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Prevalence and molecular characterization of Bartonella species from rodents and their associated ectoparasites in Kilwa District, Lindi region, Tanzania

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Abstract

Background: Bartonella are facultative Intracellular gram-negative bacteria from the Bartonellaceae family. Several rodents associated with Bartonella species have been known to be transmitted by ectoparasites to animals and humans however, limited information is available about their epidemiology, host, vector, and species specificity, particularly in southern Tanzania. This study aimed to investigate the prevalence and molecular characterization of Bartonella species in rodents and their associated ectoparasites in Kilwa district, Southern part of Tanzania.

Methods: A cross-sectional study was conducted to capture rodents using Sherman and Wire cage traps. Captured rodents were anesthetized using diethyl ether and dissected to extract spleen tissue. Ectoparasites were identified by examining their morphological features with a stereo microscope aided by ectoparasites identification keys. A total of 138 rodent spleen tissue and 93 pools of ectoparasites were subjected to conventional polymerase chain reaction (PCR) and sequencing targeting the gltA gene of Bartonella species.

Results: The overall prevalence of Bartonella species in rodents was 3.62% with a statistically significant difference in prevalence among species ($\chi^2 = 12.951$, df = 5, $p < 0.01$) compared to 3.23% in ectoparasites with no significant differences across species ($p > 0.05$). Bartonella infections were 3.37% in M. natalensis, 3.27% in R. rattus and 50.0% in G. dolichurus. The Laelaps species and Xenopsylla cheopis were found infected by 6.67% and 2.70%, respectively, while Rhipicephalus appendiculatus was not infected by Bartonella species. Sequence analysis of rodents and ectoparasites showed that the obtained sequences in the current study were genetically closely related to Bartonella mastoids from Turkey, Bartonella species from Kenya, and uncultured Bartonella species from South Africa (99.50–99.75% identity).

Conclusion: The detection of Bartonella species in rodents and their associated ectoparasites implies the reservoir role of rodents and vectors in transmitting Bartonella species to humans and animals. The results suggest that further epidemiological studies need to be done to determine whether the identified Bartonella species could be responsible for animal and human cases of febrile illness in an area.

Keywords: Rodent, Ectoparasite, Bartonella, B. mastomydis, Tanzania

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Background

Bartonella are facultative Intracellular Gram-negative bacteria from the Bartonellaceae family [1]. Bartonella species are small pleomorphic coccobacilli bacteria, fastidious and slow-growing in vitro [2]. The Bartonella bears the name of the scientist who discovered Bartonella bacilliformis in 1905, Dr. Alberto Leonardo Barton Thomson [3]. Over 32 species of Bartonella have been formally validated across various host reservoirs, with many more yet to be discovered [4]. Bartonella can occupy a double niche, infecting erythrocytes and endothelial cells, with the ability to escape the host immune system [5]. Worldwide, rodents are reported to be the natural reservoir hosts of many disease pathogens such as Bartonella species, Leptospira species, Rickettsia species, and Yersinia pestis [6–8]. At least 22 rodents adapted species responsible for maintaining several zoonotic Bartonella species that are significant to public and veterinary health [4]. Bartonella species isolated from small mammals have public health implications, including Bartonella elizabethae, Bartonella tribocorum, and Bartonella grahamii [9,10]. The transmission of Bartonella is facilitated by blood-sucking arthropod vectors, including mites, fleas, lice, and ticks [11,12]. Additionally, Bartonella species can be transmitted directly to animals and humans through scratches from an infected reservoir host, such as cats, or by coming into contact with the infectious

feces of the animal [13]. Bartonella species have been detected in various hosts and vectors in many countries, including Israel, China, and the United States [14–16]. In Africa, Bartonella species have been detected in several countries, including Tanzania, South Africa, Nigeria, Kenya DR Congo, and Tanzania [10,17–19]. The detection of Bartonella species from rodent populations in Tanzania was first reported by Gundi et al. [19] in Mbulu District, where B. elizabethae, B. tribocorum, and B. queenslandensis were identified in field rodents. A subsequent study by Theonest et al. [20] reported the detection of B. tribocorum, B. rochalimae, B. elizabethae, and B. quintana in rodents and ectoparasites in Moshi. Additionally, Mhamphi et al. [21] reported the detection of B. elizabethae and B. tribocorum in rodents and ectoparasites in the Morogoro and Kigoma regions. Recently, there have been numerous global reports of bartonellosis in febrile individuals [22–24]. Clinical symptoms of Bartonella infections in humans include fever, chills, weakness, headache, endocarditis, myocarditis, splenomegaly, and lymphadenopathy [25,26]. In developing countries, the nonspecific symptoms associated with Bartonella infections can lead to misdiagnosis as other diseases, primarily due to the absence of a specific diagnostic test for Bartonella. This lack of specificity complicates the identification of these infections, which often resemble other febrile illnesses and chronic conditions, making accurate diagnosis challenging [20,27]. The interaction between rodents and ectoparasites creates a complex ecological web that contributes to the maintenance and dissemination of Bartonella species to animals and humans [20]. Even though various studies on bartonellosis in rodents and their ectoparasites have been carried out, the epidemiology of the disease is not well covered, especially in the southern part of Tanzania, including the Kilwa district, an area with a history of tourism [8,19,20,26]. This study aimed to determine the prevalence of Bartonella species and molecular identification of Bartonella species found in rodents and their ectoparasites from the Kilwa district, Tanzania. The findings of this study contribute to the development of epidemiological surveillance of this neglected public health disease, which can serve as a foundational resource for public health officials to create and implement effective strategies for disease control.

