Original Study

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Detection and characterization of zoonotic *Bartonella* spp. in rodents and shrews ectoparasites from Kigoma and Morogoro regions, Tanzania

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Abstract: Bartonellosis is a vector-borne disease which is increasingly threatening the health of humans and animals worldwide consequent to the growing wildlife-animals-human interactions. Little is known about the epidemiology of this disease in Tanzania. In this study we investigated and characterized *Bartonella* species in small mammals' ectoparasites from potentially high-risk areas in the country. A total of 141 ectoparasites pools of mites, fleas, ticks, and lice were analyzed using conventional PCR and sequencing. *Bartonella* DNA was detected in 34.8 % of the tested ectoparasite pools, with mites at 32.9 %, fleas at 40 %, ticks at 12.5 %, and lice at 50 %. Phylogenetic analysis showed that the *Bartonella* spp. genotypes were closely related to those found in Uganda, Kenya, and South Africa. Different genotypes with independent haplotypes were observed, although most *Bartonella* spp. from fleas shared the same haplogroup. The confirmed presence of *Bartonella elizabethae* and *Bartonella tribocorum* in field and house rodents emphasizes the prevailing transmission risk of zoonotic infections in the study areas and beyond. Screening of humans, companion animals, and livestock in potentially high-risk areas in Tanzania is necessary in order to inform the development of responsive surveillance and control strategies.

Keywords: *Bartonella*; vector-borne disease; reservoirs; rural settings; habitats

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1 Introduction

Bartonellosis is an emerging vector-borne disease affecting humans and animals worldwide. The disease is caused by intracellular rod-shaped, Gram-negative *Bartonnella* species (Oteo et al. 2017; Obiegala et al. 2021). The disease pathogens are primarily transmitted by arthropod vectors particularly those associated with rodents and other small mammals including shrews and bats (Gonçalves et al. 2020), and the transmission occurs mainly through bite/superficial scratches caused by infected animals and or feces of infected ectoparasites (Cheslock and Embers 2019). Additionally, some studies suggest the possibility of vertical and transstadial transmission of *Bartonella* spp. in ticks as observed by Wechtaisong et al. (2021) as well as transplacental transmission in rodent populations by Siewert et al. (2022). In mammalian hosts, pathogenic *Bartonella* spp. infect erythrocytes and cause minor to grave manifestations including fever which is often accompanied by neurologic disorders, anemia, splenomegaly and lymphadenopathy, endocarditis, myocarditis and neuroretinitis in immunocompromised individuals (García et al. 2014; Krügel et al. 2020; Minnick et al.

2014). These and other generalized and non-specific manifestations make the clinical diagnosis of bartonellosis rather difficult. The current state of knowledge indicates that the genus *Bartonella* comprises over 30 species and subspecies out of which 13 are incriminated to contribute to blood-borne infections in humans (Álvarez-Fernández et al. 2018). Notable examples include *Bartonella quintana*, *Bartonella henselae*, *Bartonella elizabethae*, *Bartonella vinsonii* subsp. arupensis and *B. vinsonii* subsp. berkhoffi (Andric et al. 2018). These and other species are widely distributed across many habitats and are associated with diverse mammals and arthropod vectors as reported by de Sousa et al. (2018) and Diarra et al. (2020); more so, those that are inhabiting favorable macro- and micro-climatic conditions (e.g. Klangthong et al. 2015; Theonest et al. 2019).

Like in many other disease-causing pathogens, rodents and other small mammals constitute the major natural reservoirs (Ansil et al. 2021) and have been involved in the transmission of many infectious bacteria, viruses, helminths and protozoa (e.g. El Hamzaoui et al. 2020; Lappin 2018; Oguntomole et al. 2018). The life cycle and transmission of *Bartonella* spp. are completed mainly by ectoparasites including fleas, mites, ticks and lice (Regier et al. 2016). The abundance and ability to occupy diverse and variable habitats in rodents and a number of other small mammals render them as reliable sources of feed and shelter for the vector ectoparasites (Maaz et al. 2018; Mihalca and Sándor 2013). Small mammal ectoparasites are confirmed to be efficient vectors and reservoirs for a multitude of *Bartonella* spp. (Obiegala et al. 2021; Reis et al. 2011; Regier et al. 2016). Rodent-borne fleas have been reported to be naturally infected and efficient vectors of several zoonotic *Bartonella* spp. such as *Bartonella grahamii*, *Bartonella tribocorum*, *Bartonella washoensis* and *B. elizabethae* Tsai et al. (2010), (Billeter et al. 2011; Klangthong et al. 2015). Rodent-borne mites of the order Gamasida (Mesostigmata) including chigger, oribatid, and *Laelaps* species are considered potential vectors and reservoirs of zoonotic *Bartonella* spp. (Yin et al. 2021; Yu and Tesh 2014) such as *Bartonella taylorii*, *B. grahamii* and *Bartonella tamiae* as reported by Kabeya et al. (2010) and Kaminskienė et al. (2022). Similarly, ticks particularly those of the genus *Ixodes*, *Dermacentor and Haemaphysalis* are capable of naturally transmitting *Bartonella* spp. such as *Bartonella phoceensis*, *B. grahamii*, *Bartonella melophagi* and *Bartonella rattaustraliani* (Asyikha et al. 2020; Dwużnik et al. 2019; Hao et al. 2020; Panthawong et al. 2020). Importantly, some tick species are also efficient natural reservoirs of certain *Bartonella* spp. including *Bartonella birtlesii* and *B. melophagi* (Eisen 2020; Saengsawang et al. 2021). Moreover, small mammal-borne lice, although they cannot directly transmit disease pathogens to non-host species (Reeves et al. 2006), they may circulate *Bartonella* and other microorganisms from one host to the other via direct contact when the hosts are mating and/or fighting, suckling and nest-sharing (Light et al. 2010; Martinů et al. 2018). Indirect transmission is also possible when lice are attached to other bigger arthropods especially hematophagous flies that share blood meal from the same host (Durden 2019).

