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Seasonal Variation on The Prevalence and Molecular Characterization of Trypanosoma species from Rodents Trapped in Kilombero District, Tanzania

Variasi Musim Terhadap Prevalensi dan Karakterisasi Molekuler Spesies Trypanosoma dari Rodensia yang terperangkap di Distrik Kilombero, Tanzania

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ABSTRACT

Background: Trypanosomiasis is a disease mainly encountered in tropical regions and is significant to both humans and animals. Rodents and their fleas have been found to play a major role in the transmission of trypanosomiasis to humans. **Purpose:** This study aimed to examine the seasonal variation and molecular characterization of Trypanosoma species from rodents in Kilombero. **Method:** The study employed a cross-sectional design. A total of 387 rodents were captured, and anesthetized with Diethyl Ether to collect blood, spleen, and liver. Rodent tissues were tested with conventional PCR and sequencing to target the 18S rRNA and ITS1 genes. **Results:** The most prevalent species was *Mastomys natalensis* 82.43% (319/387), followed by *Rattus rattus* 16.79% (65/387), *Gramomys spp.* 0.52% (2/387), and the least captured species *Lemnscomys spp.* 0.26% (1/387). Overall microscopic prevalence was 38.76% (n=150/387), and PCR indicated prevalence of *Trypanosoma spp.* to be 6.28% (n=10/159). Male rodents had a higher molecular prevalence of *Trypanosoma spp.*, and a higher molecular prevalence of *Trypanosoma spp.* was observed in the dry season. **Conclusion:** Detection of *T. lewisi* from the rodents shows the public health significance in the study area. As a result, it is critical to use prevention and control measures in rodents to minimize potential human exposure within the area.

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ABSTRAK

Latar Belakang: Trypanosomiasis merupakan penyakit yang banyak ditemukan di daerah tropis dan berbahaya bagi manusia dan hewan. Rodensia dan pinjal diketahui berperan besar dalam penularan Trypanosomiasis ke manusia. **Tujuan:** Penelitian ini bertujuan untuk menyelidiki karakterisasi molekuler dan variasi musiman spesies Trypanosoma dari hewan pengerat di Kilombero. **Metode:** Penelitian ini menggunakan rancangan cross-sectional. Sebanyak 387 ekor tikus ditangkap dan dibius dengan Dietil Eter untuk pengambilan darah, limpa, dan hati. Jaringan tikus diuji dengan PCR konvensional dan sekuensing untuk menargetkan gen 18S rRNA dan ITS1. **Hasil:** Spesies yang paling umum adalah *Mastomys natalensis* 82,43% (319/387), diikuti oleh *Rattus rattus* 16.79% (65/387), *Gramomys spp.* 0.52% (2/387), dan spesies yang paling sedikit ditangkap adalah *Lemnscomys spp.* 0.26% (1/387). Prevalensi mikroskopis keseluruhan adalah 38.76% (n=150/387), dan PCR menunjukkan prevalensi *Trypanosoma spp.* sebesar 6.28% (n=10/159). Hewan pengerat jantan memiliki prevalensi molekuler *Trypanosoma spp.* yang lebih tinggi, dan prevalensi molekuler *Trypanosoma spp.* yang lebih tinggi diamati pada musim kemarau. Prevalensi *Trypanosoma spp.* yang lebih tinggi diamati di Mbalaji (X^2=17.90, df=10, p=0.05). Analisis filogenetik dan sekuens menunjukkan indeks kesamaan yang tinggi dari sekuens dengan *Trypanosoma lewisi* dari negara lain yang diisolasi dari spesies hewan yang berbeda. **Kesimpulan:** Deteksi *Trypanosoma lewisi* dari hewan pengerat menunjukkan signifikansi kesehatan masyarakat di area penelitian. Oleh karena itu, sangat penting untuk menggunakan tindakan pencegahan dan pengendalian pada hewan pengerat guna meminimalkan potensi paparan manusia di area tersebut.

