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Research Reports

Detection of Leptospiral Antibodies in Rodents and Shrews in Human Residence Vicinity in Kilwa District: A Potential Public Health Risk

Deteksi Antibodi Leptospira pada Rodensia dan Curut di Sekitar Perumahan Manusia di Distrik Kilwa: Potensi Risiko Kesehatan Masyarakat

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ABSTRACT

Background: Leptospirosis is a worldwide neglected zoonotic infection that affects both humans and animals in tropical and subtropical countries. **Purpose:** The objective of this study was to determine the presence of leptospiral antibodies in rodents and shrews, as evidence of public health threat. **Method:** Sera from 202 rodents and shrews were prepared and tested against five live antigens to detect leptospiral antibodies by using microscopic agglutination test. Leptospiral serovars commonly reported in Tanzania namely; Sokoine, Pomona, Hebdomadis, Lora, and Grippotyphosa were used in this study. Face-to-face interviews about cause, clinical signs, transmission, treatment and risk practices were conducted. **Results:** The overall prevalence of leptospiral antibodies in rodents and shrews was 14.36% (95% CI:0.0983-0.1996), whereby mastomys natalensis was a more predominant positive host species. Serovar Sokoine was more prevalent compared to other tested serovars. The antibody titers obtained in this study ranged from 1:20 to 1:80, which suggests a long-standing exposure of rodents and shrews to different leptospiral serovars. Awareness of respondents about leptospirosis was below 50% in all aspects of enquiry. **Conclusion:** Given the human economic activities and the ecology of rodents and shrews in study area, the findings of this study suggest a public health threat. Therefore, rodents and shrews control should be encouraged but also public and institutional efforts to prevent outbreaks are recommended.

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ABSTRAK

Latar Belakang: Leptospirosis adalah infeksi zoonosis terabaikan di seluruh dunia yang mempengaruhi manusia dan hewan di negara-negara tropis dan subtropis. **Tujuan:** Untuk menentukan keberadaan antibodi leptospira pada hewan pengerat dan curut, sebagai bukti ancaman kesehatan masyarakat. **Metode:** Serum dari 202 hewan pengerat dan tikus tanah disiapkan dan diuji terhadap lima antigen hidup untuk mendeteksi antibodi leptospiral dengan menggunakan uji aglutinasi mikroskopis. Serovar leptospiral yang umum dilaporkan di Tanzania yaitu; Sokoine, Pomona, Hebdomadis, Lora, dan Grippotyphosa digunakan dalam penelitian ini. Wawancara tatap muka tentang penyebab, tanda-tanda klinis, penularan, pengobatan dan praktik risiko dilakukan. **Hasil:** Prevalensi keseluruhan antibodi leptospiral pada tikus dan curut adalah 14,36% (95% CI: 0,0983-0,1996), di mana mastomys natalensis adalah spesies inang positif yang lebih dominan. Serovar Sokoine lebih prevalen dibandingkan dengan serovar lain yang diuji. Titer antibodi yang diperoleh dalam penelitian ini berkisar antara 1:20 hingga 1:80, yang menunjukkan paparan tikus dan curut yang lama terhadap serovar leptospira yang berbeda. Kesadaran responden tentang leptospirosis di bawah 50% dalam semua aspek penyelidikan. **Kesimpulan:** Mengingat aktivitas ekonomi manusia dan ekologi tikus dan curut di area penelitian, temuan penelitian ini menunjukkan ancaman kesehatan masyarakat. Oleh karena itu, pengendalian tikus dan curut harus didorong tetapi juga upaya publik dan kelembagaan, untuk mencegah wabah sangat direkomendasikan

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Kata kunci: Antibodi; Curut; Leptospira; Rodensia; Zoonosis