A handful studies conducted by Gundi et al. (2012); Theonest et al. (2019) and Mhamphi et al. (2023) have documented high prevalence of *Bartonella* spp. in different small mammal species in certain parts of Tanzania. Furthermore, the country has high abundance and wide distribution of small mammals and competent vector ectoparasites, which are increasingly coming to contact with humans as a result of several factors including, inter alia, climate change, land use change and ever-growing human/animal population mobility (Alila 2020; Gebrezgiher et al. 2022). As such, the country is threatened by the potentially growing spread and burden of rodent-borne pathogens including *Bartonella* spp. Yet, our knowledge of epidemiology, distribution as well as genetic diversity and relatedness of circulating *Bartonella* spp. in the country is limited. Besides, the diagnosis with routine classical methods, such as culture and serology, remain challenging (Okaro et al. 2017). They require special media and a long incubation period because of the fastidious nature of *Bartonella* species (Okaro et al. 2017). Different molecular assays including conventional and non-conventional PCRs have been used successfully and therefore offer desirable complementary options (Gutiérrez et al. 2017). Several housekeeping genes including the citrate synthase gene (*gltA*) and the RNA polymerase b-subunit gene (*rpoB*) have been deployed widely for detection and characterization of *Bartonella* spp. since they are relatively stable and extensive in the GenBank database according to La Scola et al. (2003). Likewise, *gltA* gene is appropriate to differentiate *Bartonella* species based on its accuracy and specificity as described by Huang et al. (2019).

In view of the aforesaid, we investigated and characterized *Bartonella* species circulating in small mammals' ectoparasites from potentially high-risk areas of Kigoma and Morogoro regions-Tanzania, through conventional PCR and sequencing. Also, the ectoparasites that could be playing a role as transmitters and natural reservoirs of *Bartonella* spp. were morphologically identified. The findings herein provide useful information and insights will inform and stimulate further studies towards understanding the risk of identified *Bartonella* species to humans as well as the disease ecology and transmission dynamics within and beyond the current study regions.

2 Materials and methods

2.1 Study area

Rodents and shrews were live trapped across the selected areas of Kibondo and Kakonko districts in Kigoma region (Figure 1) as well as Kilosa and Morogoro Rural district in Morogoro region (Figure 2). From each district, two villages were purposively selected. One village had reserved bush/forest/game reserves where traps were set from indoor, peridomestic, farm/fallow to the natural habitats (forest/bush land/game reserves). Another village had no natural habitats therefore traps were set from indoor, peridomestic and farm/fallow land habitats only. In Kigoma region, the study villages were Itumbiko (3°17′16.7″S 30°58′38.9″ E) Kihomoka (3°11′46.5″S 31°2′24.5″ E), Kumhama (3°35′16.4″S 30°40′40.6″E) and Kigendeka (3°46′24.0″S 30°41′33.2″E) villages. In Morogoro region, the study sites were Mamboya (6°18′11.5″S 37°06′23.9″ E), Magubike (6°14′52.884″S 37°9′48.084″E), Kibuko (6°57′12.4236″ S 37°50′46.98492″ E) and Mwarazi (7°0′44.94276″ S 37°48′51.27084″ E). Subsistence farming is the main economic activity throughout the study villages. Secondarily, there is also livestock keeping in some of the study villages both in Kigoma and Morogoro region.

2.2 Trapping of rodents and shrews

The trapping and handling of rodents and shrews protocol was approved by the Institutional Review Board of Sokoine University of Agriculture, and the Tanzania Wildlife Research Institute (TAWIRI). Briefly, rodents and shrews were live trapped in different habitats including indoor, peridomestic, farms and natural habitats (forests and game reserves) from both districts (Figures 1 and 2). Rodents and shrews were trapped using Live Sherman Traps (LFA 7.5 × 9 × 23 cm) and modified wire cage traps (locally made at the Institute of Pest Management) baited with 2:1 (1000 g of peanut butter mixed with 500 g of maize flour) as described by Mhamphi et al. (2023). The traps were set for three consecutive days in each study site from 2020 to 2021 during the wet and dry seasons. In each month a total of 200 traps were used in each study village for up to six months making the total capture effort of 3600.

2.3 Collection and identification of ectoparasites

Rodents and shrews were removed from the traps, and then euthanized following Sikes et al. (2016) guidelines. After death the ectoparasites were brushed off the animals using a shoe shine brush into a basin.

