Journal of Applied Veterinary Science and Technology
http://e-journal.unair.ac.id/javest

OPEN ACCESS

Research Reports

Molecular Detection and Characterization of *Leptospira spp* in Rodents from Different Habitats in Mgeta Ecosystem, Morogoro - Tanzania

Deteksi dan Karakterisasi Molekuler *Leptospira spp.* pada Rodensia dari Berbagai Habitat di Ekosistem Mgeta, Morogoro – Tanzania.

Gladness Alfred Mritha[1*], Eliakunda Mafie[2], Ernatus Mkupasi[1], Apia Wilbaldi Massawe[3,4], Jean N.Hakizimana[5], Gerald Misinzo[2], Abdul Selemani Katakweba[3]

[1]Department of Veterinary Medicine and Public Health, College of Vet. Med. and Biomed. Sciences, Sokoine University of Agriculture, Morogoro-Tanzania
[2]Department of Veterinary Microbio., Parasit. and Biotech., College of Vet. Med.and Biomed. Sciences, Sokoine University of Agriculture, Morogoro-Tanzania
[3]Institute of Pest Managements, Sokoine University of Agriculture, Morogoro-Tanzania
[4]African Centre of Excellence for Innovative Rodent Pest Manag. and Biosensor Techn. Development, Sokoine University of Agriculture, Morogoro-Tanzania
[5]SACIDS Africa Centre of Excellence for Infectious Diseases, Sokoine University of University of Agriculture, Morogoro-Tanzania

ABSTRACT

Background: Leptospirosis, a neglected zoonotic disease caused by *Leptospira spp.,* poses a major public health threat in tropical regions, including Tanzania. Rodents are the principal reservoirs responsible for environmental contamination and disease transmission to humans and domestic animals. However, information on Leptospira infection in rodents inhabiting undisturbed and restored forests in Tanzania remains scarce. **Purpose:** This study aimed to detect and characterize *Leptospira spp.,* infecting rodents and shrews in the Mgeta ecosystem, Morogoro, Tanzania, to better understand their potential role in maintaining and transmitting the pathogen across habitats. **Method:** A cross-sectional study was conducted from January to March 2024. Rodents and shrews were live-trapped from undisturbed forests, farms, fallow lands, naturally restored forest corridors, and human dwellings. DNA was extracted from kidney tissues and screened using semi-nested PCR targeting the secY gene. **Results:** A total of 207 small mammals were captured, consisting of 98.55% rodents and 1.45% shrews. *Leptospira spp.,* were detected in 9 (4.3%) rodents, with Praomys (44.4%), Lophuromys (33.3%), and *Mastomys natalensis* (22.2%) showing the highest prevalence. Infections were mainly detected in farm, forest, and corridor habitats. Phylogenetic analysis identified all isolates as *Leptospira borgpetersenii*, exhibiting 98.44–98.99% similarity with strains from Spain, Portugal, South Africa, and the United States. **Conclusion:** The detection of *L. borgpetersenii* across multiple habitats highlights its ecological persistence and potential zoonotic risk in the Mgeta ecosystem. Strengthened surveillance and integrated public health strategies are essential to mitigate leptospirosis transmission in agricultural and forest-edge communities.

ARTICLE INFO

Received: 13 August 2025
Revised: 10 September 2025
Accepted: 29 October 2025
Online: 31 October 2025

***Correspondence:**
Gladness Alfred Mritha
E-mail: gladnessmritha@gmail.com

Keywords: Leptospirosis; Molecular Characterization; Rodents; Shrews; Zoonosis

ABSTRAK

Latar Belakang: Leptospirosis merupakan penyakit zoonosis yang terabaikan, disebabkan oleh *Leptospira spp.,* dan menjadi ancaman kesehatan masyarakat utama di wilayah tropis termasuk Tanzania. Rodensia berperan sebagai reservoir utama yang dapat mencemari lingkungan dan menularkan penyakit ini kepada manusia serta hewan domestik. Namun, informasi mengenai infeksi Leptospira pada rodensia yang hidup di hutan alami dan hutan yang mengalami pemulihan alami di Tanzania masih terbatas. **Tujuan:** untuk mendeteksi dan mengkarakterisasi *Leptospira spp.,* yang menginfeksi rodensia dan curut di ekosistem Mgeta, Morogoro, Tanzania, guna memahami peran potensialnya dalam mempertahankan dan menularkan patogen di berbagai tipe habitat. **Metode:** Penelitian *cross-sectional* studi dilakukan pada Januari hingga Maret 2024. Rodensia dan cecurut ditangkap hidup-hidup dari hutan alami, lahan pertanian, lahan bera, koridor hutan yang mengalami pemulihan alami, dan pemukiman manusia. DNA diekstraksi dari jaringan ginjal dan diperiksa menggunakan PCR semi-nested dengan target gen secY. **Hasil:** Sebanyak 207 mamalia kecil berhasil ditangkap, terdiri atas 98,55% rodensia dan 1,45% cecurut. *Leptospira spp.,* terdeteksi pada 9 ekor (4,3%) rodensia, dengan prevalensi tertinggi pada Praomys (44,4%), Lophuromys (33,3%), dan *Mastomys natalensis* (22,2%). Infeksi terutama ditemukan di lahan pertanian, hutan, dan koridor hutan. Analisis filogenetik menunjukkan semua isolat tergolong *Leptospira borgpetersenii* dengan kemiripan genetik 98,44–98,99% terhadap strain dari Spanyol, Portugal, Afrika Selatan, dan Amerika Serikat. **Kesimpulan:** Deteksi *L. borgpetersenii* pada berbagai habitat menunjukkan keberlangsungan ekologis dan potensi risiko zoonosis di ekosistem Mgeta. Penguatan surveilans dan strategi kesehatan masyarakat terpadu diperlukan untuk mengurangi risiko penularan leptospirosis, khususnya di komunitas sekitar lahan pertanian dan tepi hutan.