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Kata kunci: Musim; PCR; Prevalensi; Rodensia; Musim; Trypanosoma

INTRODUCTION

Trypanosomiasis is a disease primarily seen in tropical regions of the world and is significant to both humans and animals. The illness is caused by hemogellate protozoan parasites which are spread by insects that bite people (Pumhom, *et al.,* 2014). *Trypanosoma cruzi* causes American Chagas disease, and *T. brucei* gambiense causes African sleeping sickness, a chronic disease in West and Central Africa (Kasozi, *et al.,* 2021$_a$). The genus Trypanosoma is categorized into two distinct groups: stercorarian parasites, which mature in the posterior section of the digestive tract and are generally non-pathogenic, except *T. cruzi*, and salivarian parasites, which develop in the anterior section of the vector's digestive tract and encompass the majority of pathogenic trypanosomes (Kumar, *et al.,* 2022). In 144 rodent species, 44 Trypanosoma spp. have been described, the majority of which are from the stercoraria section (Magri, *et al.,* 2021). A growing number of unusual human instances of *T. lewisi*, or *T. lewisi-like*, and *T. evansi* infections have been documented in Malaysia, Sri Lanka, India, and Thailand (Kumar, *et al.,* 2022).

Trypanosoma lewisi belongs to the non-pathogenic subgenus Herpetosoma and is predominantly located in rodent populations. It is a global parasite that is spread by fleas and affects rodents. Metatrypanosome infections in rodents are most likely caused by fleas or their excretions. Rattini rodents, including commensal species such as the black rat *(Rattus rattus)*, the Pacific rat *(Rattus exulans)* and the Norwegian rat *(Rattus norvegicus)*, are the ancestral reservoirs of *T. lewisi*. Human migration and trade, these rodents have expanded beyond their natural range (Pumhom, *et al.,* 2014). That has enabled *T. lewisi* and its rodent hosts to travel the globe. The other atypical trypanosome that has been isolated from human patients is *Trypanosoma evansi* (Salivaria; subgenus Trypanozoon). It was described from Egypt and most recently in India (Pumhom, *et al.,* 2014). *Trypanosoma evansi* can infect a wide range of animals, including domestic animals, wild animals, and livestock. Besides cattle, rats can be an alternative reservoir of *Trypanosoma evansi* posing risk of human and animal infections (Kasozi, *et al.,* 2021$_b$).

Chagas disease results from the protozoan parasitic pathogen *Trypanosoma cruzi*, with triatomine bugs serving as the main vectors for its transmission. Rodents serve as crucial reservoirs for this parasite and are integral to the transmission cycle of the parasite (Ocaña-Mayorga, *et al.,* 2021). Ghersi *et al.,* (2020) conducted research and reported that 11% of rodents have *T. cruzi* infectivity, particularly in urban settings such as New Orleans. In southeast Mexico, the prevalence of *T. cruzi* was positive in 9.75% of blood samples taken from synanthropic rodents in rural zones, with higher rates observed in specific species such as *Rattus rattus* and *Peromyscus yucatanicus* (Hernández-Cortazar, *et al.,* 2018). *T. cruzi* is primarily transmitted via contact with the feces of infected triatomine bugs. However, by engaging with these vectors, rodents can also contribute to the spread (Avalos-Borges, *et al.,* 2022). In rodent other mammals, congenital transmission of *T. cruzi* occurs when infected mothers also transmit the parasite to their offspring (Avalos-Borges, *et al.,* 2022). There are serious health hazards associated with *T. cruzi* in rodent populations, especially in endemic regions where people frequently come into touch with these animals (Lima-Neiva, *et al.,* 2021). In sub-Saharan African nations, the threat of sleeping sickness significantly endangers public health. The cause is a trypanosome that belongs to the complex of *Trypanosoma brucei*. According to traditional knowledge, there are three subspecies of *T. brucei: T. brucei gambiense*, which produces the chronic form of the disease in West and Central Africa; *T. brucei rhodesiense*, which causes the acute form in East Africa; and *T. brucei brucei*, an animal parasite that is safe for humans only (Kasozi, *et al.,* 2022). Previous studies have reported the presence of *T. evansi* and *T. lewisi* in rodents. This study's findings will contribute to the scientific understanding of Trypanosoma infections and have significant implications for public health and wildlife conservation efforts. By identifying the seasonal variation in the prevalence of *Trypanosoma spp.,* we can better assess the risk of transmission to humans and domestic animals, ultimately leading to more targeted and effective control measures.