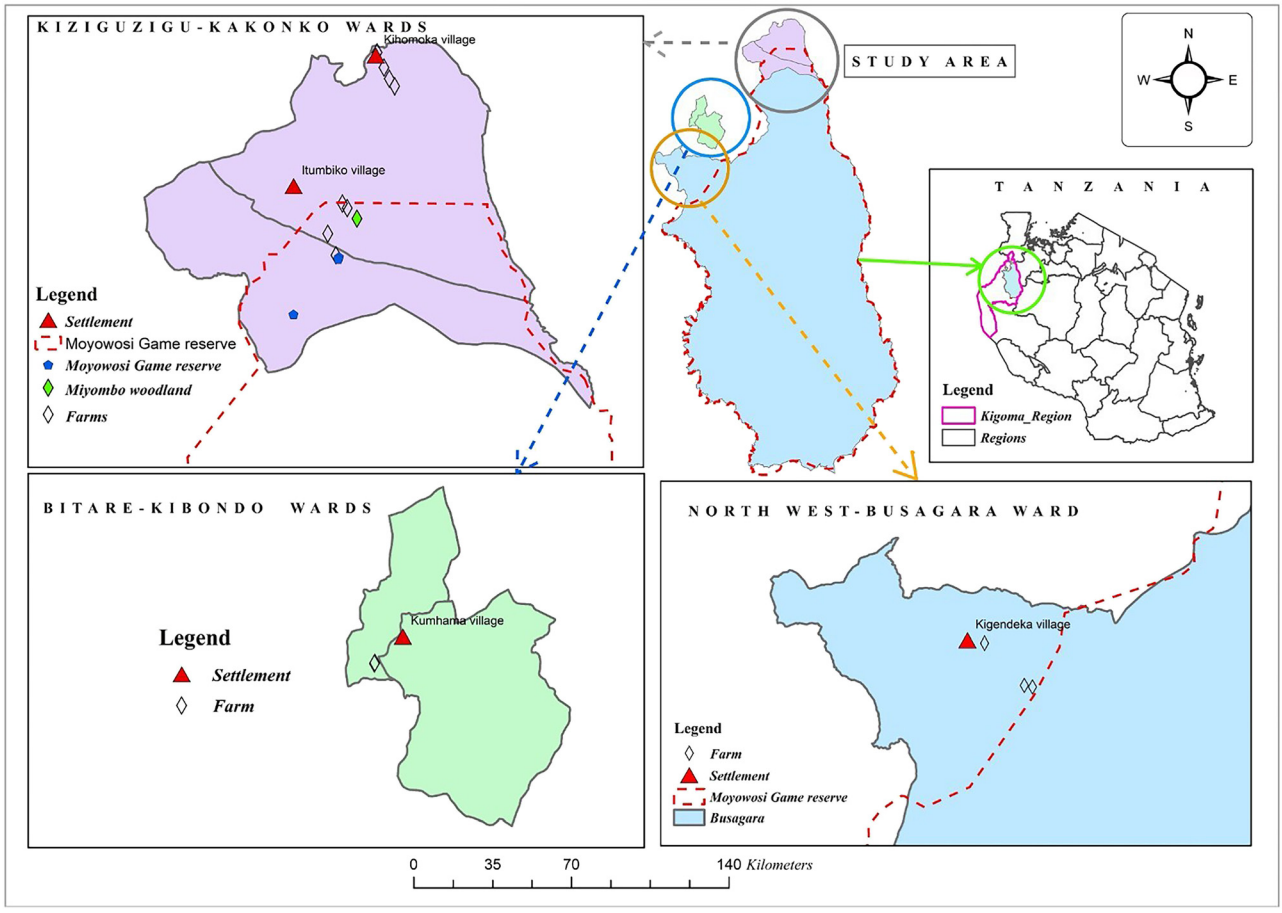

Figure 1: Map of Kakonko and Kibondo Districts in Kigoma region. Source: Authors.