INTRODUCTION

Leptospirosis stands as an under addressed global public health concern, affecting both humans and animals across the world (Holt, *et al.,* 2006; Kimari, 2016). This disease is caused by pathogenic gram-negative spirochete bacteria which belong to the Leptospira genus. The genus encompasses over 250 pathogenic serovars (Alinaitwe, *et al.,* 2020; Biscornet, *et al.,* 2021). Some of these serovars are restricted to specific geographic regions and are harbored exclusively by particular species of wild and domestic animals. The incidence rate of the disease in humans is estimated to affect 1.03 million individuals annually and results in approximately 58,900 deaths, with the majority of cases being reported in tropical and subtropical regions (Costa, *et al.,* 2015; Torgerson, *et al.,* 2015; Boey, *et al.,* 2019). Leptospirosis continues to be a neglected disease despite its substantial impact, primarily because it disproportionately affects the most impoverished populations and the presence of nonspecific symptoms, which makes its diagnosis even more challenging (Picardeau, 2015; Biscornet, *et al.,* 2021). The bacteria have the capacity to infect all mammals, taking up residence in the renal tubules and urogenital tracts, where they can multiply and eventually be expelled in the host animals' urine (Bharti, *et al.,* 2003; Kimari, 2016). In humans, infection can occur through direct contact with the urine or body fluids of infected animals, or indirectly through exposure to contaminated water or soil (Allan, *et al.,* 2015; Ngugi, *et al.,* 2019). Both wild and domestic animals serve as reservoirs for microorganisms, playing a crucial role in transmitting these pathogens to humans. The principal reservoir hosts are wildlife, particularly rodents, which can continuously shed leptospires throughout their lifespans. Additionally, animals such as cattle, goats, sheep, donkey, pigs, dogs, horses, dogs and rodents are also an important source of human infections (De Vries, *et al.,* 2014).

Leptospiral infection in cats has been linked with consumption of contaminated prey, involving serovars of the Autumnalis and Ballum serogroups. Due of their direct contact with reservoir hosts, outdoor cats are more likely to contract leptospires. Cats in rural regions might also contract the disease from pig and cow urine (Murillo, *et al.,* 2020). The presence of a cat in the household significantly increases the risk of seropositivity for leptospirosis (Adler and De la Peña Moctezuma, 2010; Murillo, *et al.,* 2020). The common serovars affecting dogs includes Canicola, Icterohaemorrhagiae, Grippotyphosa and Pomona, which lead to manifestation ranging from asymptomatic, acute leptospirosis, and chronic kidney and liver disease (Costa, *et al.,* 2022).

Wild animals especially rodents and shrews acquire chronic infection, without clinical disease and remain permanent carrier and shedder of leptospires. Rodents are therefore considered as the major reservoir of leptospira infection globally (Holt, *et al.,* 2006). With increasing urbanization, changes in land use and climate change, interaction between humans, livestock and wildlife has increased (Cheng, *et al.,* 2022). The increased interaction leads to close contact among the host components. The increased contact may lead to transmission of diseases including leptospirosis (Hassell, *et al.,* 2017; Ekwem, *et al.,* 2021). Land cultivation, rodents entering human houses, sharing of surface water, movement of livestock and wildlife and consumption of rodents may expose humans to diseases (Suwannarong, *et al.,* 2022). Direct physical contacts with rodents such as hunting, killing, preparing rodents as food, consuming rodent meat, cleaning feces and clearing carcasses are associated with rodent-borne leptospiral infection in human (Suwannarong, *et al.,* 2022). Despite being neglected in Tanzania, human leptospirosis has been reported for long time in different geographical settings such as Rukwa, Kilimanjaro, Morogoro, and Dodoma (Mach-ang'u, *et al.,*1997; Biggs, *et al.,* 2011; Crump, *et al.,* 2013; Allan, 2016; Mgode, *et al.,* 2021). In 2022, following an outbreak of leptospirosis in Ruangwa district, Lindi region, three people died and 20 leptospirosis cases were confirmed (Masunga, *et al.,* 2022).

Leptospirosis in human beings, which is called “Mgunda” in local language, was characterized in the outbreak by high fever, headache, chills, vomiting, jaundice, muscle aches, rash and abdominal pains, nasal bleeds and fatigability (Masunga, *et al.,* 2022; WHO, 2011). These signs resemble clinical presentation of other diseases like malaria, typhoid fever, dengue, and others. With reference to the most recent outbreak in Ruangwa district, Lindi region, Tanzania, leptospirosis in humans can be controlled through a combination of approaches. Early detection and treatment of cases by using doxycycline (200 mg orally) is reported to be effective, while other measures such as raising public awareness, improved surveillance, improvement of reliable diagnostics, use of personal protective equipment and improved sanitation are recommended (Masunga, *et al.,* 2022).