Cite This Article:
Mritha, G.A., Mafie, E., Mkupasi, E., Massawe, A.W., Hakizimana, J.H., Misinzo, G., and Katakweba, A.S., 2025. ***Molecular Detection and Characterization of Leptospira spp in Rodents from Different Habitats in Mgeta Ecosystem, Morogoro - Tanzania*** Journal of Applied Veterinary Science and Technology.6(2):118- .https://doi.org/10.20473/javest.V6.I2.2025.118-

Kata kunci: Curut; Karakterisasi Molekuler; Leptospirosis; Rodensia; Zoonosis

INTRODUCTION

Leptospirosis is a globally neglected zoonotic disease caused by spirochetes of the genus Leptospira, which affects humans, domestic animals, and wildlife (Haake and Levett, 2015). This disease is of particular public health importance in tropical and subtropical regions, where environmental conditions favor the pathogen's survival (Machang'u *et al.,* 2004). The disease estimates annual incidence of up to 100 cases per 100,000 people in Tanzania (Maze *et al.,* 2016). The disease in humans ranges from mild to lethal courses in its clinical spectrum and probably has a high proportion of sub-clinical and asymptomatic infections (Allan *et al.,* 2020; Maze *et al.,* 2023). Rodents are recognized as primary reservoirs of *Leptospira spp.,* with species such as *Rattus rattus, Mastomys natalensis,* and *Cricetomys gambianus* shedding the bacteria in their urine, facilitating transmission through contaminated water and soil (Kapia, 2018). Leptospira species penetrates the host through lesions on the skin and mucous membranes, invading the bloodstream and spreading throughout the body of the animal. After this initial phase, leptospires are installed in the renal tubules of infected animals, being shed for long periods through the urine, promoting the contamination of the environment, particularly implicated in the transmission cycle (Motto *et al.,* 2021; Yadeta *et al.,* 2016). Rodents not only plays a role as reservoirs of Leptospira, but also serve as critical links in the epidemiology of leptospirosis. In regions like East Africa, leptospirosis in rodents remains underexplored, despite the documented risk to individuals with occupational exposure, including farmers and researchers, as well as the close association with human habitation (Wainaina *et al.,* 2024). Recent studies in Tanzania highlight the presence of pathogenic Leptospira in agro pastoral communities and wildlife, underscoring the need for expanded surveillance and control measures (Masanja and Van, 2022).

In humans, leptospirosis presents with a spectrum of symptoms, ranging from mild, flu-like illness to severe, life-threatening complications such as jaundice and renal failure (Karunarathna *et al.,* 2024). Despite its global prevalence, the disease remains underdiagnosed and misclassified, often mistaken for other febrile illnesses like malaria and dengue (Toyokawa *et al.,* 2011). Leptospirosis imposes direct economic burdens on humans, including lost productivity due to illness, increased treatment costs, and contributing to poverty among the population in affected countries. In animals, it causes miscarriages among cattle, stillbirths, loss of milk, infertility, death, and related veterinary consequences (Bradley and Lockaby, 2023; Grace and Cook, 2023). Diagnostic methods, such as culture, are time-consuming and impractical in clinical settings, making serological and molecular techniques, including the microscopic agglutination test (MAT) and polymerase chain reaction (PCR), essential for detection (Karpagam and Ganesh, 2020).

Despite leptospirosis being a significant zoonotic disease, it remains underreported in Tanzania due to diagnostic challenges and inadequate reservoir surveillance (Costa *et al.,* 2015). Rodents and shrews are key reservoirs of *Leptospira spp.* (Machang'u *et al.,* 1997), yet molecular data on *Leptospira spp.* in these hosts are limited in the natural and restored forest corridor, such as Bunduki in Mgeta ecosystem, with a human-wildlife interface. Interaction among humans, domestic animals, and rodents facilitates the spread of zoonotic pathogens in this ecosystem, characterized by natural and restored forests, as well as anthropogenic environments such as farms and settlements. The presence of rodents in each habitat increases the risk of Leptospira transmission. Also, bacteria can survive in humid environments, which is relevant to the Mgeta ecosystem. This study addresses this gap by employing PCR and sequencing to detect and characterize Leptospira in rodents and shrews (Allan *et al.,* 2018). Results will reveal genetic diversity and zoonotic potential of strains, informing targeted prevention strategies in Morogoro and similar regions.