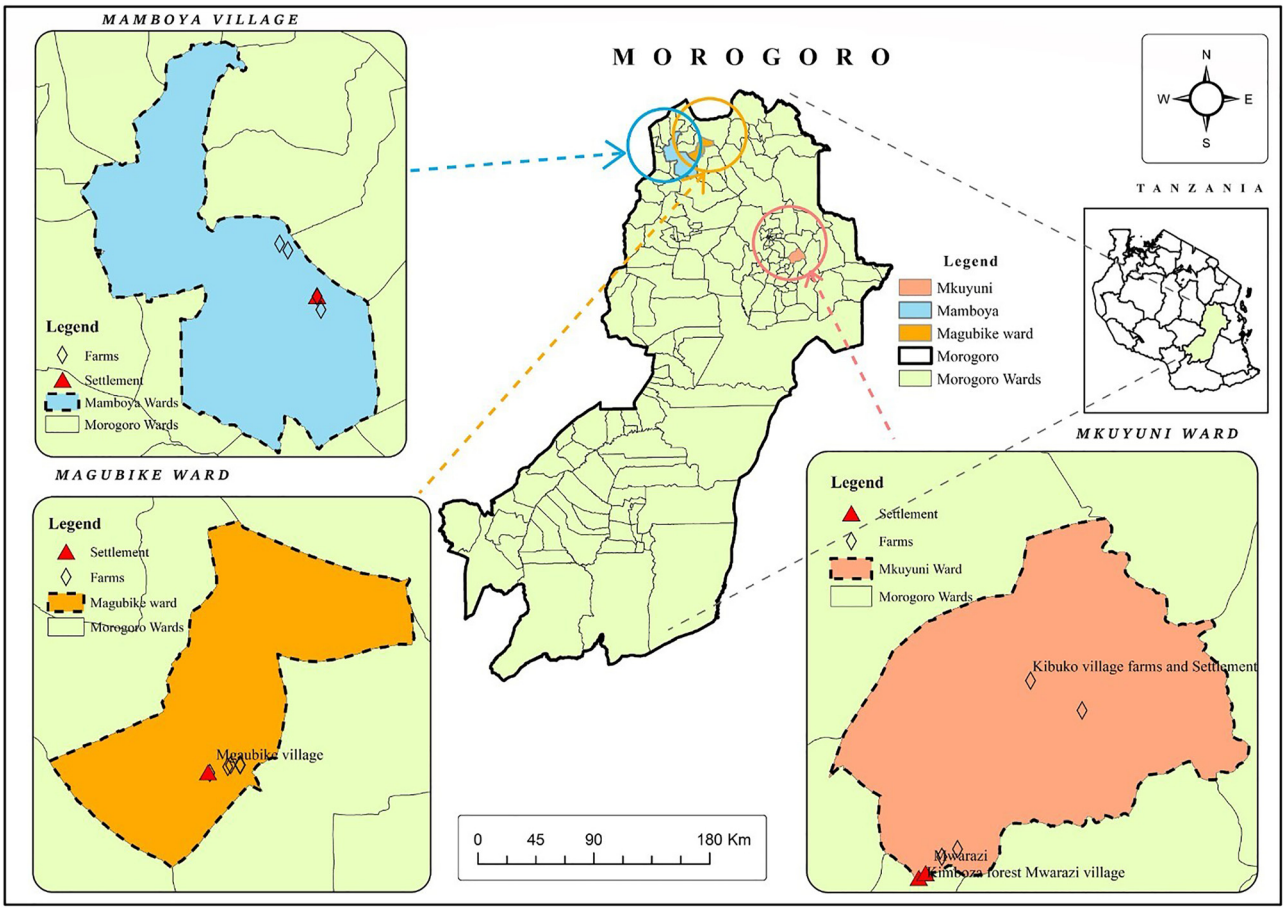

Figure 2: Map of Kilosa and Morogoro rural Districts in Morogoro region. Source: Authors.

Thereafter, the animals were morphologically identified to genus and or species level according to Happold (2013). The ectoparasites were then gently picked and stored in a separate micro vial containing 70 % ethyl ethanol according to the host they were collected from. All carcasses were preserved in 10 % formalin at the Institute of Pest Management of the Sokoine University of Agriculture. The morphological identification of the ectoparasites was done in the laboratory through a combination of various published keys and illustrations according to Pratt and Wiseman (1962); Pratt (1963), and Zumpt et al. (1966). The ectoparasites were all screened microscopically before being sorted into their genera/species. Subsamples of fleas were processed according to Paulraj et al. (2021) to confirm their genus and or species because some distinctive features in several fleas were not clear for identification to genus and/or species level. Examination of the ectoparasites was done under a bright-field digital microscope using 0.5 objective (Zeiss Primor Star Axiocam ERc 5S). The identified ectoparasites from each host of the same genus and or species were pooled (1–5 ectoparasites per pool) according to their genus or species. A total of 141 pools were obtained including 55 pools of fleas, 76 pools for mites, eight pools of ticks and two pools of lice.

2.4 DNA extraction and sequencing of ectoparasite pools for *Bartonella* spp.

Each pool of unprocessed ectoparasites was subjected to DNA extraction using Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol after washing with 70 % ethanol and followed by sterile phosphate buffered saline Gutiérrez et al. (2017). Each ectoparasites pool was crushed separately, in a sterile motor and pestle containing 200 µl of tissue lysis buffer. The subsequent procedures were done according to the manufacturer's instructions. The extracted DNA products were stored at –20 °C before the PCR test according to the method developed by (Norman et al. 1995) with some amendments. Forward primer BhCS871.p (5′-GGGGACCAGCTCATGGTGG-3′) and reverse BhCS1137.n (5′AATGCAAAAAGAACAGTAAACA-3′) targeting a 379 bp of the genus-specific citrate synthase (*gltA*) gene were used. Three microliters of DNA templates were added into the PCR reaction tube containing 12.5 µl of 2× master mix, 0.5 µl of 10 µM of each primer, and 8.5 µl of nuclease-free water. Amplification was done by a thermal cycler by the following parameters: an initial denaturing at 95 °C for 5 min, and 35 cycles of denaturation at 95 °C for 1 min, annealing at 56 °C for 1 min and elongation at 72 °C for 1 min. Amplification was finalized by holding the reaction mixture at 72 °C for 10 min. The amplified products were confirmed for the proper size of the amplicon using electrophoresis in 1.5 % agarose gel. Then after, sequenced for the partial *gltA* gene by using the same primers used to generate the PCR products. The sequencing reactions were performed in the DNA Master cycler pro-384 (Eppendorf) using BigDye(R) Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems Forster City, CA) per the manufacturer`s protocol. The fluorescent-labeled fragments were purified from the unincorporated terminators with the BigDyeXTerminatorsR Purification Kit (Applied Biosystems, Foster City, CA). The samples were injected to

electrophoresis in an ABI 3730xl DNA Analyser (Applied Biosystems, Foster City, CA).