Methods

Study Area

This study was conducted in the Kilwa district, one of the six administrative districts of Lindi region, Tanzania (Figure 1). As reported by the National Bureau of Statistics of Tanzania in 2022, Kilwa district covers an area of 14,999 square kilometers and has a total population of 297,676 people. Kilwa district is located between latitudes 8° 20′ and 9° 56′ and between longitudes 38° 36′ and 39° 50′ East of Greenwich [28]. It shares borders with the Rufiji district to the north (Pwani region), the Indian Ocean to the east, and Lindi, Nachingwea, and Ruangwa districts to the south, while Liwale district lies to the west. The district experiences a tropical climate with a hot, humid rainy season from November to May, with peak rainfall in February and March. Annual rainfall ranges between 800 and 1,400 mm, and temperatures along the coast average between 22°C and 30°C, with 98-100% humidity and an annual rainfall of 1,034 mm [28]. The land cover and vegetation in the Kilwa district are defined by a low population density and natural vegetation, featuring Miombo woodlands, scattered trees, shrubs, and dense foliage [29].

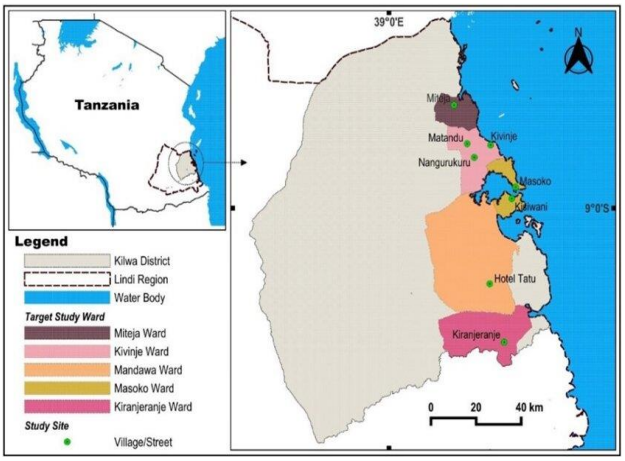

Figure 1: A map of the study area created in the Quantum Geographic Information System (QGIS).

Study design and sampling strategies

A cross-sectional study was conducted from January to February 2023 using both random and purposive sampling methods of data collection. Five wards out of twenty-three, namely Masoko, Kivinje, Miteja, Kiranjeranje, and Mandawa were purposively selected to participate in this study based on the availability of rodents. Eight villages (Masoko, Kisiwani, Kivinje, Nangurukuru, Matandu, Hoteli tatu, Kiranjeranje, and Miteja) were purposively selected based on the presence of rodents. In each study village, trapping was conducted indoors in peridomestic, fallow land, and agricultural fields.

Sample size determination

The sample size for establishing the prevalence of Bartonella species in rodents was calculated using the formula: n=Z2 p (1-p)/ d2) [18,19]. Where n= sample size, P= expected prevalence (set at 10%), d= desired precision 5%, and Z= statistics corresponding to the level of confidence (1.96 for 95% confidence). Therefore, the sample size for this study was 138 rodents.

Trapping procedures for rodents

Sherman (LFA 7.5 × 9 × 23 cm) and wire cage traps were used to capture rodents outdoors. A total of 100 Sherman traps were used, arranged in a line, with a spacing of 5 to 10 meters apart [30]. For indoor trapping, 30 wire cage traps were used, targeting 10 houses per village while considering the distance between the houses. In each selected house, 2 to 3 wire cage traps were placed, depending on the size of the house. Traps were baited with a mixture of peanut butter and maize flour with a 2:1 ratio (1000 g of peanut butter mixed with 500 g of maize flour) [26]. Traps were placed at each site for three consecutive nights and inspected every morning at 7:00 AM before moving to a different location. The captured rodents were humanely anesthetized with cotton wool soaked in diethyl ether and then placed in a lidded bottle [31]. For each captured rodent, the weight, sex, and external body measurements, including body length, tail length, and hindfoot length were recorded. Identification of the rodents followed the guidelines of [32].

Collection of ectoparasites and spleen from rodents

Each rodent was inspected for ectoparasites and combed using a fine comb and brushes. The ectoparasites from each trapped rodent were collected in a dish, separately counted, and placed in a labeled microvial containing 70% ethanol [33]. Each anesthetized rodent was pinned ventral side up on a clean dissection board. The ventral area of the rodent was cleaned with cotton wool soaked in ethanol before dissection. Clean forceps and scissors were then used for dissection to remove the spleen. Before dissecting a new animal, the dissection board, forceps, and scissors were thoroughly cleaned with bleach, water, and ethanol, as described by [34]. Both rodent spleen tissue and ectoparasite samples were transported in a cold chain to the Pest Management Laboratory for storage at a –20°C freezer, waiting for further analyses.

Identification of ectoparasites

Ectoparasites were identified in the SUA parasitology laboratory by examining their morphological characteristics with a Stereo microscope using a 10X objective lens, aided by ectoparasite identification keys [35,36]. Fleas were initially cleared by soaking them in a 10% potassium hydroxide (KOH) solution for 24 hours. They were then rinsed in distilled water for 20 minutes and subjected to a series of ethanol concentrations (70%, 80%, 95%, and absolute) for 30 minutes each to achieve gradual dehydration. After dehydration, the specimens were cleared with xylene for one hour. Finally, the fleas were mounted on microscope slides using dibutyl phthalate polystyrene-xylene (DPX) as the mounting medium, and a coverslip was applied for microscopic observation. The identified ectoparasites from each rodent of the same genus and or species were pooled (1-5 ectoparasites per pool) according to their genus or species [21]. A total of 93 pools were obtained, including 74 pools of Laelaps species, 15 pools of X. cheopis, and four pools of R. appendiculatus.