MATERIAL and METHOD

Study Area

This study was conducted in Mgeta ecosystem situated in Mvomero District, Morogoro Region, on the western slopes of the Uluguru Mountains, its geographical coordinates are 7° 2' South and 37° 34' East (Msalya *et al.,* 2021). In this study, samples were collected from Vinile and Bunduki villages (Fig. 1). It has tropical wet and dry or savanna climate with temperatures ranging between 11°C and 23°C. It experiences bimodal rainfall characterized by short and long rain, an annual rainfall of about 1400 mm, with a range of elevations from 1100 to 1900 meters above sea level. The climate is sub-tropical with regular rainfalls, which favor intensive cropping of rain-fed or irrigation-based vegetables, which are combined with livestock, especially dairy and meat goats, pigs, and poultry (Nziku *et al.,* 2016). The area's vegetation is made up of sections of grassland, farms, and mountain forests. Mgeta experiences dry periods lasting around four months, typically occurring from June to September (Eik *et al.,* 2008).

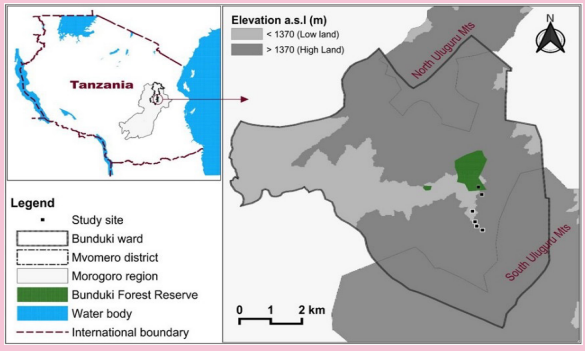

Figure 1. A map of Mgeta ecosystem within Morogoro region. The map was created using QGIS software version 3.26.1 and shape files from DIVA-GIS.

Study Design, Sampling Strategy, and Sample Size

A cross-sectional study design was carried out from January to March 2024 in Mgeta ecosystem, to assess the presence of Leptospira infection in rodents and shrews. To achieve this, rodents and shrews were captured from two villages, namely

Vinile and Bunduki where traps were placed in selected habitats, including fallow lands, farms, houses, natural forest, and around the Bunduki forest restoration corridor. These villages were selected based on their closeness to the natural and restored forest corridor, and the habitats from where the rodents were captured were selected based on the reported presence of rodents. The sample size for rodents and shrews was determined by the formula n=Zα2 p (1-p)/d2 (Thrusfield et al., 2018), whereby n = sample size, P = previous reported prevalence of Leptospira antibodies in rodents, 17% (Mgode et al., 2021). d= desired precision, 5% Zα= statistics corresponding to 5% level of significance, 1.96. This resulted in 216 rodents and shrews. Due to unfavorable climatic conditions during sampling, only 207 rodents and shrews were captured.

Study Design, Sampling Strategy, and Sample Size

Rodents and shrews were trapped by using Sherman (H.B. Sherman, Tallahassee, FL, USA) and wire cage traps. The traps were placed indoors, in the forest corridors, natural forest, farms, and fallow lands. All traps were baited with peanut butter mixed with maize flour at the ratio of 2:1 (1000 g of peanut butter mixed with 500 g of maize flour). For indoor captures, modified wire cages were used, while Sherman traps were placed outdoors. In each study village, 10-15 houses were purposefully selected based on the reported presence of rodents, and 2 to 3 wire cages were distributed across each house depending on size (Mhamphi *et al.,* 2023). In the selected habitats, a maximum of 50 Sherman traps were set in five trap lines of ten traps in each habitat. The traps were set at an interval of five meters apart from each trap station and trap lines. Traps were checked at 8:00 am and re-baited once per day for three consecutive days. In total, 200 Sherman traps were deployed in all habitats. During inspection, all traps with animals were labelled at each trapping station prior to transfer to the field temporary laboratory.

Sample Collection and Identification of Captured Animals

The captured rodents and shrews were humanely killed by excessive inhalation of halothane-soaked cotton wool. Animal data, including sex and sexual conditions (male: scrotal or abdominal testes; female: a perforated or closed vagina, and pregnant or lactating), and body measurements (body weight, head and body length, and tail length) were recorded for identification of each animal according to (Happold, 2013). Thereafter, rodents were aseptically dissected using sterilized scissors and forceps, then the kidney was aseptically sectioned, and a piece was placed into a microtube of 2.5 ml containing absolute ethanol for molecular analysis. Cross-contamination between animals was avoided through sequential dissection of single animals and stringent disinfection measures after each dissection.

Method of Analysis

A total of 207 kidneys from rodents and shrews were subjected to the extraction of DNA. DNA extraction was done using DNeasy Blood and Tissue Kit (cat# 69506) from Qiagen (Hilden, Germany), according to the manufacture's instructions. The final DNA extract was subsequently refrigerated at -20^{0}C awaiting further analysis.