2.5 Statistical analysis

The number of ectoparasite pools of each genus or species collected from different hosts and habitats in both regions was summarized and calculated as percentage proportions of the positive pools using Microsoft Excel. To evaluate the associations between *Bartonella* DNA positivity and various variables including habitats, locality, host species, and ectoparasites, a binomial generalized linear model (GLM) in R software version 4.2.1 was employed.

2.6 Phylogenetic analysis

The obtained sequences were manually edited using BioEdit Sequence Alignment Editor Software suite, version 7.2.6.1 to determine their consensus for the amplified region of *gltA* genes. The ClustalW program of the MEGA 11 software was used to align the obtained sequences before submission to Basic Local Alignment Search Tool (BLAST) for species identification using the National Center for Biotechnology Information (NCBI) database in the GenBank. Thereafter, aligned together with the selected DNA sequences with the highest similarity from the GenBank using ClustalW for multiple sequence alignment. The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (1993). According to the Akaike Information Criterion (AICc), the best substitution model was T92+G with an AICc value of 3780.747. Phylogenetic analyses were conducted in MEGA 11 with the robustness of 1000 bootstraps (Tamura et al. 2021).

3 Results

3.1 Collected small mammals and ectoparasites

Out of 1147 rodents and shrews trapped, 616 (53.7 %) were captured in Kigoma and 531 (46.3 %) in Morogoro. A total of 341 (29.7 %) rodents and shrews were infested with ectoparasites including fleas, mites, ticks, and lice (Table 1). In Kigoma, the infested rodents were 211 (62.4 %) of the total infested small mammals, no shrews were found infested from Kigoma. In Morogoro, 127 (37.2 %) rodents and 3 (0.9 %) shrews were infested with ectoparasites. The identified ectoparasites from rodent species including *Mastomys natalensis*, *Aethomys* spp., *Lemniscomys rosalia*, *Lemniscomys striatus*, *Praomys* spp., *Rattus rattus*, *Arvicanthis* spp., *Lophuromys* spp., *Gerbilliscus* spp., and shrews of the genus *Crocidura*, consisted of five species of fleas; *Dinopsyllus* cf. *lypusus*, *Ctenophthalmus* cf. *calceatus*, *Xenopsylla* cf. *brasiliensis*, *Xenopsylla* cf. *cheopis* and *Nosopsylla* cf. *fasciatus* (Figure 3) as well as *Haemophisalis* ticks and *Haplopleura* lice.

3.2 Detection of *Bartonella* DNA in ectoparasite pools

Bartonella DNA was amplified in 49 pools (34.8 %) out of the 141 pools of all ectoparasites. For individual types of ectoparasite pools, *Bartonella* DNA was detected in 25 (32.9 %) out of 76 pools of mite, 22 (40 %) out of 55 pools of flea species, 1 (12.5 %) out of eight pools of ticks and 1 (50 %) out of two pools of lice (Table 2). Among the habitat variables, the farm habitat detected 39.6 % slightly higher than 33.3 % of the indoor habitat. The natural habitat detected 22.2 % and the peridomestic habitat detected 7.1 %. Statistically, there were significant differences in the prevalence of *Bartonella* DNA among two variables, namely habitats (χ^2 = 8.589, df = 3, p = 0.035) and ectoparasites species (χ^2 = 16.5646, df = 7, p = 0.0243).

3.3 Phylogenetic analysis of *gltA* sequences

Out of 49 *Bartonella* DNA confirmed by sequencing, 22 sequences were recovered, however, only 16 consensus sequences were successfully assembled. After being submitted to BLAST in the NCBI database, seven sequences; five from mites' pools and two from fleas' pools showed low similarities (75–85 %) when compared to other *Bartonella* spp. in the GenBank database. Therefore, only nine DNA sequences

Table 1: Total ectoparasite types collected from Kigoma and Morogoro regions.

Regions	Fleas					Mites	Ticks	Lice	Total
	Dinopsyllus cf. *lypusus*	*Ctenophthalmus* cf. *calceatus*	*Xenopsylla* cf. *brasiliensis*	*Xenopsylla* cf. *cheopis*	*Nosopsyllus* cf. *fasciatus*	*Laelaps* spp.	*Haemaphysalis* spp.	*Hoplopleura* spp.	
Kigoma	55	60	5	3	3	126	2	2	259
Morogoro	0	0	8	18	0	198	8	6	238
Total ectoparasites	55	60	13	21	3	324	10	8	497

Source: Authors.

