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Epidemiological survey on the identification of Leishmania spp in rodents and lizards from selected areas across the Rift Valley ecosystem, Tanzania: a pilot study

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Abstract

Background: Rodents and lizards are reservoirs of various infectious pathogens including Leishmania. This study aims to understand species composition of rodents and lizards in relation to leishmaniasis.

Methods: A cross-sectional study was conducted between April and May 2022. Sherman live traps were used to collect small mammals, whereas pitfall traps and hand catching were used to collect lizards. Small mammals and lizards were taxonomically identified. Spleen samples were collected separately from each specimen. Spleen impregnated smears were made on microscopic slides, fixed, and stained with 10% Giemsa stain before the cytological assay. Ecological indices were computed using paleontological statistical software.

Results: A total of 303 small mammals were collected, including six rodent genera/species: Mastomys natalensis 198(65.3%); Arvicanthis spp. 73(24.1%); Rattus rattus 9 (3%); Grammomys spp. 3 (1%); Tatera spp. 2 (0.7%); Lemniscomys spp. 1 (0.3%); and one shrew, Crocidura spp. (17, 5.6%). Species diversity was significantly higher in fallow land ($p=0.003$) and kraal ($p<0.0001$) than in farmland. Mastomys natalensis demonstrates spatial omnipresence and dominates farmlands. Nine lizards were collected, including Hemidactylus mabouia 7 (77.8%) and Trachylepis striata 2 (22.2%). A total of 21 (6.9%) rodents and 5 (55.5%) lizards demonstrated the presence of Leishmania spp. amastigote-like forms, notably M. natalensis 17(5.6%), Arvicanthis spp. 4(1.3%), Hemidactylus mabouia 4(44.4%) and Trachylepis striata 1(11.1%).

Conclusion: The presence of Leishmania in rodents and lizards indicates a potential reservoir role. Therefore, further research on molecular characterization of Leishmania spp. is warranted.

Keywords: Abundance, Cytology, Diversity, Leishmania, Reservoirs, Tanzania

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Background

Human leishmaniasis is a re-emerging sandfly borne neglected tropical disease caused by protozoan parasites of the genus Leishmania. The disease remains a threat to human life in four eco-epidemiological foci, namely America, East Africa, North Africa and West and Southeast Asia [1]. Nearly 1.6 billion people are at risk, with 12 million cases of infection, and over 1 million new cases are estimated to occur annually [1,2]. Nevertheless, the East African eco-epidemiological foci represent the burdened foci with 57% of visceral leishmaniasis cases followed by the Indian sub-continent [1]. Moreover, there are incidences that end up undetected and underreported thereof, as the disease co-occurs with other tropical diseases, such as malaria, tuberculosis, typhoid and schistosomiasis, which show similar initial clinical manifestations, thus increasing the chances of misdiagnoses [2]. Human leishmaniasis occur in four forms, notably, visceral (also, known as kala-azar), cutaneous, mucocutaneous, and post kala-azar dermal leishmaniasis (PKDL), dependent on the Leishmania species and host immune response [1]. Consequently, humans suffer from stigmatization to life threatening symptoms, based on the disease form. Unlike cutaneous leishmaniasis (CL), visceral leishmaniasis is the most prevalent form of disease in Eastern Africa, particularly Kenya [3,4]. Visceral leishmaniasis (VL) is a chronic systemic disease characterized by fever, (hepato) splenomegaly, lymphadenopathy, pancytopenia, weight loss, weakness, and, if left untreated, death. Additionally, VL can result in PKDL as a skin sequela, occurring mainly within 3–12