Amplification of secY gene was done using primer sequences shown in Table 1. Outer PCR was performed in a 25 µL 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), 0.5 µL of each forward primer (secYFd), and reverse primer (secYR3), and 9.5 µL of nuclease-free water. Cycling conditions consisted of initial denaturation at 94^{0}C for 3 min followed by 40 cycles of 94^{0}C for 20 s, 62^{0}C for 25 s and 68^{0}C for 40 s. Final extension at 68 ^{0}C for 5 min was performed to complete the elongations. Nested PCR was performed with 2 µL of 10-fold diluted outer PCR amplicon, 12.5 µL of OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (from New England Biolabs, Inc), 0.5 µL of each forward primer (secYFnest), and reverse primer (secYR3), and 9.5 µL of nuclease-free water in a 25 µL reaction volume. Nested PCR was performed under the touch-down cycling conditions, which consisted initial denaturation at 94 ^{0}C for 3 min followed by 5 cycles of 94 ^{0}C for 20 s, 60 ^{0}C for 25 s and 68 ^{0}C for 40 s; 5 cycles of 94 ^{0}C for 20 s, 58 ^{0}C for 25 s and 68 ^{0}C for 40 s and 20 cycles of 94 ^{0}C for 20 s, 56 ^{0}C for 25 s and 68 ^{0}C for 40 s. Final extension at 68 ^{0}C for 5 min was performed to complete the elongations.

The amplicons were separated using a 1.5% agarose gel dissolved in Sodium borate buffer and stained with 10 µL of SafeView™ Classic DNA staining dye (Applied Biological Materials Inc.). Each well of the gel was loaded with 4 µL of each PCR product. And 4 µL of 100 bp DNA ladder was loaded into one well in order to indicate the size of any fragments. Electrophoresis was allowed to run for 60 minutes at 100V. The gel was visualized using a gel documentation machine (Gel Doc™ EZ Imager from Bio-Rad Laboratories).

Positive samples were sent to Macrogen Europe (Meibergdree 57, 1105 BA, Amsterdam, the Netherlands) for sequencing. The PCR products were purified and sequenced directly using BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and a genetic analyzer (ABI 3730xl System from Applied Biosystems). Raw nucleotide sequences (ab1 files) from samples sent for sequencing were acquired. The raw sequences from both forward and reverse primers were cleaned and assembled using the Geneious Prime (2025.0.3) software to generate consensus sequences. Good consensus partial coding sequences of the secY gene with about 419 base pairs were obtained. The obtained consensus sequences were compared with the published sequences in the GenBank using the Basic Local Alignment Search Tool (BLAST), in order to confirm the taxonomic identity. The phylogenetic tree was constructed using the Maximum likelihood method based on the Kimura 2-parameter model using MEGA 11 software.

Table 1. Primer Sequences Used for The Detection of Leptospira species

Primer Name	Orientation	Primer sequence (5' to 3')	Amplicon Size (bps)
secYFd	Forward	ATGCCGATCATYTTYGCTTC	527
secYR3	Reverse	TTCATGAAGCCTTCATAATTTCTCA	
secYFnest	Forward	AATCCATTYTCYCARATYTGGTA	419

RESULTS

Rodents and Shrews

A total of 207 animals (rodents and shrews) were captured from five different habitats. Out of them, 3 (1.45%) were shrews (*Crocidura spp.*) and 204 (98.55%) rodent species. The highest number of rodents and shrews were captured from the forest Corridor 85 (41.06%), Forest 68 (32.85%), and farm 47 (22.71%). The rodents belong to eight species, as indicated (Table 2).

Table 2. Rodents and Shrews Captured from Different Habitats

Animal Species	Corridor	Fallow	Farm	Forest	House	Total (%)
Aethomys	0	0	1	0	0	1 (0.48%)
Crocidura	2	0	0	1	0	3 (1.45%)
Grammomys	2	0	2	0	0	4 (1.93%)
Lophuromys	23	2	7	16	0	48 (23.19%)
Mastomys natalensis	11	1	23	1	0	36 (17.39%)
Mus	0	0	5	0	0	5 (2.42%)
Otomys	1	0	0	0	0	1 (0.48%)
Praomys	46	0	9	50	0	105 (50.72%)
Rattus rattus	0	0	0	0	4	4 (1.93%)
Total (%)	**85**	**3**	**47**	**68**	**4**	**207**

Leptospiral Genome Detection

DNA extracted from the kidneys of 207 captured small mammals was analyzed using semi-nested PCR, revealing that 9 samples (4.3%) tested positive for Leptospira spp. The highest prevalence rate was found in Praomys (44.4%), followed by Lophuromys (33.3%), and the lowest was *M. natalensis* (22.2%). All of the positive samples were for rodents captured from the forest and farm (Praomys), forest and along the corridor (Lophuromys), farm and fallow land (*M. natalensis*). The PCR gel electrophoresis analysis for the detection of Leptospira species demonstrates distinct amplicons at 419 base pairs (bp) in multiple sample lanes, indicating successful target amplification. The positive control (PC) exhibits a clear band at 419 bp, validating the specificity and efficiency of the PCR reaction. In contrast, the negative control (NC) shows no amplification, confirming the absence of contamination. The DNA ladder (M) provides an accurate size reference, ensuring proper interpretation of results. The presence of consistent bands in the tested samples suggests the detection of Leptospira DNA, confirming successful amplification and the reliability of the assay for pathogen identification.