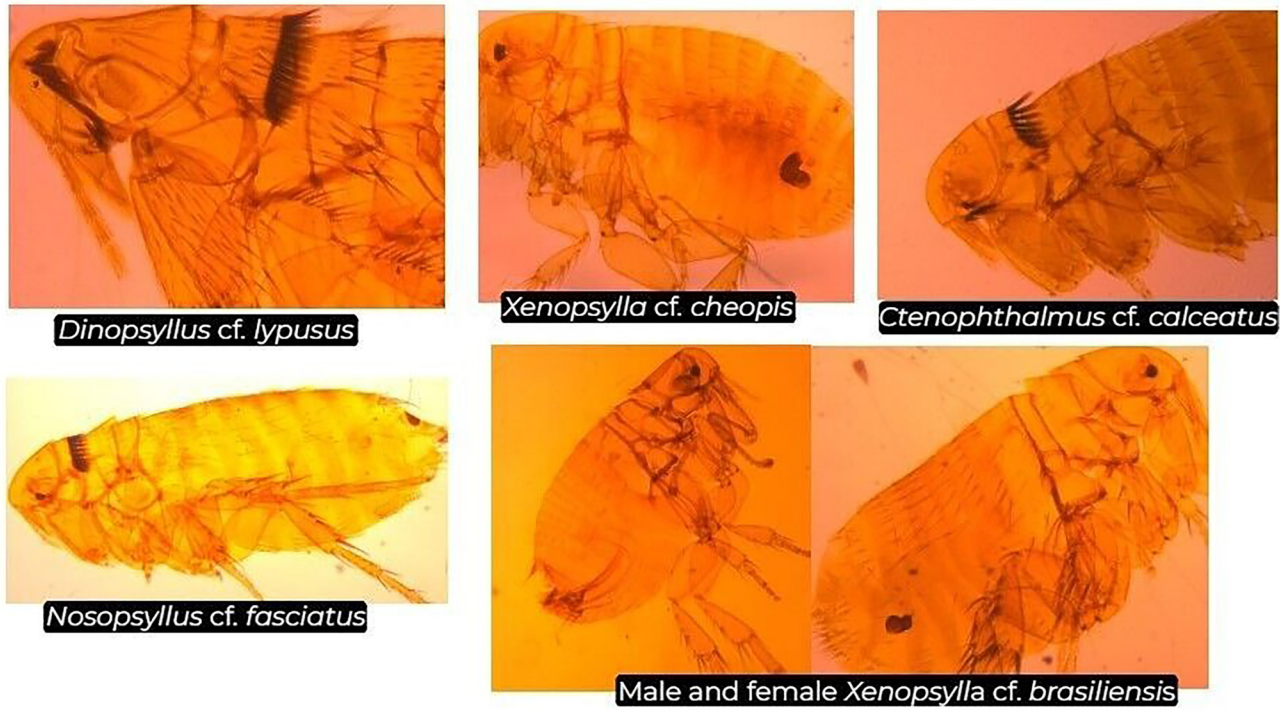

Figure 3: Fleas' species identified from rodents, as photographed using a Zeiss Primor Star Axiocam ERc 5S microscope. Source: Authors.

Table 2: Number of ectoparasites tested pools in brackets () and percentage of *Bartonella* DNA positive pools as determined by PCR targeting a fragment of *gltA* gene.

Locality	Ectoparasites								
	Mite species	Fleas species					Tick sp.	Lice sp.	Total
	Laelaps	*Dino*	*Noso*	*Xch*	*Xbr*	*Ctenoph*	*Haema*	*Hoplo*	
Kigoma	29.2 % (39)	33.3 % (27)	33.3 % (3)	0 (1)	100 % (2)	83.3 % (12)	0 (6)	–	36.7 % (90)
Morogoro	37.8 % (37)	–	–	0 (7)	0 (1)	0 (2)	50 % (2)	50 % (2)	29.4 % (51)
Overall	32.9 % (76)	33.3 % (27)	33.3 (3)	0 (0/8)	66.7 % (3)	71.4 % (14)	12.5 % (8)	50 % (2)	34.8 % (141)

Dino, *Dinopsyllus* cf. *lypusus*; *Noso*, *Nosopsyllus* cf. *fasciatus; Xch*, *Xenopsylla* cf. *cheopis*; *Xbr*, *Xenopsylla* cf. *brasiliensis; Ctenoph*, *Ctenophthalmus* cf. *calceatus; Haema*, *Haemaphisalis; Hoplo*, *Hoplopleura.* Source: Authors.

were submitted and accepted to the GenBank database (NCBI) with accession numbers (OQ382888-OQ382892 and OQ504174-OQ504177). The phylogenetic tree of the *Bartonella* sequences and the previous identified *Bartonella* genotypes retrieved from the NCBI GeneBank were used to evaluate the evolutionary relationship (Figure 4). Based on the maximum likelihood phylogeny and reference sequences of the *gltA Bartonella* gene obtained from the GenBank database; phylogenetic analysis identified one *B. elizabethae* (accession number OQ504174) from *Xenopsylla brasiliensis* with 98.71 % similar to previously identified *B. elizabethae* from Thailand with accession number JX158353.1. *Bartonella* sp., with accession number OQ504177 in this study, is phylogenetically related to *B. tribocorum* with accession number OP382454.1 previously identified from China. Five genotypes with accession numbers OQ382891; OQ382888; OQ382889; OQ382890 and OQ382892 from flea species (*Dinopsylla lypusus*, and *Ctenophthalmus calceatus*) were closely related to Ugandan and Kenyan *Bartonella* spp. with accession numbers JX428746.1, MF443361.1, and KM233491.1 with more than 99 % identity from blasting. Phylogenetically, Ugandan and Kenyan *Bartonella genotypes* were closely related to the South African *Bartonella* genotypes with GeneBank accession number AJ583114.1 as described in Figure 4. *Bartonella* genotype from lice (OQ504175) phylogenetically is related to JX428760.1 from Uganda and is closely related to genotype FJ686050.1 from Israel with 98.89 % similarity to *Bartonella elizabathae* with accession number LR134527.1 from the