Genomic extraction of DNA from rodent spleen and ectoparasite pools

DNA extraction from both rodent spleens and ectoparasites was done using the Quick-DNA™ Miniprep Plus Kit (Zymo Research), following the manufacturer's protocol. A piece of spleen tissue from each rodent was cut into small pieces, approximately 10 milligrams [20]. Pieces of spleen and pooled ectoparasite samples were crushed separately using a sterile mortar and pestle. The crushed samples were individually incubated at 56 ºC to complete the lysis of tissue for three hours in a microcentrifuge tube containing 180 µl of tissue lysis buffer and 20 µL proteinase K [37]. In order to remove insoluble debris, the mixtures were centrifuged at 12,000 x g for 1 minute and the aqueous supernatant was transferred to a clean microcentrifuge tube. 400 µl of Genomic Binding Buffer was added to each tube and mixed thoroughly. The mixture was transferred to a Zymo-Spin™ IC-XM Column in a collection tube and centrifuged at 12,000 x g for 1 minute. The collection tubes with the flow through were discarded. 400 µl of DNA Pre-Wash Buffer was added to the column in a new collection tube and centrifuged for 1 minute. The flow-through was discarded. 700 µl of g-DNA Wash Buffer was added and centrifuged for 1 minute, and the collection tube was emptied. 200 µl of g-DNA Wash Buffer was added and centrifuged for 1 minute. The collection tubes with the flow through were discarded. To elute the DNA, the Zymo-Spin™ IC-XM Column was transferred to a clean microcentrifuge tube, and 50 µl DNA Elution Buffer was added, incubated for 5 minutes at room temperature, and then centrifuged for 1 minute. The DNA's purity and concentration were measured using a Nanodrop spectrophotometer at 260 and 280 nm wavelengths. The extracted DNA was stored at −20 °C until PCR testing.

PCR amplification

Conventional PCR amplification of citrate synthase (gltA) was done using primer sequences shown in Table 1. Positive control (Ectoparasite Bartonella DNA extract) was obtained from a previous study by Mhamphi et al. [21] and nuclease-free water was used as a negative control in each PCR run. PCR was done using One Taq® Quick-Load 2X Master Mix with Standard Buffer from BioLabs. Two microliters of DNA templates were added into the microtube containing a mixture of 4 µL of 5 x HOT FIREPol® Blend Master Mix Ready to Load (from Solis Biodyne, Riia 185a, 51014 Tartu, Estonia), 0.5µL of forward primer (Bart-F), 0.5 µL of reverse primer (Bart-R) and 15µL of nuclease-free water, resulting in a total reaction volume of 20.0 µL. The cycling conditions included an initial denaturation at 95 ºC for 12 min, followed by 40 cycles of 95 ºC for 20 s, 59 ºC for 20 s and 72 ºC for 35 s. A final extension at 72 ºC for 5 min was performed to complete the extension.

Table 1: Primer sequences used for the detection of Bartonella DNA

Primer Name	Orientation	Primer sequence (5' to 3')	Amplicon size (bp)
Bart-F	Forward	CGTAATGATCTYAGTTAYGCTGCAAA	429
Bart-R	Reverse	AGAAGTGGATCATTTTGAATRTTBARYTC	

Gel electrophoresis of PCR products

Agarose gel of 1.5% was prepared by dissolving 1.5 grams of agarose in 100 mL of Tris borate EDTA buffer (TBE) in a conical flask, heating it until fully dissolved, and staining it with 10 µL of SafeView™ Classic DNA staining dye (Applied Biological Materials Inc.). A volume of 4 µL from each sample of PCR product was loaded into the gel wells, with 4 µL of 100 bp DNA ladder loaded in the first well to indicate fragment sizes. The voltage was set to 100 V and electrophoresis was run for 60 minutes. The gel was transferred to the gel documentation machine (Gel Doc™ EZ Imager from Bio-Rad Laboratories) for visualization. A sample was considered positive if a clearly defined DNA band of approximately 429 bp was visible in the gel.

Sequencing and creation of consensus sequences

PCR-positive product samples and primers used for amplification of Bartonella species were sent to Macrogen Europe in Amsterdam, Netherlands, for sequencing to confirm for Bartonella species. Forward and backward reads from sequencing were cleaned, edited, and assembled using Genious Primer software version 2024.0.7 to make consensus sequences

for analyses. The assembled consensus sequences with about 429 base pairs were compared with Bartonella species sequences deposited in the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST), to confirm the sequence similarity and taxonomic identity. The taxonomic identification was based on BLAST results having both the highest percentage identity, query cover, and the minimum E-value.

Multiple Sequence Alignment and Phylogenetic Analysis

Sequential alignment was generated using a built-in ClustalW implementation in Molecular Evolutionary Genetics Analysis (MEGA) 11.0.13 software. The phylogeny test was used to assess the reliability of the phylogenetic tree using a neighbor-joining method for tree construction based on the Tamura-Nei model, with 1000 bootstrap replicates [38]. The candidate sequences of this study with accession numbers PQ685658, PQ685659, PQ685660, PQ685661, PQ685662, PQ685663, and 5 sequences of 7 different Bartonella species retrieved from the National Centre for Biotechnology Information (NCBI) nucleotide database, were used to construct a phylogenetic tree. One Rickettsia felis downloaded from GenBank with accession number JF448473 was used as the outgroup for rooting the tree in the analysis [39].

Data analysis

Data were entered, organized, and cleaned using Microsoft Excel before actual analysis. Descriptive statistics was used to calculate the prevalence of Bartonella species in rodents and ectoparasites using SPSS statistical software version 4.2.2. The chi-square test was used to determine the difference in Bartonella prevalence in rodents and ectoparasites between locations, sex, age, species, as well as habitats. The findings were considered statistically significant at $p<0.05$.