A BLAST search for SecY gene nucleotide sequences revealed the sequence identity of 98.44 to 98.96% with *Leptospira borgpetersenii* species sequences published in GenBank, as shown in Table 3. The phylogenetic tree constructed supported the BLAST results for species identification. Phylogenetic analysis shows that all secY gene partial nucleotide sequences from this study clustered with published *Leptospira borgpetersenii* species sequences isolated from different geographical locations. The analysis also shows a clear difference between *Leptospira borgpetersenii* and other Leptospira species, for example, *Leptospira interrogans* and *Leptospira kirschneri*, which were selected as outgroup Leptospira species (Figure 3).

Table 3. Basic Local Alignment Search Tool (BLAST) Results of SecY Gene Partial Nucleotide Sequences from This Study Against Sequences from GenBank

Description	Nucleotide Identity	Accession
Leptospira borgpetersenii/New Caledonia/2016	98.96%	HQ328722.1
Leptospira borgpetersenii/Portugal/2017	98.94%	KU219487.1
Leptospira borgpetersenii/Spain/2024	98.99%	PP372554.1
Leptospira borgpetersenii/Spain/2024	98.92%	PP372555.1
Leptospira borgpetersenii/USA/2022	98.69%	MZ241244.1
Leptospira borgpetersenii/Tahiti/2017	98.69%	KY357123.1
Leptospira borgpetersenii/South Africa/2020	98.44%	MH795468.1

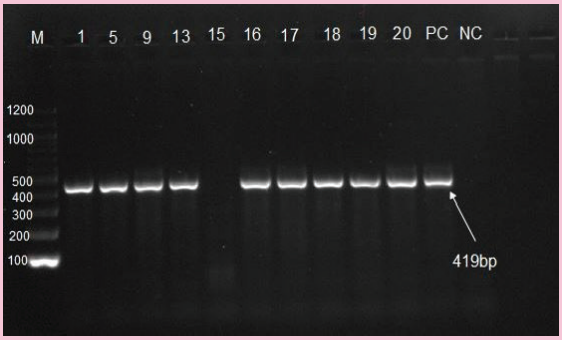

Figure 2. Gel-electrophoresis PCR results showing a band for positive samples at 419 bp for Leptospira species. Lane M, 100-bp DNA ladder; Lanes 1–20, tested samples laboratory identification numbers; Lane PC, positive control, and Lane NC, negative control

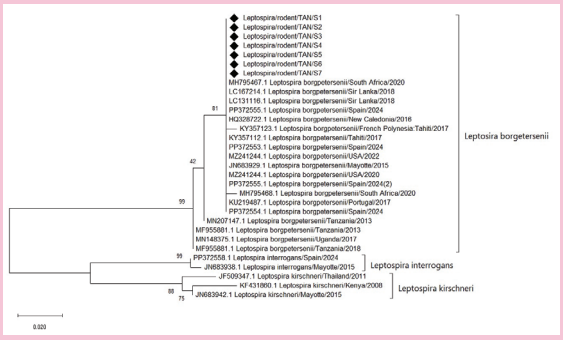

Figure 3. Phylogenetic tree of the secY gene partial sequences of Leptospira species nucleotide sequences from this study and selected sequences from GenBank.◆Sign indicates nucleotide sequences from this study.

DISCUSSION

The study findings reveal significant insights into Leptospira infection patterns among rodents in Mgeta ecosystem, Mvomero, Tanzania; emphasizing their role as potential reservoirs in the transmission cycle of leptospirosis. From a total sample of 207 captured small mammals, rodents constituted the vast majority at 98.55% (n=204), while shrews represented only 1.45% (n=3). The study captured a diverse range of species, indicating a rich small mammal community. The spatial distribution of captures showed a notable abundance in three primary habitats: corridors, forests, and farms, which accounted for 41.06%, 32.85%, and 22.71% of captures,

respectively, compared to the indoor habitat. The reason could be that outdoor habitats comprise a greater number of rodent species than indoor habitats, which are infested with only commensal rodents, including *Rattus spp.* and *Mus spp.* Additionally, these commensal rodents are sometimes poisoned by humans, resulting in a low abundance. This distribution pattern suggests the outdoor habitat, especially the three primary habitats are particularly important for rodent populations in Mgeta ecosystem. The predominant capture of animals in corridors and forests supports the findings of Shilereyo *et al.,* (2023) on the role of habitat heterogeneity's role in supporting small mammal diversity in Tanzania. The dominance of Praomys species in these captures reflects Lema and Magige (2018) observations about this genus's prevalence in African forest ecosystems. Also, the presence of *M. natalensis* in agricultural areas validates Mayamba et al., (2019) findings on its significance as both an agricultural pest and disease vector.

Using semi-nested PCR targeting the secY gene, 9 (4.3%) samples tested positive for *Leptospira spp.* The SecY gene was used as it's a conserved gene; it does not undergo mutation as it's a structural transmembrane gene. All positive cases identified exclusively in rodent species. The absence of Leptospira in shrews' contrasts with Mgode *et al.,* (2014), suggesting potential regional variations in host species importance, and maybe due to low numbers of shrews captured in the study area. The relatively low prevalence observed may reflect differences in host susceptibility, seasonal factors, or micro-environmental conditions that influence transmission dynamics. Nevertheless, even a small proportion of infected individuals can sustain environmental contamination, underscoring the epidemiological importance of continuous surveillance in wildlife populations. Among the infected populations, Praomys emerged as the most significantly affected genus with a 1.9% infection rate, followed by Lophuromys at 1.5% and *M. natalensis* at 0.9%. This pattern of infection suggests varying susceptibility or exposure rates among different rodent species, with *Praomys spp.* potentially serving as highly exposed and primary reservoir for Leptospira in Mgeta ecosystem. Infections were mainly found in rodents captured from farm, forest, and corridor habitats.