MATERIAL and METHOD

Study area

The study was conducted in Kilombero district in southwestern Tanzania (Figure 1). The district lies between 08° 00′- 16° South and 36° 04′-36° 41′ East and covers an area of 14,246 km and elevation ranges from 262 to 550 m above sea level (Magesa, *et al.,* 2023). Kilombero district is situated within a large floodplain, bordered by the Kilombero River to the southeast and the Udzungwa Mountains to the northwest (Thonfeld, *et al.,* 2020). Southeast of the Kilombero River, the floodplain extends into the Ulanga District. The majority of residents are subsistence farmers growing maize and rice. In addition, Kilombero and Ulanga districts feature significant teak wood plantations.

The majority of the low-lying areas in the northwest of the district are occupied by sugar cane plantations (Alavaisha, 2020). The Kilombero Valley has significant potential for diverse livelihood activities. The wetland supplies water for domestic use, livestock, agriculture, and fishing (Alavaisha, 2020). This region's tropical climate is conducive to cattle rearing and crop cultivation (Mkonda, 2021). Due to ideal soil, water, and climatic conditions, the area supports a range of pastoral, agricultural, and agro-pastoral enterprises (Nindi, *et al.,* 2014). Additionally, rice cultivation contributes to increased food availability for small animals like rodents (Msofe, *et al.,* 2019). The study was conducted in eleven villages namely; Sagamaganga, Mkula A, Mkula B, Sonjo, Mlimani, Ichonde, Misufini, Kanolo, Mbalaji, Nyamwezi, and Mkasu as shown in the map of the study area.

Study Design and Sampling Strategies

This study used a cross-sectional design, involving eleven randomly selected villages. Rodent trapping took place during two seasons: November 2022 (dry season) and Febru-

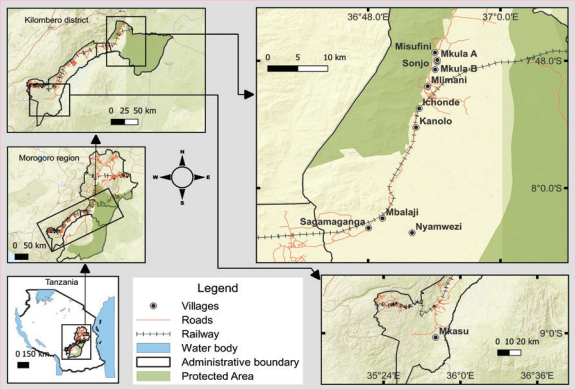

Figure 1. Map of Tanzania showing the study area. Map created in Quantum Geographic Information System (QGIS)

ary 2023 (wet season). The villages were selected based on their classification as residential and peridomestic areas. The sampling units for rodents included human residences and surrounding peridomestic areas, such as fallow lands near these settlements. The sample size for establishing the prevalence of Trypanosoma spp. in rodents was calculated according to the formula; $N=Z^2pq/e^2$ as previously described by (Katakweba, *et al.,* (2012).

Rodent Trapping and Identification

Sherman LFA live traps (7.5 × 9.0 × 23.0 cm; HB Sherman Traps, Inc., Tallahassee, FL) and modified box traps (9 x 10 x 23 cm) with an aluminum box with a wire mesh window on one side and a snap-back door on the other were used to catch rodents (Katakweba, *et al.,* 2012). The bait, which was a concoction of peanut butter and maize flour, was dropped into the traps. Small mammals were caught during the dry season (November 2022) and the rainy season (February 2023) in crop vegetation, peridomestic areas, and houses. Eight traps (four Shermans and four modified boxes) were placed in peridomestic regions in trap lines five meters apart. A similar method was used for crop vegetation. Two traps were set up inside the houses: a Sherman and a modified box. Traps were inspected and rebaited each morning if it was found that other insects and snails had eaten the bait. A Sherman or modified box trap was replaced with a new one after an animal was trapped in it. Traps were established at each significant location to enhance the capture rate throughout three successive nights of trapping. Body length, fur, ears, and tails were among the physical characteristics used to identify the seized rodent (Guibinga Mickala, *et al.,* 2021).