months after treatment [4,5]. The disease involves a complex transmission cycle due to the various species involved, sandfly vectors, and animal reservoirs beyond the human host [6]. Leishmania are obligatorily dixenous, possessing zoonotic or anthroponotic life-cycles [7]. Several Leishmania species, such as, L. infantum and L. tropica, and L. major are involved in sylvatic transmission, with the consequence of VL and CL infections, respectively [8]. Recently, L. donovani, which is primarily involved in anthroponotic transmission, was detected in rodents [9]. Indeed, animal reservoirs play a key role in maintaining endemicity. Rodents are hazardous, hyper reservoirs of zoonotic infectious pathogens, such as Yesinia spp., Leishmania, Leptospira, Trypanosomes, Hanta and Lassa viruses just to name but a few [10–12]. The role of rodents in human diseases is very clear in sub-Saharan Africa [13]. In Tanzania, several rodent-borne diseases have been documented. Nevertheless, rodents are only remarkable for the history of plague, which claimed thousands of lives [14,15]. This alerts our attention to emerging diseases such as leishmaniasis. As long as rodents and sandflies share nocturnal activity in the same ecological niche, this phenomenon possibly simplifies the availability of blood meal hosts for sandflies; hence, possible Leishmania transmission [6]. Consequently, human-rodent-vector interactions often accelerate the potential risk of contracting diseases in humans, especially in rural settings [10].
Lizards are well known to harbor Sauroleishmania, which is non-pathogenic to humans, but recently pathogenic Leishmania DNA has been reported in lizards [16,17]. Therefore, rodents and lizards are of particular concern in maintaining sylvatic transmission of zoonotic diseases, among others. Animal reservoirs efficiently harbor and serve as sources of Leishmania and play a crucial role in disease epidemiology. In Eastern Africa the disease is endemic in most semi-arid areas of Rift Valley ecosystem, where outbreaks are often associated with human-induced changes such as urbanization and extension of agriculture activities [18]. Indeed, Tanzania is part of the eastern Africa great Rift Valley ecosystem (EAGRE), with reported disease incidence [19], nevertheless, the epidemiology of leishmaniasis is grossly unknown. Similar to other endemic countries, epidemiological surveys are vital to validate the presence or absence of a pathogen within reservoirs in a specific area [20]. Moreover, despite the limited sensitivity of cytological examination of amastigotes, it remains the gold standard in routine hospitals for the confirmation of leishmaniasis [3,21].
The goal of this study was to analyze species composition of rodents and lizards in relation to Leishmania parasites. The findings herein establish a roadmap for the surveillance and understanding of leishmaniasis epidemiology toward disease prevention and control.

Methods

Study area

This study was conducted in selected areas along the eastern African great Rift Valley ecosystem in the Arusha and Manyara regions of Tanzania. The Arusha region is located at latitudes of 2° S to 6° S and longitudes of 35° E to 38° E, with an elevation of 1400 m above sea level. Manyara is located at a latitude of 3° 40’ S to 6° S and a longitude of 33° E to 38° E, and its elevation varies. The mean annual rainfall ranges from 800–1200 mm and 450–1200 mm in Arusha and Manyara, respectively. Both regions exhibit bimodal rainfall patterns. In Arusha, the long rainy season runs from March to May and short rainy season runs from October to January, whereas in the Manyara region, the long rainy season runs from January to May and short rainy season runs from October to December. The dry season runs from June to October in both regions. Overall, the temperature varies from 13 °C to 28 °C in the Arusha region and from 13 °C to 33 °C in the Manyara region [22,23]. These regions are generally considered semi-arid. The main economic activities in both regions are tourism, semi-pastoralism, and subsistence farming. In both regions, human-wildlife interactions are obvious because of their proximity to national parks and other forms of natural reserves [22,23].

Study design

A cross-sectional study design was used whereby three wards were selected for sampling. Notably, Migungani and Selela wards from the Monduli District in the Arusha region, and Dareda ward from the Babati District in the Manyara region “Fig. 1”. In addition, wards were purposively selected based on their proximity to protected areas and geographical features such as, elevation.