Results

Rodent species composition and abundance of ectoparasites

A total of 138 rodents were captured inside houses, peridomestic, fallow land, and agricultural habitats, with M. natalensis being the most prevalent rodent species (64.49%), more than other rodent species (Table 2). A total of 81 rodents were found to be infested by ectoparasites such as mites, fleas, and ticks. The ectoparasites identified from M. natalensis, R. rattus, and G. leucogaster included Laelaps species, X. cheopis, and R. appendiculatus, with Laelaps species being the most abundant ectoparasite species 94.00% than other species (Table 3).

Table 2: Species composition of captured rodents and their habitats

Rodent species	Habitats				Total	Proportion (%)
	Inside houses	**Peridomestic**	**Fallow land**	**Agriculture field**		
M. natalensis	0	22	23	44	89	64.49
R. rattus	31	0	0	0	31	22.46
G. leucogaster	0	10	0	1	11	7.97
A. wilsoni	0	1	0	3	4	2.90
G. dolichurus	0	0	1	1	2	1.45
A. chrysophilus	0	0	0	1	1	0.72
Total	**31**	**33**	**24**	**50**	**138**	**100**

Table 3: Abundance of ectoparasite species on each rodent species

Rodent species	Ectoparasites			Total	Proportion (%)
	***Laelaps* species**	***X. cheopis***	***R. appendiculatus***		
M. natalensis	341	11	4	356	92.95
R. rattus	9	8	0	17	4.44
G. leucogaster	10	0	0	10	2.61
A. wilsoni	0	0	0	0	0
G. dolichurus	0	0	0	0	0
A. chrysophilus	0	0	0	0	0
Total	**360**	**19**	**4**	**383**	**100**
Proportion (%)	**94.00**	**4.96**	**1.04**		

Prevalence of Bartonella DNA in rodent tissues

The prevalence of Bartonella species DNA in rodent tissues was 3.62 % (n=5/138). The study found M. natalensis, G. dolichurus, and R. rattus to be infected with Bartonella species among the six rodent species. Specifically, Bartonella species DNA was 3.37% (n=3/89) in M. natalensis, 50% (n=1/2) in G. dolichurus, and 3.23% (n=1/31) in R. rattus. The prevalence of Bartonella species DNA in rodents differed significantly among rodent species ($\chi^2 = 12.951$, df = 5, $^{**}p < 0.01$). However, no significant differences ($p > 0.05$) were found in Bartonella prevalence between different sexes, ages, habitats, and villages in rodents.

Prevalence of Bartonella DNA in ectoparasite pools

The prevalence of Bartonella species DNA in ectoparasite pools was 3.23 % (n=3/93). The prevalence of Bartonella species DNA based on ectoparasite species was 2.70 %, (n=2/74) and 6.67% (n=1/15) in Laelaps species and X. cheopis pools, respectively. No Bartonella DNA was detected in pools of R. appendiculatus. No significant differences ($p > 0.05$) were observed in the prevalence of Bartonella DNA between different ectoparasite species or among rodent ectoparasites across all sexes and ages.

Results of BLAST and phylogenetic analysis

All sequences were identified as B. mastomydis with percentage identity ranging from 99.50% to 99.75% from those found in GeneBank. Sequences were similar to those previously identified by B. mastomydis, with accession numbers OQ305212.2 and OQ305213.2 from Turkey. Also, candidate sequences were phylogenetically related to Bartonella species with accession number KM233487.1 from Kenya by 99.50% and Uncultured Bartonella species with accession number HM749297.1 from South Africa by 99.71% (Figure 2).

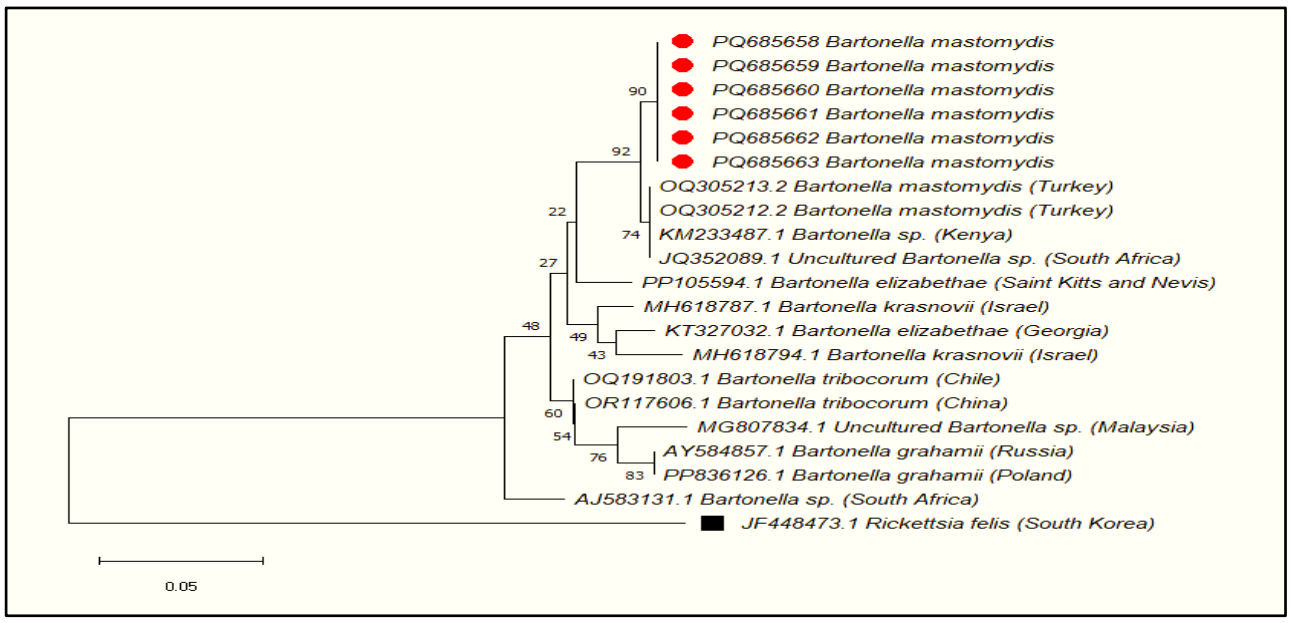

Figure 2: Phylogenetic tree showing the relatedness of the *Bartonella gltA* gene sequences (429 bp gene fragments) derived from rodents and ectoparasites along with reference sequences from the GenBank database. The phylogenetic tree was constructed using the neighbor-joining method based on a Kimura 2-parameter substitution model [38]