Sample Collection

Live rodents were anesthetized by using Diethyl ether. Samples collected from rodents were blood, liver, and spleen. Using a glass capillary, 20–25 µl of blood was extracted from the supraorbital vein (Katakweba, *et al.,* 2012). For every animal, a thick and thin blood smear was made on a microscope slide in the field and transported to the ACE II laboratory located at Sokoine University of Agriculture for further microscopic identification of trypanosomes. In the laboratory, they were submerged in 10% Giemsa stain (1:10 dilutions) for half an hour, followed by a 10-second rinse under running water, drying, and examination under a light microscope (×100 magnification with immersion oil). Also, impression smears were taken from spleen and liver samples for microscopic examination. On the other hand, the liver and spleen were extracted from anesthetized rodents, preserved in absolute ethanol, and transported to the Genome laboratory located at Sokoine University of Agriculture for further molecular analysis.

DNA Extraction

The extracted DNA was amplified using conventional PCR using 25 µl reaction volume that included 12.5 µl of Quick–Load Taq 2X master mix, a common 1µl of both forward and reverse B1 primers, 2.5 µl of BSA, 5 µl of nuclease-free water and 4 µl of purified DNA into partial coding regions of 18S rRNA gene and internal transcribed spacer 1 (ITS1) gene of *Trypanosoma spp.* (Mafie, *et al.,* 2019). Primers for PCR Amplification were as follows: for forward primer 5–CGTCCCTGCCATTTGTACACAC–3 and reverse primer 5–GGAAGCCAAGTCATCCATCG–3. The following amplifications conditions were implemented: 5 min of initial denaturation at 95°C, 35 subsequent cycles of denaturation at 95°C for 30 seconds, annealing for 45 seconds at 60°C, extension for 2 mins at 72°C, and a final elongation at 72°C for 10 mins. About 5 µl of each PCR product was run on 1.5% agarose gel stained with 5 µl of 10-mg/ml ethidium bromide for 40 minutes at 80 volts using 0.5X Tris borate-EDTA (TBE) as running buffer, followed by imaging with a UV transilluminator (Sigma Chemical USA). A Quick-load 100bp DNA ladder was used as a molecular size marker.

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Sequencing

PCR products were submitted in a volume of 20 µl to the Macrogen Europe facility in the Netherlands for Sanger dideoxy sequencing. PCR products were purified and then

Table1. Species of Rodents Caught in The Villages and Habitats

Villages	*Rattus rattus*				*Mastomys natalensis*				*Lemniscomys spp.*				*Gramomys spp.*			
	CV	H	PD	Total	CV	H	PD	Total	CV	H	PD	Total	CV	H	PD	Total
Ichonde	2	9	0	11	56	4	23	83	0	0	0	0	0	0	1	1
Kanolo	0	3	0	3	22	13	18	53	0	0	0	0	0	0	0	0
Mbalaji	0	5	1	6	24	8	21	53	0	0	0	0	0	0	0	0
Mkasu	0	4	0	4	24	0	6	30	0	0	0	0	0	0	0	0
Mkula A	0	0	0	0	1	2	1	4	0	0	0	0	0	0	0	0
Mkula B	0	1	0	1	7	0	2	9	0	0	0	0	0	0	0	0
Nyamwezi	2	0	1	3	16	2	20	38	0	0	0	0	0	1	0	1
Sagamaganga	1	2	0	3	11	3	18	32	0	0	0	0	0	0	0	0
Sonjo	0	4	0	4	6	0	2	8	0	0	0	0	0	0	0	0
Misufini	0	17	0	17	2	1	6	9	0	0	1	1	0	0	0	0
Mlimani	0	10	3	13	0	0	0	0	0	0	0	0	0	0	0	0
Total	5	55	5	65	169	33	117	319	0	0	1	1	0	1	1	2

