Detection and determinants of Leptospira infection in rodents, cattle and humans in Muheza District, Tanzania: A One Health Appeal

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Abstract

Interaction between humans, livestock, and wildlife plays an important role in zoonotic disease transmission. The emergence of Leptospira in humans, rodents, and cattle remains relatively understudied. A cross-sectional study was conducted between February and May 2023 in Muheza to determine evidence of Leptospira infection and associated risk factors in rodents, cattle, and humans. A total of 479 serum samples from rodents (n=201), humans (n=198), and cattle (n=80) were examined by using the Microscopic Agglutination Test (MAT) to detect antibodies against six live Leptospira stock culture serovars, including Pomona, Hebdomadis, Canicola, Grippotyphosa, Sokoine, and Lora. Additionally, a questionnaire survey was conducted on 140 respondents to establish potential risk factors for transmission of Leptospira. The overall Leptospira seroprevalence in rodents, cattle and humans was 6.0% (12/201; 95% CI: 3.12%-10.20 %), 12.5% (10/80; 95% CI: 6.16%-21.79%) and 13.13% (26/198; 95% CI: 8.76%-18.65%), respectively and the most predominant serovars were Grippotyphosa, Sokoine, and Hebdomadis. A significant difference in the seroprevalence was observed in the occupation category, with farmers likelier to be infected with leptospirosis than those in other categories ($\chi^2 = 9.19$, df = 3, p = 0.027). This study showed co-agglutination between rodents, cattle, and humans with serovars Hebdomadis, Sokoine, and Grippotyphosa. People aged 36-59 had the highest seropositivity, suggesting they are the most at-risk group. Our study shed light on pathogenic serovars circulating among humans, rodents, and cattle and their associated risk factors. It also highlights the importance of employing a One Heath approach in controlling zoonotic disease.

MATERIALS AND METHODS

Study design and Sampling

The study, conducted from February to May 2023, enrolled 479 participants (humans, rodents, and cattle) for serum testing and 140 respondents for a questionnaire survey. Rodent trapping involved setting live traps in houses, farms, and peridomestic environments. Rodents were identified by species, and blood samples were collected from both rodents and cattle for serum separation.

Inclusion and exclusion criteria

The study included patients of all ages with a fever who visited Teule Hospital and Ubwari Health Centre, live rodents, and cattle older than one year. Humans unwilling to consent were excluded from the study.

Rodents trapping

Rodent trapping was carried out in houses and around human dwellings, farms proximal to human settlements such as cultivated cropland by using Sherman live traps (7.5 × 9.0 × 23.0 cm) and locally made live traps

with wooden box wire mesh window (12 ×15 ×20cm) (Mlowe *et al* ., 2023). Captured rodents were collected, anesthetized with Diethyl Ether preserved with ethanol, and identified to species level using the established taxonomic nomenclature. Rodent morphometric data including weight, total length, tail length, hind foot length, and ear length were then recorded (Mgode *et al* ., 2019; Mgode *et al* ., 2021).

Blood sampling

From rodent captured, 1 to 2 ml of blood was aseptically collected from the heart puncture or plexus by using sterile syringes and needles. In cattle, which were randomly sampled from different herds, 4 to 10 ml of blood was aseptically collected from the jugular using sterile syringes and needles after proper manual animal restraint. In humans, the study involved individuals with febrile illness who were visiting Teule Hospital and Ubwari Health Center. A 2 to 4-ml blood sample was aseptically collected from each study subject by medical personnel using sterile syringes (*Mgode et* al., 2019). Socio-demographic information of study participants, including sex, occupation, location, and age was recorded.

The samples were immediately transferred into plain vacutainer tubes and allowed to clot for serum separation at room temperature for at least 30 minutes. The samples were centrifuged for 10 minutes at 3000 rpm to increase the serum volume. The harvested sera were subsequently transferred into well-labeled Eppendorf tubes and then stored at -20?C until subjected to MAT. (Mgode *et al* ., 2019).

Testing

The Microscopic agglutination test (MAT) was used to detect antibodies against six Leptospira serovars: Pomona, Hebdomadis, Canicola, Grippotyphosa, Sokoine, and Lora. Serum samples were tested to determine seroprevalence, and the chi-square test was employed for statistical analysis.

Ethical approval

The research ethical clearance for conducting this study was approved by the Research Ethics Committee at Sokoine University of Agriculture (Ref. No. SUA/ADM/R.1/8/967) issued on 29th December 2022. The permission to conduct research in Muheza was obtained from the Medical Research Coordinating Committee of the National Institute for Medical Research with reference number NIMR/HQ/R.8a/Vol. IX/4269 issued on 18th April 2023. Moreover, before commencing data collection in the study area, authorization was granted from all local authorities including Muheza district's medical officer (DMO), and village chairmen. Human participants were verbally informed that their blood might be collected and tested for leptospirosis and verbal consent was obtained from them.

