular Characterization of Rickettsia spp. from Wild Small Mammals in Public Parks and Urban Areas of Bangkok Metropolitan, Thailand. Trop Med Infect Dis. 2021 Nov 11;6(4):199. doi: 10.3390/tropicalmed6040199.