Note: CV= Crop vegetation, H= House, PD= Peridomestic

Table 2. Microscopic examination of blood smears

Rodent Species		Screened	Positive	Prevalence (%)
Gramomys spp.	Male	0	0	0
	Female	2	2	100
	Subtotal	2	2	100
Mastomys natalensis	Male	201	34	16.91
	Female	118	60	50.85
	Subtotal	319	94	29.47
Lemniscomys spp.	Male	1	1	100
	Female	0	0	0
	Subtotal	1	1	100
Rattus rattus	Male	26	20	76.92
	Female	39	33	84.62
	Subtotal	65	53	81.54
	Total	387	150	38.76

sequenced in both forward and reverse orientations with the Applied Biosystems 3730xl DNA Analyzer (Thermo Fisher Scientific, Carlsbad, CA, USA). A total of 20 raw nucleotide sequences from both forward and reverse primers were cleaned and trimmed using Bioedit software. This software was also used to generate consensus sequences. To verify each sample's taxonomic identity, all consensus sequences were matched to published sequences in the GenBank nucleotide database using the Basic Local Alignment Search Tool (BLAST). Based on the minimum E-value, highest percentage identity, and greatest query coverage (Winterhoff, *et al.,* 2020).

Multiple Sequence Alignment and Phylogenetic Analysis

The sequences from this study were aligned with those of different Trypanosoma spp. obtained from GenBank, utilizing Molecular Evolutionary Genetics Analysis (MEGA) version 11 software. The ClustalW statistical algorithm utilized the Neighbor-Joining Method along with 1000 bootstrap replications to construct a phylogenetic tree (Tamura, *et al.,* 2021). Additionally, one *Babesia spp.* sequence (Accession KY250473) was downloaded from GenBank and included in the analysis as an out-group for tree

Data analysis

The gathered data were input into Microsoft Excel 2017 and later analyzed using Rstudio software. A chi-square test was conducted to assess differences in the prevalence of trypanosome infections across different species of rodents, villages, rodent sexes, seasons, and habitats. Differences were deemed statistically significant when the p-value fell below 0.05 (Goodrich, *et al.,* 2020).

RESULTS

Rodent Species

A total of 387 rodents were captured representing four genera of rodents namely *M. natalensis, Rattus rattus, Lemniscomys spp,* and *Gramomys spp*. *Mastomys natalensis* was the most prevalent species in the study area, accounting for 82.4% (n=319) of the total captures. *Rattus rattus* was the second most frequently captured species (n = 65, 16.8%), followed by *Gramomys spp.* (n = 2, 0.5%) and *Lemniscomys spp.* (n =1, 0.3%) (Table 2). Most of the *Mastomys natalensis* was caught in crop vegetation (n=169, 43%) while the majority of *Rattus rattus* was captured in houses (n=55, 14.2%), summarized in Table 1.

Trypanosomes in Blood Smears of Rodents

Blood smears from 387 rodents were screened for trapanosomes using a microscope, while 159 were further screened by PCR. Microscopic screening of the blood smears revealed a prevalence of 38.76% (n=150/387). The prevalence among different rodent species was as follows: *Gramomys spp.* (100%, n=2/2), *Mastomys natalensis* (29.47%, n=94/319), *Lemniscomys spp.* (100%, n=1/1), and *Rattus rattus* (81.54%, n=53/65) as summarized in Table 2.

Prevalence of Trypanosoma spp. according to sex, villages, habitat and season

The overall molecular prevalence of *Trypanosoma spp.* in rodents was 6.28% (n=10/159). Among eleven villages, the highest prevalence was found in Mbalaji at 50%), (n= 3/6), followed by Ichonde at 13.33% (n= 2/15), Sagamaganga at 10% (n= 1/10), Misufini at 6.45% (n= 2/31), Mkula B at 6.25%

Table 3. Molecular prevalence of Trypanosoma spp.

Variables	Categories	No. of Rodents Examined	No. of Positive Rodents	Prevalence	X^2 p-value
Villages	Overall	159	10	6.28	0.05
	Ichonde	15	2	13.33	
	Kanolo	10	0	0	
	Mbalaji	6	3	50	
	Misufini	31	2	6.45	
	Mkasu	18	0	0	
	Mkula A	3	0	0	
	Mkula B	16	1	6.25	
	Mlimani	14	0	0	
	Nyamwezi	19	1	5.26	
	Sagamaganga	10	1	10	
	Sonjo	8	0	0	
Sex	Male	80	6	7.5	0.86
	Female	70	4	5.71	
Season	Dry	70	6	8.57	0.66
	Wet	80	4	5	
Habitat	Crop vegetation	33	2	6.06	0.68
	House	65	5	7.69	
	Peridomestic	52	3	5.76	
Species	*Gramomys spp.*	2	0	0	0.08
	Lemniscomys spp.	1	0	0	
	Mastomys natalensis	94	3	3.33	

(n= 1/16), and Nyamwezi at 5.26% (n= 1/19). The remaining villages namely, Kanolo, Mkasu, Mkula A, Mlimani, and Sonjo, recorded a prevalence of 0%. Male rodents had a higher prevalence of *Trypanosoma spp.* at 7.5% (n=6/80) compared to female rodents which had a prevalence of 5.71% (n=4/70).