Discussion

The study highlighted the epidemiology of Bartonellosis by detecting Bartonella DNA from M. natalensis, R. rattus, and G. leucogaster rodents and ectoparasites such as Laelaps species and X. cheopis. The overall prevalence of Bartonella species in rodents and ectoparasites was 3.62% and 3.23% in rodents and ectoparasites, respectively. Detection of Bartonella DNA from rodent and ectoparasite populations suggests a potential risk for domestic animal and human infections through bites from infected ectoparasites or when they come into contact with infected rodents, especially in urban areas where rodents and humans coexist closely. The prevalence of Bartonella DNA from this study is lower in comparison to other studies. Theonest et al. [20] reported a prevalence of Bartonella of 15% from rodents and 27.5% from ectoparasites in Moshi. The study of Lipatova et al. [40] reported a 23.7% prevalence of Bartonella in small mammals,29.1% in fleas, and 3.7% in ticks. Kamani et al. [10] found a 26% prevalence of Bartonella in rodents and 28% in ectoparasites of Nigerian rodents. The variation in the detection of Bartonella from rodents and ectoparasites can be attributed to different host species, habitat geographical differences, and the distribution of arthropod vectors. The prevalence of Bartonella species was significantly higher in G. dolichurus compared to other rodent species. This can be attributed to the lower number of G. dolichurus captured, though more studies are required to prove this assumption. The findings correlate with Halliday et al. [19] who reported a higher prevalence of 100% of Bartonella species in G. dolichurus compared to other species in the Mbulu district in Tanzania. The study reported a relatively low prevalence of Bartonella species observed in R. Rattus. This is in line with several recent studies suggesting that the prevalence of Bartonella in Rattus found in Africa could be low compared to the population of Rattus found in Asia [18,19,41]. This could be attributed by host escape during colonization result from host escape during colonization, where relatively small founding populations of invading species can leave their parasites behind when settling in new areas [18,42]. The study shows that rodent sex does not influence the prevalence of Bartonella species, indicating that both males and females have an equal likelihood of being exposed to and infected by Bartonella species. This study is in line with Jian et al. [43] and Yao et al. [44] reported no significant difference in the detection rate of Bartonella species associated with either gender of the rodents. The study contradicts Mariën et al. [45] found female M. natalensis to be more prone to Bartonella species infection, possibly due to their unusual social behavior of grooming. Although Laelaps species were the most abundant ectoparasites, as they spend most of their life on hosts study found no statistically significant difference in the prevalence of Bartonella DNA among ectoparasite pools, suggesting all ectoparasite species play an equal role in transmitting Bartonella infections in rodent populations. Previous research reported Bartonella prevalence rates of 1.7% in mites and 25.8% in X. cheopis in Thailand [46], 29.1% in fleas and 3.7% in ticks in Lithuania [40], and 27.5% in X. cheopis in northern Tanzania [20]. This study found no significant differences in the prevalence of Bartonella DNA among ectoparasites collected from rodents of various sexes and ages. This suggests that ectoparasites from both male and female rodents, regardless of their age, have similar levels of exposure and play an equal role in the transmission of Bartonella infections. These results are consistent with those of Thomas et al. [8], who also reported no significant differences in Bartonella species prevalence among ectoparasites from rodents of different sexes. However, their findings differed regarding age, as they noted that ectoparasites from adult male rodents had a higher exposure to Bartonella infections compared to those from juvenile rodents in Morogoro. The prevalence of Bartonella DNA in rodents and ectoparasites was found to be mostly similar, highlighting the role of ectoparasites as potential vectors in spreading Bartonella infections among rodent populations of the Kilwa district. The detection of Bartonella DNA from rodents and ectoparasites from rodents found inside houses and Agricultural fields suggests a potential risk for transmission of

Bartonella infection to domestic animals and humans, either directly through contact with rodents or indirectly through vectors such as fleas and mites [26]. It also provides a need for ongoing surveillance and monitoring of rodents and their ectoparasites to better understand the epidemiology of Bartonella and its potential impact on domestic animals and human health. The phylogenetic analysis of 22 nucleotide sequences, including six candidate sequences from this study and 16 reference sequences from NCBI, revealed significant evolutionary relationships, with all candidate sequences of rodents and ectoparasites clustered within a single clade, suggesting common ancestry of that clade. Sequence analysis indicated that all sequences in GenBank were closely related to Bartonella mastomydis (OQ3052212.2 and OQ305213.2) from Turkey, Bartonella species (KM233487.1) from Kenya [18], and uncultured Bartonella species (JQ352089.1) from South Africa [47]. Identified Bartonella strains were observed in M. natalensis G. dolichurus and R. rattus rodents. The clustering of Tanzanian Bartonella candidate sequences with those from Turkey, Kenya, South Africa, Israel, Egypt, and Ghana suggests a shared evolutionary history and common ancestry among these geographically distant populations. This could be attributed to host migration or human activities like tourism that may have contributed to the spread of this pathogen. The sequences showed over 99% similarity to B. mastomydis, which was discovered for the first time in rodents of the genus Mastomys in Benin [48]. This study identified B. Mastomydis in M. natalensis, G. dolichurus, and R. rattus rodents as well as in ectoparasites such as Laelaps species and X. cheopis. This finding suggests that B. Mastomydis can cut across different rodent species through the ectoparasite they harbor and increasing evidence for a lack of host specificity as the same Bartonella species can be identified from a diverse range of rodent hosts and their ectoparasites [43]. This aligns with findings from a study by Abreu-Yanes et al. [9], which reported the presence of B. mastomydis in Mus musculus domesticus in Spain. This study employed short sequences of a single gene target only(gltA) for molecular detection and characterization. The gltA gene has shown potential as a useful tool, as multiple studies have demonstrated that it is more specific and capable of displaying significant variation [10,18,21,41]. However, other studies suggest that using multiple genes enhances genotype identification and characterization [19,20,48]. Therefore, Longer sequences from multiple genes would be required to robustly identify and confirm Bartonella species.