The ecological distribution of infected rodents suggests that environmental factors, such as habitat type and land use, may play a significant role in Leptospiral transmission. Praomys, the most infected species, was primarily found in forest and farm habitats, reinforcing the hypothesis that agricultural areas serve as hotspots for Leptospiral transmission. The capture of Praomys in farm areas, despite being traditionally considered a forest species, suggests possible habitat shifts or increased adaptability to human-modified environments. This observation aligns with Masanja and Van (2022), who noted that agro-pastoral communities in Tanzania are at high risk of leptospirosis due to increased human-rodent interactions. Additionally, our findings support the conclusions of Wainaina *et al.,* (2024), who emphasized the role of forest-adjacent areas in harboring Leptospira-infected rodents. Several species tested negative for Leptospira, suggesting potential differences in susceptibility, exposure patterns, or ecological niches that might influence infection risk. From a phylogenetic perspective, the study confirms that *Leptospira borgpetersenii* isolates from this study share high genetic similarity (98.44%–98.99%) with strains from diverse geographic regions, including Spain, South Africa, and the United States of America. The genetic similarity with strains from distant locations may indicate possible historical intercontinental spread of Leptospira strains or the presence of globally conserved evolutionary strains within rodent reservoirs. The clustering of Tanzanian isolates with those from New Caledonia and the USA also implies that certain Leptospira strains may have a broad ecological niche and adaptive capacity. Also, sequences obtained in this study were genetically distant from other *L. borgpetersenii* strains previously reported in Tanzania, suggesting they may represent different clonal lineages or genotypes circulating within the country. This indicates that multiple genetically distinct lineages of *L. borgpetersenii* are present in Tanzania, possibly with epidemiological links beyond national borders. The tree distinctly separates *Leptospira borgpetersenii* from other species like *Leptospira interrogans* and *Leptospira kirschneri*, which were used as outgroup species. This clear distinction reinforces the accuracy of species identification.

The phylogenetic clustering of Tanzanian isolates with strains from Spain, Portugal, South Africa, and the United States raises important questions about transmission and dispersal pathways. While the observed genetic similarity may reflect long-term evolutionary conservation of certain clonal lineages, it is also possible that human-mediated movement of animals (e.g., livestock trade, commensal rodents transported via shipping routes or agricultural exchanges) has historically contributed to the transboundary spread of Leptospira. Migratory wildlife species, including birds, could also facilitate indirect dissemination by transporting infected ectoparasites or acting as mechanical carriers across regions. Alternatively, conserved strains of *L. borgpetersenii* may have persisted in parallel in multiple regions due to their adaptation to similar ecological niches, such as humid environments that support rodent reservoirs. Although our study cannot confirm the exact mechanisms, these scenarios highlight how local strains may be epidemiologically linked to global lineages. Broader genomic studies across wildlife, livestock, and environmental samples will be essential to clarify whether the similarities reflect recent introductions, historical spread, or ecological conservation of strains.

This study has several limitations that should be acknowledged. First, sampling was limited to two villages during a single season, which restricts the generalizability of the findings and precludes assessment of seasonal variation. The uneven capture success across species and habitats, along with the very small sample of shrews, also reduced power to detect host- and habitat-specific differences. Host identification relied on morphology alone, which may misclassify cryptic species. Methodologically, reliance on a single locus (secY) with a short amplicon constrained phylogenetic resolution, while the lack of culture isolation or multi-locus

resolution, while the lack of culture isolation or multi-locus sequence typing further limited strain characterization. Finally, the study did not incorporate environmental samples or data from domestic animals and humans, limiting the ability to infer ecological transmission pathways. Future work addressing these limitations through broader temporal and spatial sampling, multi-locus or whole-genome approaches, inclusion of multiple host and environmental reservoirs, and integration of One Health frameworks would provide a more comprehensive understanding of Leptospira dynamics in this ecosystem.

CONCLUSION

This study provides new insights into the molecular epidemiology of Leptospira in Mgeta ecosystem, reinforcing the role of rodents in its transmission cycle. At the same time, our findings align with previous research, which also extends the current knowledge by identifying additional rodent hosts and linking environmental factors to Leptospiral prevalence.

ACKNOWLEDGEMENT

We would like to extend our sincere appreciation to BEPREP Project (Identification of Best Practices for Biodiversity Recovery and Public Health Interventions to Prevent Future Epidemics and Pandemics Project) funded by European Union for financial support. And special thanks go to the following people Ms. Mwajabu Suleiman, Mr. Mwinyi Masala, and Ms. Mariam Makange for their contribution in this study.

CONFLICT of INTEREST

All authors declare that they have no conflict of interest regarding the content and publication of this manuscript.

FUNDING INFORMATION

BEPREP Project (Identification of Best Practices for Biodiversity Recovery and Public Health Interventions to Prevent Future Epidemics and Pandemics Project) funded by European Union.