The prevalence of the *Trypanosoma spp.* in the dry season showed a higher prevalence of 8.57%) (n= 6/70) compared to the wet season at 5% (n= 4/80). Additionally, the prevalence of the *Trypanosoma spp.* varied by habitats: houses had the highest prevalence at 7.63% (n=5/65), followed by crop vegetation at 6.06% (n=2/33), and peridomestic areas at 5.76% (n=3/52). The highest prevalence of *Trypanosoma spp.* based on the rodent species was recorded in *Rattus rattus* at 13.21% (n= 7/53), followed by *Mastomys natalensis* at 3.33% (n= 3/94).

The lowest prevalence was recorded in *Gramomys spp.* and *Lemniscomys spp.* both at 0%. Additionally, the Chi-Square test showed that the prevalence of *Trypanosoma spp.* was significantly higher in rodents captured from Ichonde compared to other villages with a $p< 0.05$. In contrast, no significant differences were found between sex, seasons, habitats, and rodent species ($p>0.05$), as summarized in Table 3.

Molecular identification of Trypanosoma spp.

Of the 150 positive samples for *Trypanosoma spp.* from the microscopic examination, only 10 samples tested positive on conventional PCR analysis (Figure 2).

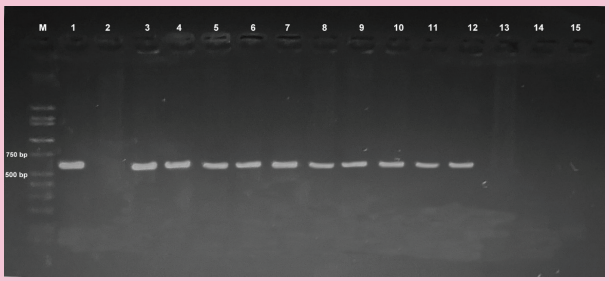

Figure 2. PCR amplification of partial coding region of the 18S rRNA gene & ITS1 gene of Trypanosoma spp. M is a DNA ladder while lane number 1 and 2 are positive and negative controls respectively. Lanes 3-12 are positive samples (623 bp), while lanes 13-15 are negative samples.

Sequencing/BLAST results

To verify each sample's taxonomic identity, 10 excellent sequences were compared with published sequences found in the National Centre for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). The percentage of sequences that were recognized as Trypanosoma lewisi ranged from 92% to 98%.

Phylogenetic analysis results

The phylogenetic tree was generated from four representative nucleotide sequences which were identified as *T. lewis* clustered together with *T. lewis* sequences from previous studies thus confirming the identity of isolates from this study to be *T. lewis*. The output of phylogenetic analysis (phylogenetic tree) is shown in Figure 3.

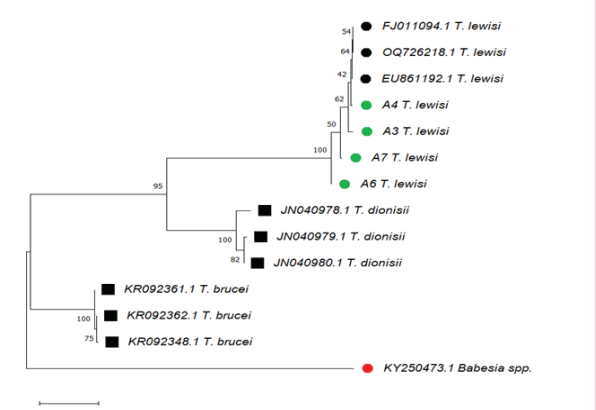

Figure 3. A phylogenetic tree constructed using the Neighbor-Joining Method (1000 bootstrap values) based on 623 bp of the 18S ribosomal RNA gene and internal transcribed spacer 1 (ITS1) gene partial coding regions of *Trypanosoma spp. Babesia spp.* sequence was used as an out-group. The sequences with green dots are isolates from this study while the ones with black icons are sequences retrieved from NCBI database.