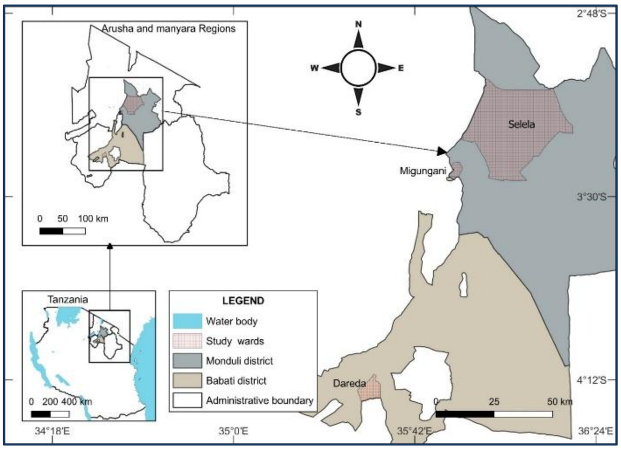

Figure 1: Map showing study wards along the eastern Africa great rift valley ecosystem, Babati and Monduli districts, Tanzania. The map was developed on February 2024 using QGIS software version 3.26.1

Trapping and processing of small mammals and lizards

Small mammals (rodents and shrews) were captured by using Sherman LFA live traps (7.5 × 9.0 × 23.0 cm: HB Sherman traps, Inc, Tallahossee, FL). The traps were baited with peanut butter mixed with maize flour, three Sherman traps were placed inside houses (indoors) for three consecutive nights, at strategic points (corridors and kitchen) in order to increase capture rate [10]. In farmland, fallow land and kraal (boma enclosure) Sherman traps were placed in line transect whereby each line trap had 10 traps situated 7 m apart and 5 m apart between line traps [10]. In addition, lizards were collected by pitfall traps, and hand catching in various habitats as mentioned above. Captured rodents, shrews, and lizards were euthanized using Diethyl Ether for blood and tissue biopsies from the spleen to screen for Leishmania infections. To prevent cross-contamination, a sterile syringe was utilized to obtain 1 ml of blood from the heart for blood smears preparation. A sterile scalpel was used to collect visceral samples (spleen) from the specimens. All collected tissues were stored in 70% ethanol, organized by species, and labeled accordingly. Spleen-impregnated smears were prepared by gently pressing the biopsy specimen with forceps onto a clean slide [24].

Identification of small mammals and Lizards

Captured small mammals were taxonomically identified at the species/genus levels according to established taxonomic key [25]. Morphological taxonomic features used included weight, fur characteristics, and body measurements. Additionally, the sex of the small mammals was identified (male or female), as described [26]. Lizards were identified according to existing valid taxonomic key [27].

Identification of Leishmania from small mammals and Lizards

Blood and spleen-impregnated smears were made on microscopic slides, allowed to air dry before fixing with methanol, stained with 10% Giemsa stain for 20 min, washed with tap water, and air-dried for 15 min. Amastigotes were carried under a X100 oil emersion objective. All examinations were performed at the laboratories of the Institute of Pest Management and Department of Microbiology, Parasitology, and Biotechnology.

Data analysis

The data were organized and cleaned using Microsoft spreadsheet version 2019. Relative abundance (RA%) was calculated as the ratio of the number of specimens for each species to the total number of specimens caught ×100. Ecological indices, species richness, evenness and diversity were computed using Paleontological Statistics software (PAST) [29]. A Student's t-test was used to compare diversity indices between habitats [30]. Statistical tests were considered significant when the p-value <0.05.

Results

Species composition and abundance

A total of 303 small mammals and nine lizards were captured at different localities in the Monduli and Babati districts. Of the 303 small mammals, representing six rodent genera/species: Mastomys natalensis 198 (65.3%); Arvicanthis spp. 73 (24.1%); Rattus rattus 9 (3%); Grammomys spp. 3 (1%); Tatera spp. 2 (0.7%); Lemniscomys spp. 1 (0.3%); and one shrew, Crocidura spp. 17 (5.6%). Male and female constituted 170 (56.1%) and 133 (43.9%) respectively (Table 1). The most predominant species was M. natalensis (65.3%), which was collected from all wards, whereas the least dominant species was Lemniscomys spp. (0.3%). Unlike the former, the latter were collected from a single ward at a low number. Additionally, nine lizards were collected, comprising two species: Trachylepis striata 2 (22.2%) and Hemidactylus mabouia 7 (77.8%) (Table 2). Hemidactylus mabouia was captured on termite hills, whereas Trachylepis striata was trapped in rocks. The composition and abundance of rodents and Crocidura varies across wards. Migungani represented higher abundance, followed by Selela, and Dareda (Table 1). Across habitats, farmland shown high abundance, followed by kraal, fallow land, and indoors (Table 3). Despite the differences in location, Mastomys natalensis was the most abundant species in all wards and dominated farmland and fallow land. Arvicanthis spp. were more dominant in kraal, whereas Rattus rattus was the only species found indoors. Moreover, a small number of pregnant M. natalensis and Arvicanthis spp. females were observed (Table S1).