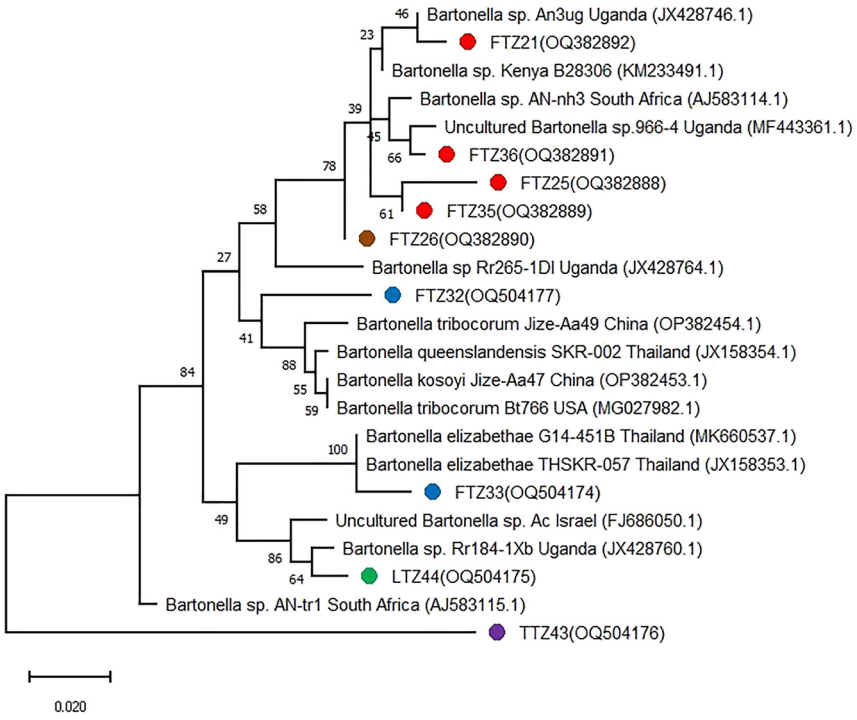

Figure 4: Phylogenetic tree showing the relatedness of the Bartonella gltA gene sequences detected from rodents and shrews ectoparasites; fleas (FTZ), lice (LTZ), and ticks (TTZ) along with reference sequences from the GenBank database. The phylogenetic tree was constructed using the maximum likelihood method. The tree with the highest log likelihood (−1773.15) is shown. Evolutionary analyses were conducted in MEGA 11 (2021). The detected Bartonella genotypes and their sources in this study are indicated by nodes of different colors.

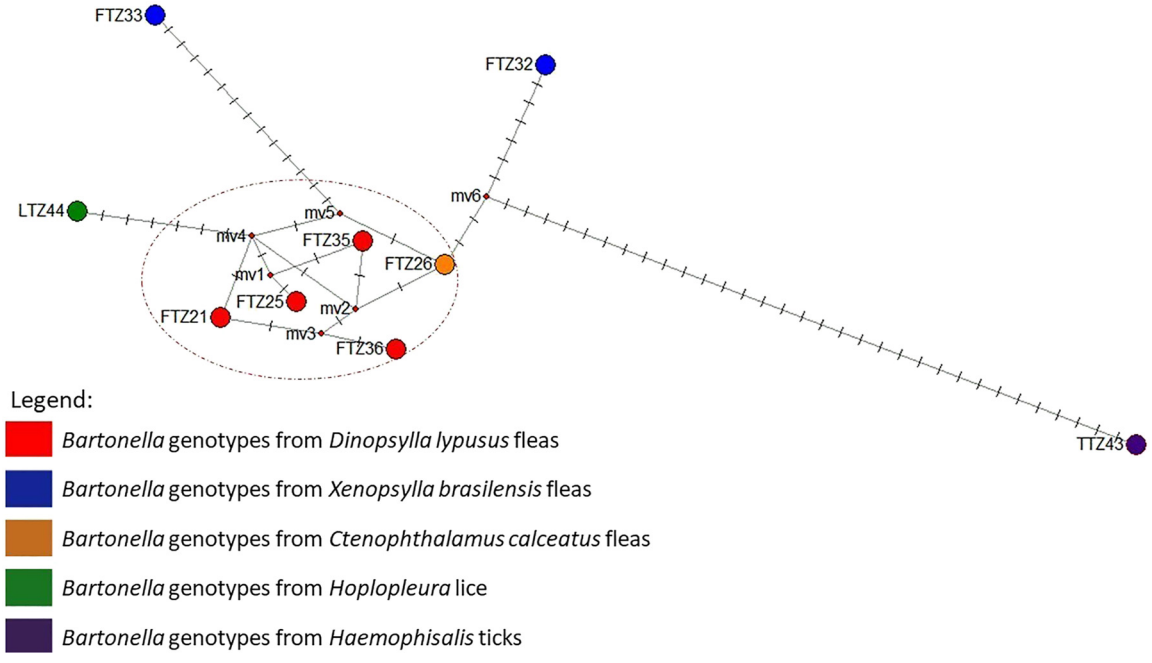

Figure 5: Median-joining network showing the evolutionary relationships and likely ancestral networks among *Bartonella* haplotypes based on the 379-bp sequence of *gltA* gene from fleas (FTZ), ticks (TTZ), and lice (LTZ) hosts.