Conclusion

This study highlighted the prevalence of Bartonella species and the genetic characterization of Bartonella genotypes in rodents and ectoparasites from the Kilwa district using the gltA gene. The findings suggest that M. natalensis, G. dolichurus, and R. rattus serve as significant reservoir hosts for B. mastomydis, with Laelaps species and X. cheopis acting as important vectors facilitating the transmission of Bartonella infections to both animals and humans. These results suggest that further epidemiological studies need to be done to determine whether the identified Bartonella species is a zoonotic species and if it could be responsible for animal and human cases of febrile illness in an area and also employ other genes to identify and characterize Bartonella species.

Abbreviation

ACE: African Center of Excellence; BLAST: Basic Local Alignment Search Tool; bp: base pairs; BTD: Biosensor Technology Development; DNA: Deoxyribose-Nucleic Acid; DPX: Dibutyl phthalate polystyrene-xylene; gltA: citrate synthase; IRPM: Innovative Rodent Pest Management; KOH: Potassium Hydroxide; MEGA: Molecular evolution genetics analysis; NBS: National Bureau of Statistics; NCBI: National Center for Biotechnology Information; PCR: polymerase chain reaction; QGIS: Quantum Geographic Information System; Rpm: revolution per minute; Spp: Species; SPSS: Statistical Package for the Social Sciences; SUA: Sokoine University of Agriculture and µl: Microlitre.

Declaration

Acknowledgment

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Availability of data and materials

Data will be available by emailing wayapilato@gmail.com

Authors' contributions

Pilato Waya (PW), the primary investigator, contributed to the conception, data collection, analysis, interpretation, and drafting of the manuscript. Martin John (MJ) and Christopher Sabuni (CS) supervised the field, and laboratory work and contributed to the critical review, editing, and rewriting of the manuscript. Elisa Mwega supervised laboratory work and contributed to the critical review, editing, and rewriting of the manuscript. All authors have read and approved the final version of the manuscript for publication.

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. The study adhered to the necessary research clearance and ethical protocols to protect participant rights and ensure compliance with regulations. Research clearance was granted by SUA, with reference number DPRTC/R/186/26.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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References