ETHICAL APPROVAL

The study adhered to the necessary research clearance and ethical protocols to protect rights and ensure compliance with regulations. Research clearance was granted by Sokoine University of Agriculture, with reference number DPRT-C/R/186/47

AUTHORS' CONTRIBUTIONS

GAM contributed to the conceptualization and design of the study, conducted the field investigation, performed data curation and analysis, and prepared the original manuscript draft. EM and ASK provided supervision and critically reviewed and edited the manuscript. AWM was responsible for funding acquisition and overall supervision of the research activities. GM provided technical assistance in molecular analyses. EMk contributed to project supervision and participated in manuscript review and editing. JNH provided supervisory support and assisted with data analysis.

REFERENCES

Allan, K.J., Halliday, J.E.B., Moseley, M., Carter, R.W., Ahmed, A., Goris, M.G.A., Hartskeerl, R.A., Keyyu, J., Kibona, T., Maro, V.P., Maze, M.J., Mmbaga, B.T., Tarimo, R., Crump, J.A., and Cleaveland, S., 2018. Assessment of Animal Hosts of Pathogenic Leptospira in Northern Tanzania. *PLOS Neglected Tropical Diseases,* 12, e0006444.

Allan, K.J., Maze, M.J., Galloway, R.L., Rubach, M.P., Biggs, H.M., Halliday, J.E.B., Cleaveland, S., Saganda, W., Lwezaula, B.F., Kazwala, R.R., Mmbaga, B.T., Maro, V.P., and Crump, J.A., 2020. Molecular Detection and Typing of Pathogenic Leptospira in Febrile Patients and Phylogenetic Comparison with Leptospira Detected among Animals in Tanzania. *The American Journal of Tropical Medicine and Hygiene*, 103, 1427–1434.

Bradley, E.A., and Lockaby, G., 2023. Leptospirosis and the Environment: A Review and Future Directions. *Pathogens,* 12, 1167.

Costa, F., Hagan, J.E., Calcagno, J., Kane, M., Torgerson, P., Martinez-Silveira, M.S., Stein, C., Abela-Ridder, B., and Ko, A.I., 2015. Global Morbidity and Mortality of Leptospirosis: A Systematic Review. *PLOS Neglected Tropical Diseases,* 9, e0003898.

Eik, L., Kifaro, G., Kiango, S., Nordhagen, Ø., Safari, J., and Mtenga, L., 2008. Productivity of Goats and Their Contribution to Household Food Security in High Potential Areas of East Africa: A Case of Mgeta, Tanzania. *African Journal of Food, Agriculture, Nutrition and Development* 8(3), 278–290.

Grace, D., and Cook, E., 2023. Zoonoses and Poverty: The Multiple Burdens of Zoonoses in Low- and Middle-Income Countries. In: Sing, A. (1st Ed), Zoonoses: Infections Affecting Humans and Animals. Cham, Switzerland: Springer.

Haake, D.A., Levett, P.N., 2015. Leptospirosis in Humans, in: Adler, B. (Ed.), Leptospira and Leptospirosis, Current Topics in Microbiology and Immunology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 65–97.

Happold, D., 2013. Mammals of Africa Volume III - Rodents, Hares and Rabbits. Bloomsbury.

Kapia, S., 2018. Detection of Leptospira and Seasonal Prevalence of Fleas Collected from Small Mammals in Mukwe Constituency, Kavango East Region of Namibia [Thesis]. University of Namibia.

Karpagam, K.B., Ganesh, B., 2020. Leptospirosis: A Neglected Tropical Zoonotic Infection of Public Health Importance-An Updated Review. *European Journal of Clinical Microbiology & Infectious Diseases,* 39, 835–846.

Karunarathna, I., Gunasena, P., Aluthge, P., Perera, N., Gunathilake, S., Alvis, K.D., Gunawardana, K., Rajapaksha, S., Warnakulasooriya, A., Athulgama, P., Dius, S., Ranwala, R., Vidanagama, U., Godage, S., Pn, R., Fernando, C., Ekanayake, U., Hapuarachchi, T., Jayawardana, A., Bandara, S., and Rathnayak, B.,

2024. Leptospirosis Clinical Presentation, Diagnosis, and Interprofessional Care. *Uva Clinical Anaesthesia & Intensive Care.*

Lema, R., and Magige, F.J., 2018. The Influence of Agricultural Activities on The Diversity of Rodents in Kindoroko Forest Reserve and Surrounding Areas, North Pare Mountains, Tanzania. *Tanzania Journal of Science*, 44, 97–106.

Machang'u, R.S., Mgode, G., and Mpanduji, D., 1997. Leptospirosis in Animals and Humans in Selected Areas of Tanzania. *Belgian Journal of Zoology*, 127, 97–104.

Machang'u, R.S., Mgode, G.F., Assenga, J., Mhamphi, G., Weetjens, B., Cox, C., Verhagen, R., Sondij, S., Goris, M.G., and Hartskeerl, R.A., 2004. Serological and Molecular Characterization of Leptospira Serovar Kenya from Captive African Giant Pouched Rats (Cricetomys gambianus) from Morogoro Tanzania. *FEMS Immunology & Medical Microbiology,* 41, 117–121.