Despite its potential to affect cattle, wildlife, and public health, leptospirosis is still a neglected zoonotic disease in Tanzania. The disease is often misdiagnosed due to clinical similarities with other febrile illnesses like malaria and typhoid, leading to underreporting and poor disease management. Effective control methods are hindered by a lack of epidemiological data, particularly in rural and flood-prone areas. Furthermore, little is known about how rodents and domestic animals contribute to the cycle of transmission, which leaves gaps in surveillance and intervention plans (Mgode, *et al.,* 2021).The objective of this study was to determine evidence of leptospiral infection in rodents and shrew in Kilwa district by detecting leptospiral antibodies. Kilwa district is found in Lindi region, where a recent outbreak occurred, and it is along the way to Dar es Salaam, a major commercial city of Tanzania. Information from this study provides important surveillance data regarding the presence of circulating causative agent in reservoir animals, distribution of population at risk and possibility of leptospirosis transmission to Dar es Salaam and other regions linked to it. Such information is essential for the disease prevention and control strategies.

MATERIAL and METHOD

Description of the Study Site

Kilwa district council is one of the six councils located within the Lindi Region in the southern part of Tanzania. Geographically, it is situated between latitude 8°20' to 9°56' and longitude 38°36' to 39°50' east of the Greenwich Meridian. Kilwa district shares its borders with Rufiji District in the north (located in the Coast Region), Lindi, and Ruangwa Districts in the south, Liwale District in w, and the Indian Ocean to the east (Figure. 1). The total land area of the district is 13,347.50 square kilometers, comprising of 12,125.9 square kilometers of land and 1,221.52 square kilometers of ocean (URT, 2022). According to the 2022 national census, Kilwa has a population of 297,676 individuals, with 145,343 being males and 152,333 being females. The climate in the area is characterized as hot and humid, with average temperatures ranging from 22°C to 30°C. Humidity levels are high, typically around 98-100% during the long rainy season, which brings an annual rainfall range of 800-1400 mm. This rainy period coincides with the onset of each monsoon: the long rains (masika) occur from mid-March to May, while the short rains (vuli) fall between October and December. Kilwa district's drainage pattern is shaped by three major rivers Matandu, Mavuji and Mbwemkuru as well as ten seasonal streams. The predominant soil type is brownish-grey alluvial sandy soil, although alluvial soils can also be found along the Mavuji, Matatu, and Mbwemkuru rivers. The district's primary economic activities revolve around agriculture, which engages 81% of the labor force. Other key economic pursuits include livestock farming, fishing (especially along the coastal areas), tourism, wildlife, beekeeping, and trade (Ichumbaki and Mapunda, 2017; Lubao and Ichumbaki, 2022; URT, 2022). All these features in the study area favor survival and transmission of leptospirosis to humans and animals.

Study Design and Sampling Strategies

A cross-sectional study design was carried out from January to March 2023 in Kilwa district, southern Tanzania, to assess the presence of leptospiral antibodies in rodents and shrews, public awareness and socioeconomic characteristics of respondents in relation to leptospirosis. To achieve this, a random selection of six specific locations for sampling was carried out, and Kiranjeranje, Mavuji, Kivinje, Nangurukuru, Masoko and Hoteli Tatu villages were selected. The sample size of 202 for rodents and shrews was determined by formula

$$n=Z\alpha^2 \, p \, (1-p)/ d^2$$

whereby n= sample size, p=previous reported prevalence of leptospiral antibodies in rodents, 15.5% (Mgode, *et al.,* 2021), d= desired precision, 5%, Zα= statistics corresponding to 5% level of confidence, 1.96. These 202 rodents were trapped in different locations, such as human dwellings, grain storage facilities, forest areas, agricultural land, and fallow lands located in close proximity to human residences. The setup of the traps matched a total of 130 households which were within the trapping area. These 130 households were involved in questionnaire interview, in which the household head or adult representative responded to questions.