DISCUSSION

The study aimed to assess the prevalence of *Trypanosoma spp.* in rodents in Kilombero district, Tanzania. The study reported the prevalence of *Trypanosoma spp.* in rodents by microscopic and PCR tests. This shows the potential role of rodents as reservoirs of trypanosomes. This shows the possibility of rodents carrying pathogenic and zoonotic *Trypanosoma spp.* that can have public health implications. A study conducted by Katakweba, *et al.,* (2013) on the prevalence of trypanosomes in the blood of *R. rattus* raises a public health concern that this commensal rodents could serve as a reservoir and vector for human or animal pathogenic trypanosomes, including *T. rhodesiense*, *T. gambiae*, and others. Overall microscopic prevalence of *Trypanosoma spp.* in rodents was higher than the 4% documented by Dada (2016) in Nigeria and Katakweba, (2018) in Morogoro and the 7% reported by Babyesiza *et al.,* (2024) in Uganda. The differences in geographical locations and sampling strategies could explain the differences in the prevalence of Trypanosoma. In contrast, the prevalence identified through PCR in this study was slightly lower than the 11% reported by Votýpka, *et al.,* (2022) across several sub-Saharan nations and the 13% reported by Babyesiza, *et al.,* (2024) in Uganda. This can be explained by the variations in the sensitivity of the detection techniques used in these various studies. The prevalence found in this study indicates that rodents of Kilombero are infected with Trypanosoma spp., and poses significant concerns as it can be transmitted to humans and other animals by way of vectors including ticks and fleas.

Molecular screening of *Trypanosoma spp.* was performed on liver and spleen tissues and, for microscopic screening, on blood samples. *Trypanosoma spp.* tends to replicate more often in the blood, being a fluidic milieu for optimal parasite antigenic diversity (Wardhana, *et al.,* 2024). This finding aligns with Pumhom, *et al.,* (2014) who found *T. lewisi* to be more prevalent in blood than other tissue types in Southeast Asia. In their study, about 13% of 616 rodents tested positive. Similarly, Magang, *et al.,* (2023), in Southern Cameroon reported high rates of blood infection among different rodent species, further corroborating that blood represents the preferred niche for these parasites.

The prevalence of *Trypanosoma spp.* was higher in Mbalaji than all of the other villages. That was specifically due to the number of rodents caught and found to be positive. This finding is in accord with Aleman, *et al.,* (2017) in Texas who found that the prevalence of *Trypanosoma spp.* was strongly determined by the number of rodents caught. In addition, Mbalaji possessed tall trees with thick vegetation cover that support harbored rodents which are the reservoir hosts for Trypanosoma spp., as well as the ectoparasites such as fleas and tsetse fly which are the vectors for Trypanosoma. This result is consistent with that of Yefi-Quinteros, *et al.,* (2018), who reported that rodents caught in densely vegetated regions frequently have greater Trypanosoma parasite burdens due to dense vegetation serving as a home for ectoparasites that are vectors of Trypanosoma, such as fleas and tsetse flies.

The prevalence of *Trypanosoma spp.* concerning rodent sex suggests that any sexual activity could be contagious. This is supported by the lack of statistically significant variation in Trypanosoma prevalence between rodent sexes. Although no significant difference was observed, male rodents were more prevalent than female rodents. This could be attributed to male rodents that are breeding may be the source of being more prevalent since they roam more in search of females to breed and establish habitat, which may expose them to different parasites that act as vectors for Trypanosoma. This result is in line with the findings of Babyesiza, *et al.,* (2024) in Uganda who reported that there is no significant difference in the prevalence between male and female rodents but male rodents had a higher prevalence of Trypanosoma compared to female rodents. Also, the study by Dahesh and Mikhail (2016) in Egypt reported that male rodents had a higher prevalence of *Trypanosoma spp.* than female rodents. Whereas no significant difference exists among seasons, higher prevalence was observed during the dry season than in the wet season. This is most probably because of the fact that rodents would all migrate towards areas with water and food, resulting in their nearing human and animal settlements where they are more prone to getting in contact with ectoparasites acting as vectors for *Trypanosoma spp.* This result agrees with the report by Pumhom, *et al.,* (2014) for Southeast Asia that rodents would tend to aggregate more frequently around water supplies when dry seasons increase, hence increasing rates of contact and transmissions of Trypanosoma through vectors like fleas and tsetse flies.