Table 1. Species composition across localities and relative abundance of small mammals.

Small mammals	Species	Wards			Total captured	Sex		Relative abundance (%)
		Migungani	Selela	Dareda		Male	Female	
Rodents	*Mastomys natalensis*	62	73	63	198	100	98	65.3
	Arvicanthis spp.	44	29	0	73	48	25	24.1
	Rattus rattus	3	5	1	9	5	4	3
	Grammomys spp.	2	0	1	3	3	-	1
	Tatera spp.	2	0	0	2	2	-	0.7
	Lemniscomys spp.	0	0	1	1	1	-	0.3
Shrew	*Crocidura* spp.	5	4	8	17	11	6	5.6
	Total (7 species)	118	111	74	303	170	133	100

Table 2. Species composition and prevalence of Leishmania infection in lizards.

Common name	Scientific name	Wards		Total captured	Infected lizards	Prevalence
		Migungani	Selela			
African stripped skink	*Trachylepis striata*	2	-	2	1	11.10%
Tropical house gecko	*Hemidactylus mabouia*	6	1	7	4	44.40%
Total (2 species)		8	1	9	5	55.50%

Diversity of rodents

The species diversity varied across wards. Migungani ward had a higher diversity value than Selela, and Dareda. Nevertheless, the evenness index revealed that rodent community at Selela was more even than those at Migungani and Dareda. Across habitats, diversity was significantly higher in fallow land than in farmland ($t = 3$, $df = 50.1$, $p = 0.003$), and significantly higher in kraal than in farmland ($t = 4.6$, $df = 267.6$, $p < 0.0001$), whereas there was no significant difference between fallow land and kraal ($t = -0.18$, $df = 42$, $p = 0.857$). No diversity was observed indoors in this study (Table 4).

Table 3. Species composition and abundance of rodents and Crocidura across habitats.

Species	Habitats				Total
	Farmland	Fallow land	Kraal	Indoor	
Mastomys natalensis	140	16	42	0	198
***Arvicanthis* spp.**	4	0	69	0	73
Rattus rattus	0	0	0	9	9
***Grammomys* spp.**	0	1	2	0	3
***Tatera* spp.**	0	0	2	0	2
***Lemniscomys* spp.**	1	0	0	0	1
***Crocidura* spp.**	8	9	0	0	17
Total (7 species)	153	26	115	9	303

Table S1: Number of pregnant female rodent observed

Species	Male	Female	Pregnancy	Prevalence %
Mastomys natalensis	100	98	29	29.5 (29/98)
***Arvicathis* spp.**	48	25	2	8 (2/25)
Rattus rattus	5	4	-	-
***Grammomys* spp.**	3	-	-	-
***Tatera* spp.**	2	-	-	-
***Lemniscomys* spp.**	1	-	-	-
***Crocidura* spp.**	11	6	-	-
Total (7 species)	170	133	31	

Prevalence of Leishmania

Of the 303 samples screened, none of the blood smears were positive, whereas 21 (6.9%) were found to be infected with amastigote-like forms in the spleen, which constituted M. natalensis 17 (5.6%) and Avicanthis spp. 4 (1.3%). Two M. natalensis individuals were observed exhibiting distinct clinical infestations, one presented with splenomegaly, while the other displayed ulcerative lesions on the nose. The habitat and ward from which each positive sample was recorded are presented in supplementary material 1 (Table S2). Lizards were positive for amastigote-like forms in 5/9 (55.5 %). Of these, 1 (11.1%) and 4 (44.4%) were Trachylepis striata and Hemidactylus mabouia, respectively (Table 2).