RESULTS

Seroprevalence of leptospiral antibodies in various rodent species captured in the study site

Leptospiral antibodies were detected in this study, whereby 201 rodents were tested. A seroprevalence of 6.0% (12/201; 95% CI: 3.12%-10.20 %). The highest infection rate was found in *Rattus rattus* at 4% (8/201) followed by *Mastomys natalensis* at 1.5% (3/201), and the lowest was in *Acomys* spp as shown in Table 1.

Table 1

Cattle Seroprevalence:

Out of 80 cattle sampled, 12.5% tested positive for leptospirosis, with no significant differences in seroprevalence based on sex, location, or grazing patterns.

Table 2

Human Seroprevalence:

Among 198 human participants, the seroprevalence was 13.13%. Farmers were the most affected occupation group, showing a significantly higher infection rate compared to other professions. Age was another significant factor, with individuals aged 36-59 years showing the highest rates of infection.

Table 3

Serovar Prevalence Across Species

The predominant serovars in rodents, cattle, and humans were Grippotyphosa, Sokoine, and Hebdomadis. Notably, co-infection with different serovars was observed across species. For example, humans and rodents exhibited co-infections with serovars Lora and Pomona.

Table 4

not-yet-known not-yet-known

not-yet-known

unknown

Table 5

DISCUSSION

This study provides crucial insights into the zoonotic transmission cycle of leptospirosis in the Muheza District. The detection of leptospirosis in both livestock and humans indicates that agricultural and livestock practices contribute to the endemicity of the disease. The high seroprevalence in rodents, particularly *Rattus rattus* , underscores their role as a significant reservoir, while cattle likely act as incidental hosts, furthering the transmission to humans. (Schoonman & Swai, 2009, 2010; Swai & Schoonman, 2012a; Motto *et al* ., 2023;).

In the present study, the seroprevalence of antibody-specific leptospiral antigens among humans, rodents, and cattle was examined by using MAT as the reference serological method (Niloofa *et* al., 2015). The overall seroprevalence in rodents, humans, and cattle was 5.97%, 13.13% and 12.5% respectively. The difference could be due to the previous exposure to prophylactic use of antibiotics such as amoxicillin and ampicillin to treat dry cough and the use of doxycycline to treat stomach pain, explicitly patients attending hospitals within the study locality which acts as an antibacterial lowering prevalence of leptospirosis.

These findings revealed that the *Rattus rattus* is a potential reservoir of leptospirosis in Muheza district, as evidenced by the high infection rate among the rodents collected from various habitat types within study sites. This can be attributed to the high population in the area. But also, the fact that the species cohabits with humans and cattle, as it is mostly found in houses and peri-domestic could be another reason for being a potential reservoir of leptospirosis. Comparison of the seroprevalence of *Leptospira* serovars among rodent species showed statistical differences for habitat types and locations. The current finding resembles other studies conducted elsewhere in Tanzania, which also, unveiled the existence of leptospiral antibodies in Rattus rattus *(Katakweba et al., 2012; Assenga et al., 2015; Mgode et al., 2019; Motto et al., 2021; Mlowe et al., 2023)* **.** Moreover, in rodents, the predominant serovar was Sokoine at 3.48% (n=7). The predominant of the serovar Sokoine in rodents may be attributed to their potential role as a source of leptospiral infection in humans and animals.

The variation in seroprevalence among different species and habitat types highlights the need for targeted interventions based on ecological and occupational factors. The finding that farmers are at the highest risk reinforces the importance of occupational health measures, such as protective clothing and improved hygiene practices, to reduce exposure. (Nthiwa *et al.,* 2019). This is in line with other previous studies that have highlighted farmers as being the highest risk groups for leptospirosis in parts of Tanzania and elsewhere (Schoonman & Swai, 2009; Hashemi *et al* ., 2021; Maze *et al* ., 2023). Other researchers highlighted various groups including dog keepers (Msemwa et al., 2021), livestock keepers (Motto *et al.,* 2023), fishing communities (Mgode *et al.,* 2019), slaughter pigs (Ngugi *et al.,* 2019), slaughtered cattle (Swai & Schoonman, 2012b) as well as miners and sewage workers (Hashemi *et al.,* 2021) being the highest risk group for contracting leptospirosis.

Interestingly, in humans, the predominant serovar was Grippotyphosa followed by Sokoine. Other prevalent serovars included Hebdomadis, Pomona, and Lora, with none detected in Canicola. The predominant of Grippotyphosa in humans could be due to the high contact rate between humans and cattle within the study locality, especially during the milking stage. The predominance of Grippotyphosa in cattle may be due to their role as maintenance hosts for this serovar. Cattle are recognized as natural and maintenance hosts for serovar Grippotyphosa (Soares*et al.,* 2020). (Assenga *et al.,* 2015; Mgode *et al* ., 2021; Msemwa *et al.,* 2021; Majawa *et al.,* 2023; Mlowe*et al.,* 2023).