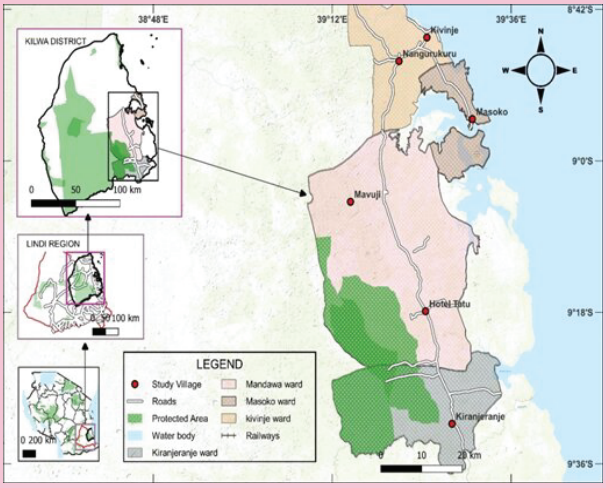

Figure 1. Map of Kilwa District Showing Study Village

Rodent Trapping

Rodent and shrew were captured from different selected places from January to March 2023. Trapping was carried out indoors and outdoors around human households. Also, capture was done in the farms that were at close proximity to human settlement (peri-domestic) such as cultivated, fallow lands, and grazing pasture lands. In the outdoor trapping, different traps were used such as Sherman live rodent traps, (FL, USA), wire cages and Hava Hart traps, while indoor, locally made live traps were used. In human houses, traps were placed in kitchens and on top of shelves where food is stored as well as in living places (Kimari, 2016). Peanut butter blended with maize flour was used as bait for the traps to attract the rodents. The traps were placed at each site for a minimum of three consecutive days, which is equivalent to three trap nights, before being relocated to a different spot. In the study area, a total of 250 trapping sites were established. In the households, a trapping site included dwelling, kitchen and store room. For the outdoor exercise, traps in forest areas, agricultural land, and fallow lands were placed at least 25 meters from one another to account for dependence between captured animals. Every morning, the traps were checked for any captured animals. At each trapping site one rodent/ shrew was randomly picked for further investigation and others were released.

Blood Sample Collection and Processing

Rodent and shrews were sent to the field laboratory for collection of blood samples. Prior to blood sample collection, external morphological features were observed and necessary measurements were taken for primary identification of the captured animals and age estimation. Such measurements included body weight, total length, tail length and ear length and hindfoot length as previously described (Mgode, *et al.,* 2021). Captured rodents and shrews were euthanized humanely by using di-ethyl ether then followed by taking blood from the supraorbital vein or by heart puncture. The collected blood was allowed to settle and serum was separated at room temperature. Serum samples obtained were stored at -20°C to be used later for serological tests (Cole, *et al.,* 1973; Goris and Hartskeerl, 2014).

Microscopic Agglutination Test

Microscopic agglutination test (MAT) was used to determine antibodies against Leptospira as described by (Faine, *et al.,* 1999; Goris and Hartskeerl, 2014). Five leptospiral serovars which are most common in Tanzania, namely *Leptospira kirschneri* serogroup Grippotyphosa serovar Grippotyphosa, *L. kirschneri* serogroup Icterohaemorrhagiae serovar Sokoine, *L. interrogans* serogroup Australis serovar Lora, *L. interrogans* serogroup Pomona serovar Pomona and *L. santarosai* serovar Hebdomadis were used as an antigen in this study as previously done (Machang'u, *et al.,* 2004; Mgode, *et al.,* 2006). Cultures were obtained using modified Ellinghausen and McCullough medium, after 5-7 days of incubation at 30°C. Cultures with a density of approximately 300 × 108 leptospires/mL were considered to be suitable to be used as live antigens. Before adding 50 µl of the full-grown antigens to all microtiter plate wells, 10 µl of sera samples were diluted with phosphate-buffered saline (PBS) to obtain 100 µl of diluted sera in microtiter plate to obtain an initial dilution range of 1:20–1:160. Then the mixtures were incubated for two hours at 30°C. After two hours the serum-antigen mixtures were visualized under a dark field microscope to determine the presence of agglutination. All samples that tested positive against antigen at ≥1:20 were further titrated to determine the end titer point from dilution of 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, 1:1,280, 1:2,560, 1:5,120, 1:10,240 and 1:20,480 (Mgode, *et al.,* 2021).

Socio-Demographic and Leptospirosis Awareness Data Collection

Structured questionnaire was reviewed by public health professionals and pre-tested and administered to 130 respondents whose areas (residence, farms, forests and fallow lands) were involved in trapping of rodents and shrews. Face-to-face interviews were conducted at residential areas of respondents. The questionnaire asked about socio-demographic information such as education, marital status, presence of livestock at home, occupation and the norm of consuming rodents. On the other hand, the questionnaire sought answers about awareness of respondents in relation to leptospirosis. In this study, awareness means the way in which a respondent relates to (or has insight into) leptospirosis as a problem. Awareness assessment is an experiential enquiry that helps to determine what strategy to employ in solving a problem (Markova´and Berrios, 2006). Questions about the cause, transmission, clinical signs, zoonotic potential, diagnosis, risky practices, treatment and preventive measures of leptospirosis, were asked. The respondent was required to give the answer either in agreement with the leptospirosis related proposition or not. The questionnaire took approximately 30 minutes to complete.