1. Gutiérrez R, Shalit T, Markus B, Yuan C, Nachum-Biala Y, Elad D, et al. Bartonella kosoyi sp. nov. and Bartonella krasnovii sp. nov., two novel species closely related to the zoonotic Bartonella elizabethae, isolated from black rats and wild desert rodent-fleas. Int J Syst Evol Microbiol. 2020 Mar;70(3):1656–65. doi:10.1099/ijsem.0.003952.
2. Minnick MF, Anderson BE. Bartonella. In: Tang Y-W, Sussman M, Liu D, Poxton I, Schwartzman J, editors. Molecular Medical Microbiology [Internet]. 2nd ed. Elsevier; 2015. p. 1911–39. Available from: https://linkinghub.elsevier.com/retrieve/pii/B978012397169200105 0
3. Alarcón GS, Alarcón RD. The Bartonellas and Peruvian Medicine: The Work of Alberto Leonardo Barton [Internet]. New Brunswick, NJ: Rutgers University Press; 2019. doi:10.36019/9780813596174.
4. Favacho ARDM, Andrade MN, De Oliveira RC, Bonvicino CR, D'Andrea PS, De Lemos ERS. Zoonotic Bartonella species in wild rodents in the state of Mato Grosso do Sul, Brazil. Microbes Infect. 2015 Nov–Dec;17(11–12):889–92. doi:10.1016/j.micinf.2015.08.001.
5. Tahmasebi Ashtiani Z, Ahmadinezhad M, Bagheri Amiri F, Esmaeili S. Geographical distribution of Bartonella spp in the countries of the WHO Eastern Mediterranean Region (WHO-EMRO). J Infect Public Health. 2024 Apr;17(4):612–8. doi:10.1016/j.jiph.2024.01.007.
6. Kessy ST, Rija AA. Knowledge and practices related to plague persistence in plague-endemic foci, Mbulu District, Tanzania. PLoS Negl Trop Dis. 2024 May;18(5):e0012202. doi:10.1371/journal.pntd.0012202.
7. 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. Parasites Vectors. 2017 Mar 23;10(1):158. doi:10.1186/s13071-017-2094-8.
8. Thomas C, Msoffe V, Van Houtte N, Mhamphi G, Mariën J, Sabuni C, et al. Molecular detection of Leptospira and Bartonella in Mastomys natalensis and its ectoparasites in Morogoro, Tanzania. Mammalia. 2023;87(5):539–44. doi:10.1515/mammalia-2023-0031.
9. Abreu-Yanes E, Abreu-Acosta N, Izquierdo-Rodriguez E, Martin-Carrillo N, Foronda P. Bartonella species and haplotypes in rodents and their fleas in Lanzarote and El Hierro in the Canary Islands, Spain. J Vector Ecol. 2020 Dec;45(2):254–61. doi:10.1111/jvec.12396.
10. Kamani J, Morick D, Mumcuoglu KY, Harrus S. Prevalence and diversity of Bartonella species in commensal rodents and ectoparasites from Nigeria, West Africa. PLoS Negl Trop Dis. 2013 May;7(5):e2246. doi:10.1371/journal.pntd.0002246.
11. Billeter SA, Levy MG, Chomel BB, Breitschwerdt EB. Vector transmission of Bartonella species with emphasis on the potential for tick transmission. Med Vet Entomol. 2008 Mar;22(1):1–15. doi:10.1111/j.1365-2915.2008.00713.x.
12. Kosoy M, Mckee C, Albayrak L, Fofanov Y. Genotyping of Bartonella bacteria and their animal hosts: current status and perspectives. Parasitology. 2018 Apr;145(5):543–62. doi:10.1017/S0031182017001263.
13. Reif KE. Bartonellaceae. In: McVey DS, Kennedy M, Chengappa MM, Wilkes R, editors. Veterinary Microbiology [Internet]. 1st ed. Wiley; 2022. p. 392–404. doi:10.1002/9781119650836.ch42.
14. McCormick DW, Rassoulian-Barrett SL, Hoogestraat DR, Salipante SJ, SenGupta D, Dietrich EA, et al. Bartonella spp. infections identified by molecular methods, United States. Emerg Infect Dis. 2023 Mar;29(3):467–76. doi:10.3201/eid2903.221223.
15. Wang Y, Li R, Yin T, He Z, Lu Z, Shao Z, et al. Prevalence of tick infection with Bartonella in China: a review and meta-analysis. Acta Parasitol. 2024; doi:10.1007/s11686-024-00893-0.
16. Marciano O, Gutiérrez R, Morick D, King R, Nachum-Biala Y, Baneth G, et al. Detection of Bartonella spp. in wild carnivores, hyraxes, hedgehog and rodents from Israel. Parasitology. 2016 Sep;143(10):1232–42. doi: 10.1017/S0031182016000603.
17. Gundi VAKB, Kosoy MY, Makundi RH, Laudisoit A. Identification of diverse Bartonella genotypes among small mammals from Democratic Republic of Congo and Tanzania. Am J Trop Med Hyg. 2012 Aug;87(2):319–26. doi: 10.4269/ajtmh.2012.12-0099.
18. Mhlanga TA, Chitanga S, Matthee S, Malatji MP, Mukaratirwa S. Prevalence and genetic diversity of Bartonella spp. in wild small mammals from South Africa. Appl Environ Microbiol. 2024 Apr 15;90(8):e00842-24. doi: 10.1128/aem.00842-24.
19. Halliday JEB, Knobel DL, Agwanda B, Bai Y, Breiman RF, Cleaveland S, et al. Prevalence and diversity of small mammal-associated Bartonella species in rural and urban Kenya. PLoS Negl Trop Dis. 2015 Mar 17;9(3):e0003608. doi: 10.1371/journal.pntd.0003608.
20. Theonest NO, Carter RW, Amani N, Doherty SL, Hugho E, Keyyu JD, et al. Molecular detection and genetic characterization of Bartonella species from rodents and their associated ectoparasites from northern Tanzania. PLoS One. 2019 Oct 15;14(10):e0223667. doi: 10.1371/journal.pone.0223667.
21. Mhamphi GG, Msoffe VT, Lyimo CM, Katakweba AS, Massawe AW, Komba EVG, et al. Detection and characterization of zoonotic Bartonella spp. in rodents and shrews ectoparasites from Kigoma and Morogoro regions, Tanzania. Mammalia. 2024;88(1):41–51. doi: 10.1515/mammalia-2023-0072.
22. Breitschwerdt EB, Maggi RG, Chomel BB, Lappin MR. Bartonellosis: an emerging infectious disease of zoonotic importance to animals and human beings. J Vet Emerg Crit Care (San Antonio). 2010 Feb;20(1):8–30. doi: 10.1111/j.1476-4431.2009.00496.x.
23. Iralu J, Bai Y, Crook L, Tempest B, Simpson G, McKenzie T, et al. Rodent-associated Bartonella febrile illness, southwestern United States. Emerg Infect Dis. 2006 Jul;12(7):1081–6. doi: 10.3201/eid1207.040397.
24. Kosoy M, Bai Y, Sheff K, Morway C, Baggett H, Maloney SA, et al. Identification of Bartonella infections in febrile human patients from Thailand and their potential animal reservoirs. Am J Trop Med Hyg. 2010 Jun;82(6):1140–5. doi: 10.4269/ajtmh.2010.09-0581.