The study's identification of co-agglutination between rodents, cattle, and humans for multiple serovars further emphasizes the complex dynamics of leptospirosis transmission in multi-host systems. The shared environment, particularly water sources, likely plays a key role in maintaining the infection cycle between these species. Mgode *et al* ., 2021; Msemwa *et al.,* 2021; Majawa *et al.,* 2023).

The seroprevalence data for rodents, cattle, and humans in Muheza District is consistent with other studies conducted in Tanzania, such as in Katavi, where a high prevalence was reported in humans (29.96%), and in Tanga, where bovine leptospirosis was also prevalent (Motto *et al* ., 2023). These results underscore the importance of considering local environmental and socio-economic factors when developing control measures.

Limitations of the study.

In Africa, the diagnosis of leptospirosis using the Microscopic Agglutination Test (MAT) involves the recommended use of 10 serovars (Mirambo *et al.,* 2022). The current study may have underestimated the seroprevalence of *Leptospira* antibodies because only six serovars were included in the panel. Further studies should enhance diagnosis techniques.

However, our study was conducted only during the rainy season, as most cases of leptospirosis occur during this time. It is recommended that further studies be conducted during the dry season to help control rodents and reduce human and animal exposure to leptospirosis. Additionally, future research should include molecular characterization of leptospiral infection to identify species levels.

Conclusion and recommendations

Finally, the study advocates for a multisectoral One Health approach to control leptospirosis in the region. This would involve collaboration between public health, veterinary, agricultural, and wildlife sectors to implement effective surveillance and intervention strategies. The authors recommend further research to improve diagnostic methods and expand studies into different seasons and geographical areas to better understand the seasonal and spatial dynamics of the disease.

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Conflict of interest

We declare that no conflict of interest exists in this article.

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Figure 1. Map of Muheza districts, Tanga region, Tanzania showing the study sites

Reference: QGIS. 3.34.1-Prizren; EPSG: 4326- WGS 84.

Figure 2. Co-infection with different leptospiral serovars in rodents, humans, and cattle

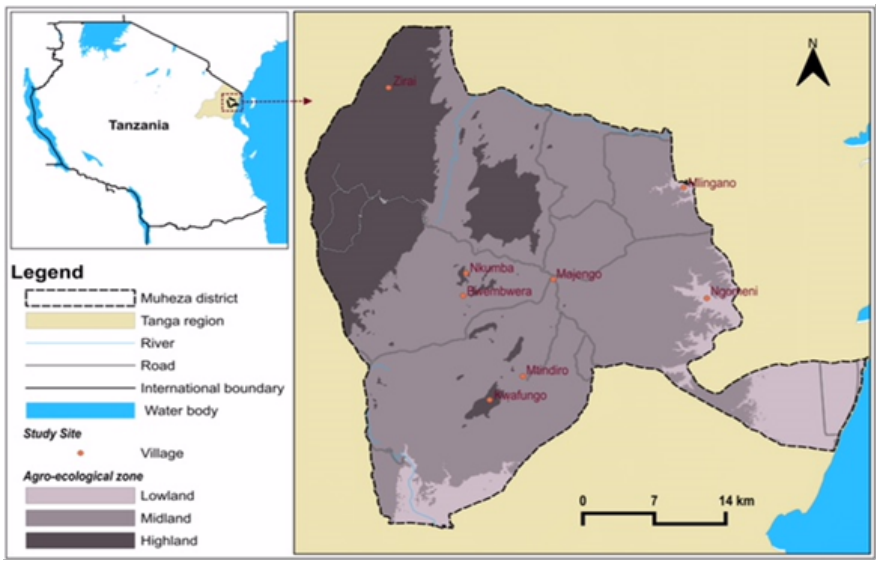
Table 1. Rodents' species captured and their seroprevalence of leptospiral antibodies in various species of rodents in the Tanga region, Tanzania (n=201)

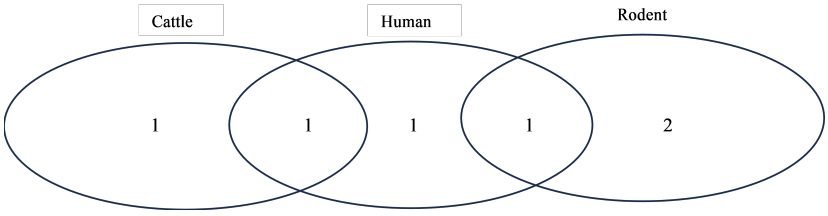
Table 2. Sero-prevalence of Leptospira antibodies in cattle in the study area (n= 80)

Table 3. Number and percentage of study subjects found seropositive to *Leptospira* bacteria with socio-demographics information of the study participants (n=198)

Table 4. Seroprevalence of different leptospiral serovars in rodents, humans, and cattle in the Tanga region, Tanzania.

Table 5. Logistic Regression to assess the association of seroprevalence of Leptospira antibodies in humans, rodents, and cattle to different variables





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`TABLES (1-5).docx` available at https://authorea.com/users/847058/articles/1234987-detection-and-determinants-of-leptospira-infection-in-rodents-cattle-and-humans-in-muheza-district-tanzania-a-one-health-appeal