Data Analysis

Data were entered and organized and cleaned using Microsoft Excel 2019 before actual analysis. Summary statistics such as means, proportions and percentages for both interview and laboratory data were computed and data are presented in text, tables and graphs. The prevalence of leptospirosis in small mammals (rodents and shrews) was computed by taking the number of samples that tested positive for leptospiral antibodies and dividing by the total number of samples examined. Plots were created using the ggplot2 package using R statistic software version 4.2.2 (Bates *et al.,* 2015). The association between the seropositivity and different characteristics such as habitat, site, sex, and serovars were determined by using Fisher's exact test or Pearson's Chi-square test. The findings were considered statistically significant at $p \leq 0.05$.

RESULTS

Rodents and Shrews Captured in the Study Area

A total of 202 animals were captured from farms, domestic, peri-domestic and fallow land areas in Kilwa district. Among them, 200 were rodents from six genera (*Mastomy natalensis*, *Aethomys s., Acomys spp., Grammomys spp., Rattus sp.* and *Tatera spp.*) and two shrews. *Mastomys natalensis* was the predominant species making 66.83 percent of the trapped animals while the least captured species was *Aethomys species* (0.5%) (Table 1).

Table 1. Composition of Captured Rodent Species and Shrew

Type	Genus/Species	Number	Percentage Composition(%)
Rodents	*Acomys spp.*	7	3.5
	Aethomys spp.	1	0.5
	Grammomys spp.	7	3.5
	Mastomys natalensis	135	66.8
	Rattus sp.	35	17.3
	Tatera spp	15	7.4
Shrews	Shrews	2	1.0
TOTAL		202	100.0

Prevalence of Leptospira Antibodies

Out of the 202 serum samples, 29 tested positive to at least one of the serovar Sokoine, Grippotyphosa, Hebdomadis, Lora, and Pomona. The overall prevalence of leptospiral antibodies in rodents and shrews was 14.36% (95% confidence interval: 9.83–19.96) (Table 2). Among 29 positive samples, 27 were from rodents and two were from shrews. Sokoine was the predominant serovar that reacted positively among the tested serovars (Table 3). On the other hand, mastomys natalensis had a large percentage of about 66.83% of the small mammal species that tested positive followed by *Rattus sp.* (17.33%) and *Tatera spp.* 7.43%. Other species like *Aethomys spp., Grammomys spp., Tatera spp.,* tested negative against all five leptospiral serovars (Figure 2). The antibody titers that reacted positively with leptospiral serovars were between 1:20 to 1:80, 14 samples reacted positively at titer 1:40, eight samples reacted positively at titer 1:80 and seven samples reacted positively at titer 1:20 (Table 3). *Mastomys natalensis* reacted positively with all titers followed by *Rattus sp.* which reacted with two titers (1:20 and 1:40) while *Acomys sp.* reacted to one titer (1:40) and shrews reacted to one titer (1:20) (Figure 2). Also, Table 4 shows the association between prevalence of leptospiral antibodies with different characteristics such as species, habitat, sites, sex and serovars. All these

Table 2. Prevalence of Leptospiral Antibodies in Rodents and Shrews

Serovars	Number of Animals Tested	Leptospirosis positive (1:20 – 1:80 titers)	Serovar Prevalence (%)
Griptyphosa	202	5	2.48
Hebdomadis	202	4	1.98
Lora	202	4	1.98
Pomona	202	6	2.97
Sokoine	202	10	4.95
Total	202	**29**	14.36

Table 3. Antibodies Titers of Small Mammals Tested With Five Leptospiral Serovars

Titer	Leptospiral Serovars				
	Griptyphosa	Hebdo	Lora	Pomona	Sokoine
1:20	1	0	0	3	3
1:40	1	3	3	2	5
1:80	3	1	1	1	2
Total	5	4	4	6	10

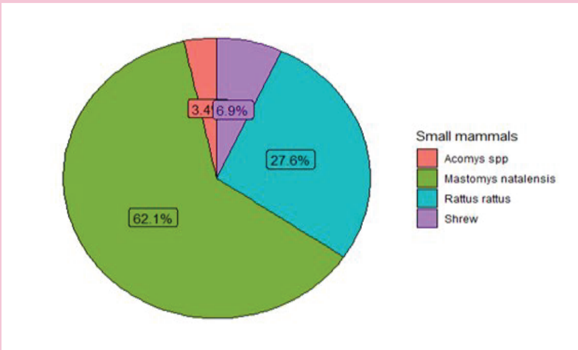

Figure 2. Percentage of Small Mammal Species that Reacted Positively with Leptospiral Serovars.