25. Cheslock MA, Embers ME. Human bartonellosis: an underappreciated public health problem? Trop Med Infect Dis. 2019 Jun 6;4(2):69. doi: 10.3390/tropicalmed4020069.
26. Mhamphi GG, Abdul SK, Apia WM, Rhodes HM, Robert SM, Erick VGK, et al. Prevalence of Bartonella spp. in rodent and shrew species trapped in Kigoma and Morogoro Regions, Tanzania: a public health concern. Afr J Microbiol Res. 2023;17(7):156–63. doi: 10.5897/AJMR2023.9835.
27. Crump JA, Morrissey AB, Nicholson WL, Massung RF, Stoddard RA, Galloway RL, et al. Etiology of severe non-malaria febrile illness in northern Tanzania: a prospective cohort study. PLoS Negl Trop Dis. 2013 Jul 25;7(7):e2324. doi: 10.1371/journal.pntd.0002324.
28. Misana SB, Tilumanywa VT. An assessment of the vulnerability and response of coastal communities to climate change impact in Lindi Region, southern Tanzania. In: Yanda PZ, Bryceson I, Mwevura H, Mung'ong'o CG, editors. Climate Change and Coastal Resources in Tanzania. Cham: Springer International Publishing; 2019. p. 117–53. doi: 10.1007/978-3-030-04897-6_7.
29. Lokina R, Tibanywana J, Ndanshau M. Environmental implication of sesame production in Tanzania: a case study of Kilwa District, Lindi Region. Tanzan Econ Rev. 2020;10(1):73–90.
30. Mulungu LS, Makundi RH, Massawe AW, Machang'u RS, Mbije NE. Diversity and distribution of rodent and shrew species associated with variations in altitude on Mount Kilimanjaro, Tanzania. Mammalia. 2008;72(3):178–85. doi: 10.1515/MAMM.2008.021.
31. Gebrezgiher GB, Makundi RH, Katakweba AAS, Belmain SR, Lyimo CM, Meheretu Y. Arthropod ectoparasites of two rodent species occurring in varied elevations on Tanzania's second highest mountain. Biology (Basel). 2023 Mar 2;12(3):394. doi: 10.3390/biology12030394.
32. Happold DCD, editor. Mammals of Africa. Vol. 3. London: Bloomsbury Publishing; 2013. p. 27–691.
33. Morick D, Baneth G, Avidor B, Kosoy MY, Mumcuoglu KY, Mintz D, et al. Detection of Bartonella spp. in wild rodents in Israel using HRM real-time PCR. Vet Microbiol. 2009 Oct 20;139(3–4):293–7. doi: 10.1016/j.vetmic.2009.06.014.
34. Herbreteau V, Jittapalapong S, Rerkamnuaychoke W, Chaval Y, Cosson JF, Morand S. Protocols for field and laboratory rodent studies. 2011:5–46.
35. Babyesiza WS, Mpagi J, Ssuuna J, Akoth S, Katakweba A. Ectoparasite fauna of rodents and shrews with their spatial, temporal, and dispersal along a degradation gradient in Mabira Central Forest Reserve. J Parasitol Res. 2023;2023:7074041. doi: 10.1155/2023/7074041.
36. Mathison BA, Pritt BS. Laboratory identification of arthropod ectoparasites. Clin Microbiol Rev. 2014;27(1):48–67. doi: 10.1128/CMR.00008-13.
37. Diarra AZ, Kone AK, Doumbo Niare S, Laroche M, Diatta G, Atteynine SA, et al. Molecular detection of microorganisms associated with small mammals and their ectoparasites in Mali. Am J Trop Med Hyg. 2020;103(6):2542–51. doi: 10.4269/ajtmh.19-0727.
38. Tamura K, Stecher G, Kumar S. MEGA11: Molecular Evolutionary Genetics Analysis version 11. Mol Biol Evol. 2021;38(7):3022–7. doi: 10.1093/molbev/msab120.
39. Ko S, Kim HC, Yang YC, Chong ST, Richards AL, Sames WJ, et al. Detection of Rickettsia felis and Rickettsia typhi and seasonal prevalence of fleas collected from small mammals at Gyeonggi Province in the Republic of Korea. Vector Borne Zoonotic Dis. 2011;11(9):1243–51. doi: 10.1089/vbz.2010.0261.
40. Lipatova I, Paulauskas A, Puraite I, Radzijevskaja J, Balciauskas L, Gedminas V. Bartonella infection in small mammals and their ectoparasites in Lithuania. Microbes Infect. 2015;17(11–12):884–8. doi: 10.1016/j.micinf.2015.06.010.
41. Billeter SA, Borchert JN, Atiku LA, Mpanga JT, Gage KL, Kosoy MY. Bartonella species in invasive rats and indigenous rodents from Uganda. Vector Borne Zoonotic Dis. 2014;14(3):182–8. doi: 10.1089/vbz.2013.1375.
42. Meheretu Y, Leirs H, Welegerima K, Breno M, Tomas Z, Kidane D, et al. Bartonella prevalence and genetic diversity in small mammals from Ethiopia. Vector Borne Zoonotic Dis. 2013;13(3):164–75. doi: 10.1089/vbz.2012.1004.
43. Jian R, Ren Q, Xue J, Xie GC, Wang J, Chen GQ, et al. Genetic diversity of Bartonella infection in residential and field rodents in Hebei, China. Front Microbiol. 2022;13:1039665. doi: 10.3389/fmicb.2022.1039665.
44. Yao XY, Liu H, Sun J, Zhang YQ, Lv ZH, Zhang XL, et al. Epidemiology and genetic diversity of Bartonella in rodents in urban areas of Guangzhou, Southern China. Front Microbiol. 2022;13:942587. doi: 10.3389/fmicb.2022.942587.
45. Mariën J, Vanden Broecke B, Tafompa PJJ, Bernaerts L, Ribas A, Mnyone LL, et al. Host related factors determine co-occurrence patterns between pathogenic bacteria, protozoa, and helminths in populations of the multimammate mouse, Mastomys natalensis. bioRxiv. 2022. doi: 10.1101/2022.01.14.476303.
46. Klangthong K, Promsthaporn S, Leepitakrat S, Schuster AL, McCardle PW, Kosoy M, et al. The distribution and diversity of Bartonella species in rodents and their ectoparasites across Thailand. PLoS One. 2015;10(10):e0140856. doi: 10.1371/journal.pone.0140856.
47. Brettschneider H, Bennett NC, Chimimba CT, Bastos ADS. Bartonellae of the Namaqua rock mouse, Micaelamys namaquensis (Rodentia: Muridae) from South Africa. Vet Microbiol. 2012;157(1–2):132–6. doi: 10.1016/j.vetmic.2011.12.032.
48. Martin-Alonso A, Houemenou G, Abreu-Yanes E, Valladares B, Feliu C, Foronda P. Bartonella spp. in small mammals, Benin. Vector Borne Zoonotic Dis. 2016;16(4):229–37. doi: 10.1089/vbz.2015.1838.