GenBank database. *Bartonella* genotype (OQ504176) from *Haemophisalis* tick detected in this study showed 98.94 % similarity to *Bartonella* strain with accession number AJ583115.1 from South Africa. Furthermore, the Median-joining network (Figure 5) showed five clades, among them four clades form different clusters, with different lineages and unique haplotypes. Five genotypes related to *Bartonella* spp. discovered in Ugandan, Kenyan and South African *Bartonella* spp. formed one haplogroup/clade, different lineages with independent haplotypes.

4 Discussion

In this study, *Bartonella* DNA was found in the four primarily ectoparasites (mites, fleas, ticks and lice) of rodents including *M. natalensis*, *Aethomys* spp., *L. rosalia*, *L. striatus*, *Praomys* spp., *Rattus rattus*, *Arvicanthis* spp., *Lophuromys* spp., and shrews of the genus *Crocidura*, collected from different habitats in Kigoma and Morogoro regions in Tanzania. The detection of *Bartonella* DNA from various habitats signifies a high prevalence of *Bartonella* species genotypes in rodent and shrew ectoparasites in the study areas. This implies that the *Bartonella* genus is more prevalent in small mammal's ectoparasites from different habitats. This could be contributed by high abundance of ectoparasites and their hosts that lead to increased interactions in the sampled habitats, resulting in the transmission of the pathogens (Voordouw 2015). Furthermore, vertical transmission between adult ectoparasites and the larvae helps to maintain the bacteria to circulate within the vector population (Brinkerhoff et al. 2010; Szubert-Kruszyńska et al. 2019).

The phylogenetic analysis detected diverse genotypes that clustered into five different clades, phylogenetically, closely related to *Bartonella* spp. from Uganda (Bai et al. 2017), Kenya (Halliday et al. 2015), and South Africa (Pretorius et al. 2004). Among them, two genotypes are identical to zoonotic *Bartonella* species; *B. elizabethae* and *B. tribocorum* discovered in *X. brasiliensis* from *Rattus rattus* and *L. striatus* respectively. The sparse genotypes detected in this study could be explained by the fact that the *Bartonella* genus has divergent evolution and high genetic variety due to distinct genetic characteristics (Han et al. 2022). This is also further supported by the haplotype analysis which showed different lineages with unique haplotypes in each clade. However, four unique genotypes from *Dinopsyllus lypusus* and *Ctenophthalmus caelcatus* formed one haplogroup. This indicate that fleas of the genus *Dinopsyllus* and *Ctenophthalmus* shared related *Bartonella* genotypes. This study also, reports for the first time unique *Bartonella* genotypes found in lice and ticks from Morogoro region. Moreover, *Bartonella* genotypes detected in mites from Morogoro and two pools of fleas from Kigoma showed low sequences similarities (75–85 %) below the cutoff point suggested by La Scola et al. (2003) when compared with other *Bartonella* spp. in the GenBank database. Such low similarities could be due to the fact that the *Bartonella* genus has high genetic diversity with distinct species and strains, therefore, the sequences available in the GeneBank may only represent a limited subset of the overall *Bartonella* diversity (Gutiérrez et al. 2017). Also, the sequences obtained from rodent ectoparasites may represent evolutionary divergence and specific regional variants that are not well-represented in the GeneBank (Li et al. 2015). Therefore, these genotypes could be new species probably or need to be further confirmed by whole genome sequencing. The zoonotic *Bartonella* spp. detected in this study have been isolated from febrile patients in Thailand by Kosoy et al. (2010), and also reported from ectoparasites in Tanzania by Theonest et al. (2019); the Democratic Republic of Congo by Gundi et al. (2012); Nigeria by Kamani et al. (2013) and Slovakia by Špitalská et al. (2022).

Bartonella DNA has been reported in different rodent and shrew ectoparasites worldwide. In Tanzania, this is the first study to report *Bartonella* DNA in mites (*Laelaps* spp.), ticks (*Haemophisalis* spp.) and lice (*Hoplopleura* spp). The high percentage of *Bartonella* DNA detected in rodents and shrews ectoparasites is comparable to other studies conducted elsewhere; for instance, Theonest et al. (2019), detected 27.5 % of *Bartonella* DNA in *Xenopsylla cheopis* from Tanzania, Kamani et al. (2013), reported an overall of 28 % of rodent ectoparasites pools collected in rodents from Nigeria, Loan et al. (2015), reported 25 % *Bartonella* prevalence in chigger mites from China, Klangthong et al. (2015), detected 1.7–57.1 % from different ectoparasites in Thailand, and Dwużnik et al. (2019), reported prevalence of 11–57 % in tick nymphs collected from rodents in Poland. The occurrence of *Bartonella* DNA and the detected zoonotic *Bartonella* species from fleas and other ectoparasites within the human habitation has an implication on public health importance on the transmission of bartonellosis to humans and domestic animals.

5 Conclusions

This study confirmed the presence of potentially zoonotic *Bartonella* spp., *B. elizabethae* and *B. tribocorum*, in ectoparasites collected from field and domestic rodents trapped from various habitats. These findings emphasize their potential role in the transmission of bartonellosis in the study areas and beyond. Consequently, call for strategic and wider screening of humans, companion animals and livestock particularly across the human-animal-wildlife interfaces since the reservoirs and vectors are widely spread. Also, regular control of ectoparasites on companion and livestock animals is recommended.

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