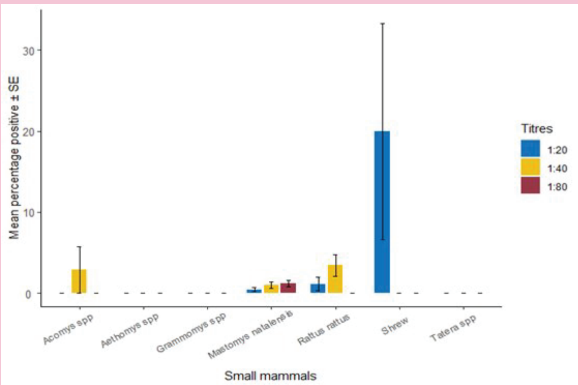

Figure 3. The Proportion of the Leptospiral Antibody Titers across Different Small Mammal Species.

characteristics were not significantly associated with the prevalence of leptospiral antibodies. The prevalence within species of rodents and shrews had a p-value of 0.058, and within the habitat, the p-value was 0.7, also within sites, sex, and serovars p-value were 0.5, 0.5 and 0.4, respectively.

Socio-Demographic Information of Respondents

Among the 130 respondents, 70 (53.8%) were males and 60 (46.2%) were females. Among the respondents, 78 (60%) were married, which suggests that majority of them live in families. The age categories (18-25, 26-35, 36-45, 46-55, and >55 years) of respondents were equally represented at around 15 to 25% as shown in Table 5. More than 50% of respondents had at least secondary education while 16.2% had informal education. Majority of the respondents, 125 (96.2%) were involved in livestock keeping, that is they kept at least one among cattle, goats or sheep species. Other economic activities in which respondents were involved include crop growing 102 (78.5%) and fishing 17 (13.1%). A total of 18 (13.8%) of respondents reported that they consume rodent as food. The summary of socio-demographic characteristics of respondents is summarized in Table 5.

Awareness of Respondents about Leptospirosis in Humans

A large proportion of respondents 76 (58.5%) have heard about leptospirosis in humans. However, 73.8% of respondents did not agree that leptospirosis is caused by microorganisms. On the other hand, about 100 (77%) of respondents did not agree that urine of infected animal can transmit leptospirosis, although 40% of them knew that rodents were responsible for transmission of the disease. Less than 25 (19.2%) of respondents knew that consumption of contaminated food and water can lead to leptospiral infection. Additionally, about 59 (45%) respondents agreed that leptospirosis can cause human death, while 101 (77.7%) denied that leptospirosis is a zoonotic disease. Moreover, less than 42 (32.3%) respondents agreed that leptospirosis patient may develop jaundice, liver problems or kidney failure and about 46 (35%) of them admitted that the disease is treatable at hospital. Furthermore, less than 31 (24%) agreed that putting on protective gear such as rubber boots and gloves and avoiding direct contact with flood water can prevent contamination and transmission of leptospirosis (Table 6).

DISCUSSION

Seroprevalence of Leptospiral Antibodies

The present study has reported seroprevalence of leptospiral antibodies of 14.36% (95% CI:0.0983-0.1996) in rodents and shrews. This is an indication that this small mammal harbors leptospiral bacteria. The reported figure is smaller than that of 15.5% (95% CI:0.0649-0.2946) reported in Dodoma and 17% (95% CI:0.0964-0.260 reported in Morogoro (Mgode, 2015, 2021). On the other hand, the finding of the current study is higher than reports from other studies in Tanzania. For instance, the seroprevalence of leptospira antibodies in rodents and shrews of 11.9% has been reported in Kakonko, Kigoma (Majawa, *et al.,* 2023), and 7.6% in Unguja Island (Ally, *et al.,* 2023). In all these studies (the current and previous studies), MAT was used to detect the presence of leptospira antibodies in rodents and shrews. The differences between these research outcomes could be due to differences in habitats and geo-climatic variations. Regardless of the variation in the proportions of positive samples, the message is that leptospira infection risk to humans and other animals is widespread across Tanzania. Detection of evidence of leptospira infection in animals other than rodents and shrews has been reported in different areas. The animal species include the African giant pouched rats *(Cricetomys spp.)* in the