Masanja, Y.L., and Van, H.T.H., 2022. Patterns of Zoonotic Diseases and Associated Socio-Economic Factors in Tanzania: A Scoping Review. *Tạp Chí Nghiên Cứu Y Học,* 154, 108–120.

Mayamba, A., Byamungu, R.M., Makundi, R.H., Kimaro, D.N., Isabirye, M., Massawe, A.W., Kifumba, D., Nakiyemba, A., Leirs, H., Mdangi, M.E., Isabirye, B.E., and Mulungu, L.S., 2019. Species Composition and Community Structure of Small Pest Rodents (Muridae) in Cultivated and Fallow Fields in Maize-Growing Areas in Mayuge District, Eastern Uganda. *Ecology and Evolution,* 9, 7849–7860.

Maze, M.J., Biggs, H.M., Rubach, M.P., Galloway, R.L., Cash-Goldwasser, S., Allan, K.J., Halliday, J.E.B., Hertz, J.T., Saganda, W., Lwezaula, B.F., Cleaveland, S., Mmbaga, B.T., Maro, V.P., and Crump, J.A., 2016. Comparison of The Estimated Incidence of Acute Leptospirosis in The Kilimanjaro Region of Tanzania between 2007-08 and 2012-14. *PLOS Neglected Tropical Diseases,* 10, e0005165.

Maze, M.J., Shirima, G.M., Lukambagire, A.-H.S., Bodenham, R.F., Rubach, M.P., Cash-Goldwasser, S., Carugati, M., Thomas, K.M., Sakasaka, P., Mkenda, N., Allan, K.J., Kazwala, R.R., Mmbaga, B.T., Buza, J.J., Maro, V.P., Galloway, R.L., Haydon, D.T., Crump, J.A., and Halliday, J.E.B., 2023. Prevalence and Risk Factors for Human Leptospirosis at A Hospital Serving A Pastoralist Community, Endulen, Tanzania. *PLOS Neglected Tropical Diseases,* 17, e0011855.

Mgode, G.F., Katakweba, A.S., Mhamphi, G.G., Fwalo, F., Bahari, M., Mdangi, M., Kilonzo, B.S., and Mulungu, L.S., 2014. Prevalence of Leptospirosis and Toxoplasmosis: A Study of Rodents and Shrews in Cultivated and Fallow Land, Morogoro Rural District, Tanzania. *Tanzania Journal of Health Research,* 16(3), 5–250.

Mgode, G.F., Mhamphi, G.G., Massawe, A.W., and Machang'u, R.S., 2021. Leptospira Seropositivity in Humans, Livestock and Wild Animals in a Semi-Arid Area of Tanzania. *Pathogens,* 10, 696.

Mhamphi, G.G., Katakweba, A.S., Massawe, A.W., Makundi, R.H., Machang'u, R.S., Komba, E.V.G., and Mnyone, L.L., 2023. Prevalence of Bartonella Spp. in Rodent and Shrew Species Trapped in Kigoma and Morogoro Regions, Tanzania: A Public Health Concern. *African Journal of Microbiology Research,* 17, 156–163.

Motto, S.K., Shirima, G.M., Bronsvoort, B.M.D.C., and Cook, E.A.J., 2021. Epidemiology of Leptospirosis in Tanzania: A review of The Current Status, Serogroup Diversity and Reservoirs. *PLOS Neglected Tropical Diseases,* 15, e0009918.

Msalya, G.M., Urassa, F.E., and Kifaro, G.C., 2021. Quality of Milk from Norwegian Dairy Goats Bred and Raised in Mgeta Division, Morogoro Region, Tanzania. *Tanzania Journal of Agricultural Sciences,* 20, 54–62.

Nziku, Z., Asheim, L.J., Eik, L.O., Mwaseba, D.L., and Kifaro, G.C., 2016. Climate Change Adaptation in Vulnerable Crop and Livestock Production Systems in Mgeta, Tanzania. *African Journal of Food, Agriculture, Nutrition and Development,* 16, 10853–10865.

Shilereyo, M.T., Magige, F.J., Ogutu, J.O., and Røskaft, E., 2023. Small-Mammal Abundance and Species Diversity: Land use and Seasonal Influences in The Serengeti Ecosystem, Tanzania. *Frontiers in Conservation Science,* 4, 981424.

Thrusfield, M., Christley, R., Brown, H., Diggle, P.J., French, N., Howe, K., Kelly, L., O'Connor, A., Sargeant, J., and Wood, H., 2018. Veterinary Epidemiology, 1st ed. Wiley.

Toyokawa, T., Ohnishi, M., and Koizumi, N., 2011. Diagnosis of Acute Leptospirosis. *Expert Review of Anti-infective Therapy*, 9, 111–121.

Wainaina, M., Wasonga, J., and Cook, E.A.J., 2024. Epidemiology of Human and Animal Leptospirosis in Kenya: A Systematic Review and Meta-Analysis of Disease Occurrence, Serogroup Diversity and Risk Factors. *PLOS Neglected Tropical Diseases,* 18, e0012527.

Yadeta, W., Michael, B.G., and Abdela, N., 2016. Leptospirosis in Animal and its Public Health Implications: A Review. *World Applied Sciences Journal,* 34, 845–853.