The higher prevalence of *Trypanosoma spp.* was recorded in rodents found in houses compared to those in peridomestic and crop vegetation areas. This could be explained by the greater concentration of rodents in houses, where they share ectoparasites and are in closer interaction with each other and other species. This agrees with the study conducted by Wardhana, *et al.,* (2024) in Indonesia, showing that the prevalence of Trypanosoma infection was higher in house-dwelling rodents. In the same line of agreement, Pumhom, *et al.* (2014) depicted that the prevalence of infection from rodents in human settlements was considerably higher due to the commonly inhabiting species favored by human-dominated habitats.

Trypanosoma spp. prevalence was greater in *Rattus rattus* than in other rodent species even though there was no significant difference in prevalence with other rodent species trapped. This is probably due to the fact that *Rattus rattus* exists in close association with human habitation and hence is more exposed to ectoparasites such as fleas and tsetse flies, which are vectors of Trypanosoma. Apart from that, *Rattus rattus* has the habit of residing in close association with other members of the same species, hence making it convenient for them to share ectoparasites. This finding is consistent with Tanthanathipchai, *et al.,* (2023), where it was noted that Rattus rattus is highly suited to co-inhabiting humans in Thailand due to the rats being prevalent within human societies, which is favorable for Trypanosoma transmission. Rodent infestation of human habitation raises the risk of transmission of trypanosomes to humans and animals.

Molecular characterization, sequencing, and phylogenetic analysis revealed *T. lewisi* in all 10 PCR-positive rodent samples. The results agree with reports by Archer, *et al.,* (2018), in South Africa and Kamaruzaman, *et al.,* (2021) in Malaysia which reported *T. lewisi* in rodents. The study contradicts, however, with Yefi-Quinteros, *et al.,* (2018), who reported *Trypanosoma cruzi* in rodents in Chile. This variation in the detection of *Trypanosoma spp.* may be due to geographical location and the presence of a vector that promotes the transmission of the parasite. Genes 18S rRNA and ITS1 were the marker genes utilized in detecting *T. lewisi* in this research, and this is in line with Garcia, *et al.,* (2019), who reported that PCR amplification and sequencing of the 18S rRNA gene and ITS1 region is an effective tool to identify *T. lewisi* in rodents. Phylogenetic analysis here used a 623 bp base pair long sequence, which was a little longer than the 483 bp (Egan, *et al.,* 2020), for detecting *T. lewisi* in Australia. *T. lewisi* detected inthe current study provides a signal of public health risk for the people of Kilombero that remains unreported. Within 24 h post-infection, the organism quickly divides into adult parasites (Zhang, *et al.,* 2019). Vertical transmission from mother to fetus is also possible (Desquesnes, *et al.,* 2022), enabling its rapid dissemination in rodent populations and, ultimately, into humans.

CONCLUSION

The study confirms rodents are infected with *T. lewisi,* with ectoparasites like fleas transmitting the infection. *Rattus rattus* is more susceptible. PCR detection is recommended for accurate detection. More studies are needed to understand the ecology and epidemiology of small mammal trypanosomes in Kilombero. Research on ectoparasites, rodents, and other small mammals is needed to determine Trypanosoma status, characterize species, and control ecological risks.

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CONFLICT of INTEREST

The authors declare that they have no conflict of interest regarding the research, authorship, and publication of this article.

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ETHICAL APPROVAL

The study complied with all legal requirements and safeguarded participant rights by adhering to ethical guidelines and the proper research approval. The research clearance was granted by SUA with reference number SUA/DPRTC/R/186/26 issued on 4 July 2024.

AUTHORS' CONTRIBUTIONS

AJ, the principal investigator (PI), took part in the first draft of the manuscript's conception, formal analysis, and data curation. AK is the primary supervisor, and JJ is the core supervisor. JJ and AK helped with the approach, supervision, review, editing, and rewriting of the manuscript. After reading the final draft of the manuscript, all authors have given their approval.

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