Tabel 4. Association Between the Leptospiral Infection and Different Characteristics

Characteristic	Overall, N = 1,010	-Ve, N = 981	+Ve, N = 29	p-value*
Small Mammals,	(%)	(%)	(%)	0.058
Acomys spp.	35 (3.5)	34 (3.5)	1 (3.4)	
Aethomys spp.	5 (0.5)	5 (0.5)	0 (0)	
Grammomys spp.	35 (3.5)	35 (3.6)	0 (0)	
Mastomys natalensis	675 (67)	657 (67)	18 (62)	
Rattus sp.	175 (17)	167 (17)	8 (28)	
Shrew	10 (1.0)	8 (0.8)	2 (6.9)	
Tatera spp.	75 (7.4)	75 (7.6)	0 (0)	
Habitat,				0.7
Domestic	95 (9.4)	91 (9.3)	4 (14)	
Fallowland	150 (15)	146 (15)	4 (14)	
Farm	420 (42)	410 (42)	10 (34)	
Peri-domestic	345 (34)	334 (34)	11 (38)	
Site,				0.5
Hoteli tatu	160 (16)	155 (16)	5 (17)	
Kiranjerange	150 (15)	147 (15)	3 (10)	
Kivinje	135 (13)	128 (13)	7 (24)	
Masoko	140 (14)	135 (14)	5 (17)	
Mavuji	110 (11)	107 (11)	3 (10)	
Nangurukuru	150 (15)	147 (15)	3 (10)	
Sex,				0.5
F	340 (34)	332 (34)	8 (28)	
M	670 (66)	649 (66)	21 (72)	
Serovars,				0.4
Griptyphosa	202 (20)	197 (20)	5 (17)	
Hebdo	202 (20)	198 (20)	4 (14)	
Lora	202 (20)	198 (20)	4 (14)	
Pomona	202 (20)	196 (20)	6 (21)	
Sokoine	202 (20)	192 (20)	10 (34)	

Note: N = Number sample tested. * Stand for significance at p ≤ 0.05, Fisher's exact test; Pearson's Chi-square test.

Tabel 5. Socio-demographic Characteristics of Respondents in Study Area.

Variable	Category	Frequency	Percentage
Sex	Male	70	53.8
	Female	60	46.2
Age (years)	18 – 25	23	17.7
	26 -35	25	19.2
	36 – 45	29	22.3
	46 – 55	33	25.4
	>55	20	15.4
Marital Status	Single	19	14.6
	Married	78	60
	Divorced	10	7.7
	Widow	12	9.2
	Widower	11	8.5
Education	Informal	21	16.2
	Primary	51	39.2
	Secondary	45	34.6
	Post-secondary	13	10.0
Livestock Keeping	Yes	125	96.2
	No	5	3.8
Cropping	Yes	102	78.5
	No	28	21.5
Fishing	Yes	17	13.1
	No	113	86.9
Merchandize	Yes	5	3.8
	No	125	96.2
Rodent Consumption	Yes	18	13.8
	No	112	86.2

Ngorongoro Conservation Area (Kahangwa, *et al.,* 2024), bats, buffalo, cats, cattle, fish, dogs, goats, lion, and pigs (Motto, *et al.,* 2021). This shows how wide the reservoir spectrum is. The detection of leptospiral antigens in more than 10 regions of Tanzania at different consecutive times in two decades suggests that the risk of circulating leptospira is endemic in Tanzania (Motto, *et al.,* 2021). Therefore, stringent measures from different disciplines may be a necessity if this neglected disease is to be contained.