Table 4. Ecological parameters of rodent species across habitats and wards.

Parameters	Habitats				Wards		
	Farmland	Fallow land	Kraal	Indoor	Migungani	Selela	Dareda
Abundance	153	19	115	9	118	111	74
Species Richness (R)	4	3	4	1	6	4	5
Shannon (H’)	0.3637	0.8629	0.8153	0	1.072	0.8857	0.552
Evenness_(E)	0.3597	0.79	0.565	1	0.4866	0.6062	0.3473

Table S2. Prevalence of Leishmania infection in rodents across habitats and wards

Species	Dareda				Migungani				Selela				
	*Fl	Fr*	K*	Indoor*	*Fl	Fr*	K*	Indoor*	*Fl	Fr*	K*	Indoor*	Total (Prevalence)
Mastomys natalensis	1/9	6/54	-	-	0/7	1/13	4/42	-	-	5/73	-	-	17/198 (8.5%)
***Arvicathis* spp.**	-	-	-	-	-	-	1/44	-	-	0/4	3/25	-	4/73 (5.4%)
Rattus rattus	-	-	-	0/1	-	-	-	0/3	-	-	-	0/5	0/9 (0.0%)
***Grammomys* spp.**	0/1	-	-	-	-	-	0/2	-	-	-	-	-	0/3 (0.0%)
***Tatera* spp.**	-	-	-	-	-	-	0/2	-	-	-	-	-	0/2 (0.0%)
***Lemniscomys* spp.**	-	0/1	-	-	-	-	-	-	-	-	-	-	0/1 (0.0%)
***Crocidura* spp.**	0/5	0/3	-	-	0/4	0/1	-	-	-	0/4	-	-	0/17 (0.0%)
Total (Prevalence)	1/15 (6.6%)	6/58 (10.3%)		0/1 (0.0%)	0/11 (0.0 %)	1/14 (7.1%)	5/90 (5.5%)	0/3 (0.0%)		5/81 (6.1%)	3/25 (12%)	0/5 (0.0%)	21/303 (6.9%)