The current study reports low leptospiral antibody titers in both rodents and shrews which are below the common cut-off point of 1:160 (Goris, *et al.,* 2012). The lower titers suggest chronic exposure or prolonged exposure of animals to the pathogen. This is associated with the low level of IgG that can be below the detection thresholds of microscopic agglutination test (Mgode, *et al.,* 2014). Despite low antibody titer, rodents and shrews can still shed leptospires in their urine due to the fact that low antibody titers from microscopic agglutination test do not indicate the absence of infection as rodents and shrews can harbor bacterial in their kidney and shed it (Boey, *et al.,* 2019). Some studies have reported higher antibody titers than the threshold. For instance, studies in Thailand (Suwannarong, *et al.,* 2022) and Southern Chile (Balcázar, *et al.,* 2024) where leptospirosis is not endemic, had antibody titers above the threshold by microscopic agglutination test. This scenario is indicative of recent carriers in those areas. On the other hand, mixed exposure status, that is chronic or recent carrier and recent/active exposure, have been reported elsewhere. Studies on seroprevalence of leptospiral antibodies in Kakonko, Kigoma, Tanzania (Majawa, *et al.,* 2023), Unguja Island (Ally, *et al.,* 2023) and China have reported a mixture of higher and lower antibody titers than thresholds. This situation shows that some subjects had a prolonged exposure while others have recent first-time encounter. This sort of result enriches the epidemiological understanding of the risk in specific study areas. On the other hand, a study in Bahi, Dodoma, Tanzania (Mgode, *et al.,* 2021) has reported lower antibody titers than threshold in all sera samples. This situation represents a long standing/ chronic exposure of the hosts to leptospires.

Economic Activities and Socio-Cltural Factors

Economic activities and socio-cultural factors in the study area may have influence for potential leptospirosis outbreak in humans. In Kilwa district, fishing is one of the important economic activities and which promotes high interaction between people and reservoirs especially in warehouses and food-selling premises. On the other hand, cultivation is common in Kilwa district. This activity leads to direct human-soil-rodent contact, and hence increased risk of leptospirosis transmission. Moreover, it is a common norm for some community members in Kilwa district to use rodents as a source of food just like in other communities reported elsewhere (Suwannarong, *et al.,* 2022). Catching, handling and consumption of rodents increases the chance of direct contact between humans and rodents. Increased contact between humans and rodents poses a high leptospiral transmission risk and this is supported by different scientific reports (Sarkar, *et al.,* 2002; Halliday, *et al.,* 2013). Therefore, control and management of rodents and shrews is inevitable for prevention of disease transmission and outbreak. The study area, Kilwa, is along the highway connecting Lindi region (where the recent leptospirosis occurred) and Dar es Salaam, the main commercial city of Tanzania. Therefore, detection of leptospiral antibodies in rodents and shrews suggests that the risk of contact to human hosts is evident, such that transmission to Dar es Salaam and other areas which are commercially and socially connected to Dar es Salaam is possible. This finding, therefore, adds to the epidemiology of leptospirosis by identifying the risk (presence of circulating leptospiral bacteria) but also shows the distribution of the population at risk. The current study assessed the evidence of leptospiral infection in rodents and shrews by using five common leptospiral serovars in Tanzania. Rodents and shrews could be exposed to other serovars as explained by other literature (Mgode, *et al.,* 2021). This is the limitation of the current study. But also many participants in Kilwa District were not familiar with leptospirosis, making it difficult to evaluate their true knowledge and willingness to participate.

CONCLUSION

Rodents and shrews in humans' living houses, forests, farms and fallow land in Kilwa district are positive for all five tested leptospiral serovars. The leptospiral antibody titers suggests a chronic carrier status. Therefore, rodents and shrews harbor leptospiral for a prolonged time period. But also there is a traditional culture of eating rodents and economic activities in study area such as livestock keeping, crop growing and fishing, potentiate human and livestock contact with rodent and shrews and their secretions. This scenario suggest a public health threat. We, therefore, recommendpublic awareness on leptospirosis among Kilwa district communities and control and management of rodents and shrews.

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CONFLICT of INTEREST

The authors declare no conflicts of interest. The funding agencies had no role in the study design, data collection, analysis, or interpretation, nor in the writing of the manuscript or the decision to publish the results.

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ETHICAL APPROVAL

Ethical clearance for this study was approved from the Institutional Review Board of the Sokoine University of Agriculture with reference number (SUA/DPRTC/R/186/39) issued on 14/08/2023.Furthermore, permission was also granted from the Kilwa district administrative authorities to allow the research activity in all respective study sites (wards and villages) prior to conducting the study with Ref No. EA.76/249/03/35 issued on 03/02/2023. Prior to collecting data, participants were informed about the study objectives and given the opportunity to provide verbal consent.

AUTHORS' CONTRIBUTIONS

Conceptualization, data curation, formal analysis, and writing of the original draft: MCK as principal investigator (PI). Methodology, supervision, review, editing, and re-writing of the manuscript: AASK and AML as the main supervisors. All authors have read and accepted to be published final version of the manuscript

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