*Fl: Fallow land; Fr: Farmland; K: Kraal: In: Indoor

Discussion

In the present study, six rodent genera/species and one shrew species were recorded. All genera identified herein are well known in Tanzania and beyond [31,32]. The male-to-female ratio was approximately 1.3, with a slightly higher number of males (170) than females (133). Similar observations have been reported elsewhere in Tanzania [10]. Male rodents have been shown to have a wider home range during the wet season compared to the dry season [33], suggesting a slightly higher number of males captured in the wet season. Additionally, a small number of pregnant M. natalensis and Arvicanthis spp. females were also observed. This observation may be due to seasonal effects; our samples were collected during the late wet season, a period characterized by sufficient feed, which mostly coincides with the rodent breeding season [33,34]. Mastomys natalensis was the predominant species, followed by Arvicanthis spp. Notably, M. natalensis is the only rodent species recorded across all wards, indicating its omnipresence. The least abundant species were Lemniscomys spp., and Tatera spp, both of which were recorded in a single ward and in low numbers. This is in line with Datiko et al. [35], who reported that Lemniscomys spp. and Tatera spp. were less distributed species. Furthermore, the occurrence of M. natalensis in higher numbers is not surprising, as it has been reported in various regions of Tanzania; Kilosa [10], Mbulu [36], Mbeya [37], and beyond [31]. This could be due to the wide distribution of the species throughout East Africa, as well as its breeding habit, with an average litter size of 11 [32,38]. Across sampled habitats, farmland and kraal not only host higher species richness but also a higher abundance compared to the other habitats. Unlike M. natalensis, Arvicanthis spp. were only found in kraal and farmland. Mastomys natalensis dominated farmland and fallow land, whereas Arvicanthis spp. dominated kraal, with few observations on farmland. The tendency of M. natalensis to dominate farmland and fallow has been widely documented [39,40], and could be attributed to their generalist feeding behavior [41]. Additionally, R. rattus was the only species captured indoors. This could be due to species nesting behavior [42]. Moreover, Tatera spp. and Lemniscomys spp. were limited to kraal and farmland, respectively. This indicated that rodent species adapt differently to different ecological settings [35,43]. The diversity index was higher at Migungani (H’ 1.07, R = 6, E = 0.486) compared to Selela (H’ 0.88, R = 4, E = 0.606) and Dareda (H’ 0.55, R = 5, E=0.347). However, a low evenness was noted at Migungani, signifying that the community was overexpressed with specific species. Additionally, Selela had low species richness while maintaining ecological diverse. This phenomenon could be due to the balance of rodent species within different microhabitats [44]. In contrast, overexpression of M. natalensis and Arvicanthis spp. in farmland and kraal altered the community diversity structure. The diverse community is said to be species-rich and evenly abundant [44]. In that context, fallow land (H’ 0.86, R = 3, E = 0.79) was found to host diverse community, with a high diversity value and evenness, compared to other habitats. However, the high abundance of M. natalensis in farmlands could be due to species nesting and feeding behavior. Mulungu et al. [41], reported that M. natalensis are specialized granivores and herbivore species and are well-known crop pests on farms. Among the screened rodents, none were detected for Leishmania in blood smears. Nevertheless, M. natalensis (8.58%, 17/198) and Arvicanthis spp. (5.47%, 4/73) demonstrated Leishmania spp. from a spleen-impregnated smear. This support other findings that imprinting and/or spleen aspirates has relative high sensitivity under microscope compared to other samples [24,45]. Leishmania have been detected in rodents in many areas beyond Tanzania. Kassahun et al. [6], reported Leishmania prevalence rates of 15.3% and 17.4% for M. natalensis and Arvicanthis spp. respectively, in Ethiopia. The difference in prevalence may be due to differences in sample size as well as the region level of endemicity [6,46]. The non-detection of Leishmania in peripheral blood smears suggests low parasite load in rodents, since early dissemination of Leishmania starts in the spleen [47,48]. In addition, the absence of parasites in other rodent species and shrews could be due to limited exposure to sandfly vectors, subsequent to species nesting behavior and activity pattern [41–43,49]. Of 9 sampled lizards, 5 (55.5%) were positive for Leishmania spp. amastigote-like forms, which constitute 4 (44.4%) H. mabouia and 1 (11.1%) T. striata. However, Mendoza-Roldan et al [17], reported a prevalence of 35.7% in lizards. The difference in prevalence could be due to the different lizard species studied, sample sizes and levels of regional endemicity. Importantly, despite the dogma that lizards carry non-pathogenic Leishmania, the coexistence of pathogenic and non-pathogenic Leishmania in lizards have been documented [16]. Therefore, the presence of Leishmania in lizards and rodents in the study area provide clues for the possible reservoir role, however, further research on molecular characterization of Leishmania species is required.

Conclusion

We demonstrated the presence of Leishmania spp. amastigote-like form in rodents and lizards in an area previously documented having potential sandfly vectors. Furthermore, the agricultural rodent pest, Mastomys natalensis accounts for the majority of infections. These findings underscore the necessity for further research on the molecular characterization of Leishmania species.

Abbreviation

CL: Cutaneous Leishmaniasis; DNA: Deoxyribonucleic acid; EAGRE: East Africa Great Rift Valley Ecosystem; PKDL: Post kala-azar Dermal Leishmaniasis; VL: Visceral Leishmaniasis

Declaration

Acknowledgment

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Availability of data and materials

Data will be available by emailing alysamiji@gmail.com

Authors' contributions

All authors contributed equally in the conceptualization, manuscript writing, and interpretation of the findings. The authors read and approved the final manuscript.

Ethics approval and consent to participate

We conducted the research following the declaration of Helsinki. Research clearance was obtained from the Sokoine University of Agriculture with reference number (SUA/ADM/R.1/8/772) issued on 18/11/2021. In addition, permission from the study regions was secured from the Arusha (FA.132/195/01'P'/375) and Manyara (FA.262/347/01K/344) regions. Verbal consent was obtained from the community who participated/allowed rodents to be trapped in their houses and farms.

Consent for publication

Not applicable

Competing interest

The authors declare that they have no competing interests.

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